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► **To cite this version:**

Fany Sardenne, Nathalie Bodin, Leo Barret, Laura Blamey, Rodney Govinden, et al.. Diet of spiny lobsters from Mahé Island reefs, Seychelles inferred by trophic tracers. *Regional Studies in Marine Science*, 2021, 42, pp.101640. 10.1016/j.rsma.2021.101640 . hal-03490581

HAL Id: hal-03490581

<https://hal.science/hal-03490581>

Submitted on 14 Oct 2022

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Diets of spiny lobsters from Mahé Island reef, Seychelles, inferred by trophic tracers

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Abstract

Spiny lobsters (*Panulirus longipes*, *P. penicillatus* and *P. versicolor*) are an important resource in Seychelles, where they inhabit coastal carbonate and granite reefs that have been impacted by multiple coral bleaching events over the past two decades. Little is known about their biology and ecology in this region. Interspecific competition for food resources was previously suggested, but no quantitative data on the diet of spiny lobsters were available. Using carbon and nitrogen stable isotope compositions and fatty acid profiles of three spiny lobster species and their potential prey, a Bayesian mixing model for diet estimation was applied to compare the diet proportions of spiny lobsters among species and between reef types (carbonate and granite reefs). Model outputs suggested the three lobster species consume mainly crustaceans (*Anomoura* hermit crabs; half of the diet), then Echinoidea (sea urchins), algae and molluscs. *P. versicolor* was found to consume slightly more molluscs and algae than the two other studied species, which was consistent with its lower trophic level (2.4 vs 2.8 for the two other species). Trophic level did not increase with carapace length of spiny lobsters, but large individuals had higher carbon isotopic values suggesting that they might feed closer to the coast or more on detritus feeders than their smaller congeners. Diets of spiny lobsters were fairly similar between carbonate and granite reefs, except that lobster inhabiting granite reefs consumed more sea urchins. While our overall findings were consistent with gut contents of *Panulirus* spp. from other world regions, they should be confirmed, as the discrimination of several prey based on trophic tracers was low, which increased mixing model uncertainty.

Keywords: Fatty acids, Stable isotopes, Bayesian mixing model, *Panulirus* sp., tropical reef habitats, Western Indian Ocean.

1. Introduction

To maintain ecosystems in a healthy, productive, and resilient condition, several elements affecting species populations should be considered, such as interactions with other species or effects of environmental changes (Pikitch et al., 2004). To reach this goal, ecosystem-based fisheries management requires fisheries research to account for relationships between target species and their habitats, including trophic interactions (Bellchambers et al., 2010; Tam et al., 2017).

Spiny lobsters *Panulirus longipes*, *P. penicillatus* and *P. versicolor* are important ecological, social and economic resources in the Seychelles Archipelago, a small island state in the Western Indian Ocean (Fig. 1). They are found on shallow coral and granite reefs and in recent years, recurrent coral bleaching events have resulted in the loss of coral cover and structural complexity on these reefs and an increase in macroalgae cover (Graham et al., 2006; Harris et al., 2014). Although variable depending on the year, approximately three metric tons of spiny lobster (*P. longipes*, *P. penicillatus* and *P. versicolor*) are landed each year by the artisanal fishery (Barret, 2019), most of it being sold to local hotels and restaurants. Declines in catch per unit effort (CPUE) (24%) in the 2016–2017 season, and continued suppressed CPUE from independent fishery surveys have resulted in a temporary closure of the lobster fishery (SFA Fisheries Research Section, 2019), and highlighted the need for an ecosystem-based approach to fishery management, particularly given the new ecological conditions, i.e. recurrent coral bleaching events and likely habitat modification.

Despite the importance of spiny lobsters for the Seychelles, little is known about their biology and ecology in the region (Barret, 2019). As part of a precautionary approach to fisheries management, a number of management measures are in place, including the prohibition of traps to catch lobsters (they may only be caught using snorkelling) (Payet and Isidore, 2002). This avoids a possible modification of the diet toward bait, as has been observed in Western Australia (MacArthur et al., 2011). However, the diet of spiny lobsters in the Western Indian Ocean remains

unknown. A recent study based on trophic tracers, i.e. carbon and nitrogen stable isotope compositions (SI; $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) and fatty acids (FAs) profiles, compared trophic niches of three spiny lobster species and found a high probability of dietary competition among these species (Sabino et al., 2020). While this study did not analyse the potential prey of spiny lobsters, results were consistent with the gut content analysis of *Panulirus* spp. in other world regions: *Panulirus* spp. are opportunists, mainly feeding on crustaceans and molluscs, and to a lesser extent on red algae (Briones-Fourzán et al., 2019; Castañeda-Fernández-de-Lara et al., 2005; Joll and Phillips, 1984; Mashaii et al., 2001). However, the proportion of algae might be underestimated due to a rapid gastric evacuation (<12 h; Waddington, 2008). Additionally, the predominant benthic habitat surrounding a reef can be an important source of variation for the diet of spiny lobsters (Blamey et al., 2019; Goñi et al., 2001; MacArthur et al., 2011). Similar variability in diet probably occurs for spiny lobsters from the Seychelles, as their potential for dietary competition was higher in granite reefs than in carbonate reefs (Sabino et al., 2020), probably in relation to the higher prey diversity in carbonate reefs (Robinson et al., 2019). However, quantitative diet studies are needed to better describe their trophic ecology while considering their different types of reef habitat in the Seychelles.

SI and FAs are intrinsic ecological tracers commonly used to infer assimilated diet in consumers (Ramos and González-Solís, 2012), including in spiny lobsters (e.g. Blamey et al., 2019; Guest et al., 2009; Sabino et al., 2020; Waddington et al., 2008). $\delta^{13}\text{C}$ values allow for the discrimination of habitat types (e.g. coastal vs offshore, the latter being characterised by depleted $\delta^{13}\text{C}$ values; (France, 1995) and $\delta^{15}\text{N}$ values inform on the species' trophic level due to a ^{15}N enrichment with increasing trophic levels (Vander Zanden et al., 1997). SI in muscle reflect the diet of spiny lobsters in the last three months (Waddington and MacArthur, 2008). FAs makeup lipids, and FAs of fat depots such as the hepatopancreas in crustaceans, reflect the FA profiles of prey assimilated by consumers in the last few days to weeks, depending on species (Antonio and Richoux, 2016; Shu-Chien et al., 2017). Both SI and FA data can be used for the quantitative diet

reconstruction through mixing models (e.g. Jankowska et al., 2018; Neubauer and Jensen, 2015; Young et al., 2018).

The objective of this study was to compare the trophic ecology of the three tropical spiny lobsters commonly caught in the Seychelles (*P. longipes*, *P. penicillatus* and *P. versicolor*) using SI and FA data from spiny lobsters and their potential prey. Specifically, we were interested in (1) what are the main prey of spiny lobster in Seychelles, (2) do diet and trophic level differ between spiny lobster species, and (3) does spiny lobster diet differ between reef types (carbonate vs granite reefs)?

2. Material and methods

2.1. Organism collection

Spiny lobsters and their potential prey were collected from the shallow coastal waters from reefs around the coasts of Mahé, the main island of the Seychelles Archipelago, home to around 90% of the country's population, and supporting 60% of the licensed lobster fishermen. Based on literature (Table S1), potential lobster prey (n=74 individuals from 13 species and 7 phylogenetic classes) were collected by scuba-divers at four sites (maximum 10 meters depth) (Fig. 1) in April 2018 and October 2019. Spiny lobsters (26 *P. longipes*, 31 *P. penicillatus* and 7 *P. versicolor*) were collected by divers on snorkel from 15 sites (12 granite and 3 carbonate reefs) in October 2018 and May 2019 (Table 1). Granite reefs are granitic rocky reefs with coral growth on the granite substrate, and carbonate reefs are continuous carbonate fringing reefs (Harris et al., 2014). Note that many carbonate reefs have deteriorated or collapsed following multiple coral bleaching events (Obura et al., 2017) and therefore most lobsters in our study were found on granitic reefs. All organisms were frozen at -80°C immediately after collection until further processing (less than seven days after collection) at the Seychelles Fishing Authority Research Laboratory. For spiny lobsters, a sample of hepatopancreas (for FA analysis) and a sample of tail muscle (for SI analysis)

were carefully dissected from the organism. Potential prey were identified to species level whenever possible and their tissues were sampled for both FA and SI analysis. For echinoderms, a sample of muscle was carefully dissected from the Aristotle's lantern; for bivalves, gastropods and anomurans (decapod malacostracans — hermit crabs), the whole-body tissue was collected after removing the shells; finally, polyps of *Palythoa* (anthozoan) were kept whole and algae (green algae of the Chlorophyta phylum; brown algae of the Ochrophyta phylum; and red algae or Rhodophyta) were kept with their small epiphytes (<1 mm).

All prey and spiny lobster samples were then kept at $-80\text{ }^{\circ}\text{C}$ for a maximum of five months, freeze-dried for 72 h, then ground to a homogeneous powder with a ball mill, and stored again at $-80\text{ }^{\circ}\text{C}$ prior to subsequent analysis.

2.2. Stable isotope analysis

For potential prey items, carbon isotope composition was analysed on a subsample acidified with HCl at 0.5 to 2 N (according to the effervescence) to remove carbonates, then rinsed three times with distilled water, and freeze-dried for 48 hours. Nitrogen isotope composition was analysed on a subsample of the bulk powder. For lobster muscle, the bulk powder was analysed for both carbon and nitrogen isotope composition, as the muscle did not contain carbonate neither high lipid content (fat content is 0.7% wet weight and C:N ratio remains < 3.5). Acidified and bulk powders were weighted into tin capsules and analysed by continuous flow on a Flash EA2000 elemental analyser coupled to a Delta V Plus isotope ratio mass spectrometer (Thermo Fisher scientific) at the Pôle Spectrométrie Océan, University of Brest, France, or on a Flash EA1112 elemental analyser coupled to a Delta V Advantage isotope ratio mass spectrometer (Thermo Fisher scientific) at the LIENSs stable isotope facility, University of La Rochelle, France. Calibrations were based on reference materials (IAEA-600, IAEA-CH-6 and IAEA-N-2). Results were reported in the δ unit notation and expressed as parts per thousand (‰) relative to the international standards

(atmospheric N₂ for nitrogen and Vienna-Pee Dee Belemnite for carbon). Analytical precision based on replicate measurements of acetanilide was < 0.15 ‰ for both δ¹³C and δ¹⁵N values at both laboratories. Inter-laboratory replicate measurements on reference materials (n=9), acetanilide (n=3), urea (n=3), casein (n=3), and lobster samples (n=3) found no significant differences in results between laboratories, with all differences below the analytical precision (< 0.15 ‰). C:N ratios were determined from % element weights.

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laboratories, with all differences below the analytical precision (<0.15 h). C:N ratios were determined from % element weights.

2.3. Fatty acid analysis

Subsamples of dry powders (n=141) were treated as described in Sardenne et al. (2019). Lipids were extracted from 50–100 mg of dry tissue with 6 mL of CHCl₃:MeOH (2:1, v/v) mixture directly added into glass vials. Extracts were flushed with nitrogen, vortexed, sonicated for 15–20 min, and stored for 24 h at –20 °C. Tricosanoic acid (23:0) was added as an internal standard to 2 mL of lipid extract. Total lipids were transesterified with 800 µL of H₂SO₄ (3.8% in MeOH) at 100 °C for 10 min then washed with hexane-saturated distilled water. Fatty acid methyl esters (FAMES) and dimethyl acetals (DMA; from vinyl ether lipids) were separated and quantified on a Varian CP8400 gas chromatography equipped with a Zebron ZB-WAX column (30 m length, 0.25 mm internal diameter, 0.25 µm film thickness; Phenomenex) and a flame ionisation detector at the Lipidocean facility, University of Brest, France. Samples were injected in splitless mode at 280 °C and carried by hydrogen gas. The oven temperature was raised from 60 °C to 150 °C at 50 °C min⁻¹, to 170 °C at 3.5 °C min⁻¹, to 185 °C at 1.5 °C min⁻¹, to 225 °C at 2.4 °C min⁻¹ and then to 250 °C at 5.5 °C min⁻¹. FAMES were identified by comparing sample retention times to those of four external standard mixtures (37-components, BAME, PUFA no. 1, and PUFA no. 3 FAME mix; Supelco) using Galaxie 1.9.3.2 software (Varian). The mean analytical variability of the method was 8.1%, based on Supelco 37-component FAME mix routinely checked. Individual FA results were expressed as a percentage of the total identified FAs, for 44 FAs accounting for > 1.5% in at least two samples. Total fat content was calculated as the sum of individual fatty acids (based on 23:0 concentration) and expressed in µg mg⁻¹ of dry tissue.

2.4. Data analysis

2.4.1. Fractionation factors (FFs) and trophic level calculation

Calculation of trophic levels and Bayesian mixing model include FFs (also called ‘trophic enrichment factors’ or ‘trophic discriminant factors’) to take into account for isotopic enrichment in heavier isotopes (^{13}C and ^{15}N) between successive trophic levels, i.e. between consumers and their prey. Although FFs are commonly set to 0.5‰ and 3.4‰ for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in marine systems, respectively, FFs are influenced by biological factors (e.g., species, tissue, age, food nutrients) (McCutchan et al., 2003; Vander Zanden et al., 2001) and a large range of FF values have been used to compute the trophic level of spiny lobsters based on $\delta^{15}\text{N}$ values: e.g. 3.5‰ for *Jasus edwardsii* (Guest et al., 2009), 2.6‰ for *P. cygnus* (Waddington et al., 2008). To better take into account FFs’ variability in the Seychelles marine food web, FFs were estimated for each potential prey of spiny lobster with simple linear regressions, as FFs tend to decrease with increasing isotopic ratios of prey (Caut et al., 2009). FFs’ estimation was thus based on the isotopic values of potential prey, and a diet experiment on *P. cygnus* (Waddington and MacArthur, 2008) (Fig. S1b). The obtained FFs were used to compute the trophic levels of spiny lobsters (see below) and were included in the Bayesian mixing model (see Section 2.4.2).

Trophic levels were calculated relative to the $\delta^{15}\text{N}$ values of macroalgae (green, brown and red algae, average $\delta^{15}\text{N} = 6.5 \pm 0.5$ ‰, n=18), according to the following equation:

$$\text{Trophic level} = \frac{\delta^{15}\text{N}_{\text{spiny lobster}} - \delta^{15}\text{N}_{\text{macroalgae}}}{\text{FF}_{\text{macroalgae}}} + 1$$

with the average FF between lobster tail muscle and macroalgae previously computed from Fig. S1b: $\text{FF}_{15\text{N}} = 2.9 \pm 0.4$ ‰). This calculation assumes the isotopic baseline to be the same for the three spiny lobster species.

2.3.2. Diet estimation using mixing modelling method

A Bayesian mixing model was run to estimate the proportional contribution of sampled prey to the diet of spiny lobsters, based on SI values and FA profiles summarized through PCA. Because the discriminatory power of mixing models starts to decline above six or seven potential prey items (Phillips et al., 2014), the prey species (n=13) were grouped as much as possible while respecting the phylogeny (n=9 classes) (Table 2). The model was run with two different groupings to compare (i) the diet among the three spiny lobster species, and (ii) the diet of spiny lobsters between reef types (granite vs carbonate reefs, regardless of the species because of low sample size and unbalanced design). The Bayesian mixing model included three types of parameters that were set as follow:

- (i) *Fractionation factors*. For SI, FFs were obtained as described in Section 2.4.1. For FA profiles, FFs were set to 0 as the bioconversion of FA was assumed to be negligible in the hepatopancreas of spiny lobster due to its high proportion of dietary lipids (Shu-Chien et al., 2017).
- (ii) *Concentration dependencies*. Disparities among prey in the concentrations of trophic tracers induce different contributions of each prey to consumers' values (Phillips and Koch, 2002). Percent of carbon and nitrogen elements and total fat content were thus included for each potential prey.
- (iii) *Priors*. Model priors on diet source proportions should be included when potential sources have similar values of trophic tracers (Franco-Trecu et al., 2013). Priors on the nine prey class proportions were thus included, based on the literature on stomach contents for *Panulirus* spp. (Table S1).

Samples for the posterior distributions were drawn by Markov Chain Monte Carlo (MCMC) simulation methods (10,000 iterations, 4 parallel MCMC chains, and 1,000 burn-in rate). Model convergence diagnostics are presented in Fig. S2. Strong negative correlations between prey

proportions may impair model estimations. To avoid this and to obtain a comprehensive overview of the lobsters' diet, proportions of prey classes were grouped a posteriori (Phillips et al., 2014) on a phylogenetic basis (i.e. Mollusc for Bivalvia and Gastropoda classes; Algae for Chlorophyta, Ochrophyta and Rhodophyta phylum).

2.4.3. Variability in trophic tracers and diet estimations

Factors explaining variability in trophic tracers were assessed using ANOVA for univariate data (trophic level, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values), and PERMANOVA for multivariate FA profiles (nontransformed data). SIMPER analysis was used to detect FAs that contribute the most to differences among groups. For potential prey, the influence of the two fixed factors 'Class' (nine phylogenetic classes) and 'Reef type' (granite vs carbonate reefs) was assessed. For spiny lobsters, the influence of 'Species' (*P. longipes*, *P. penicillatus*, and *P. versicolor*), 'Reef type' (granite vs carbonate reefs), 'Carapace length' and the interaction 'Species*Carapace length' were considered as fixed factors. Homogeneity of residuals was tested with Levene's test. Since homogeneity was found for all groups, parametric post hoc Tukey HSD tests were used to refine differences among groups. Diet estimations, i.e. outputs from the Bayesian mixing model, were compared among spiny lobster species and between reef types in the Bayesian framework (no test value, only probabilities were reported).

Data analyses were performed using R software 3.5.0 (R Development Core Team, 2018), the JAGS program, and the "simmr" package (<http://mcmc-jags.sourceforge.net>) (Parnell et al., 2013). All results are reported as means \pm SD.

3. Results

3.1. Variability in trophic tracers among potential lobster prey

Among the potential prey of spiny lobsters, the phylogenetic species class (i.e. ‘Class’) explained most of the variability in trophic tracers, while reef type explained no difference. $\delta^{13}\text{C}$ values varied between species’ class ($F=39.6$, $d=8$, $p<0.001$; 83.4% of explained variability), with values ranging from -23.8 ± 0.7 h for green algae of the Ulvophyceae class (*Chlorodesmis* spp.) to -10.8 ± 1.6 h for brown algae of the Phaeophyceae class (*Turbinaria decurrens*) (Fig. 2a). Reef type explained 0.5% of the variability in $\delta^{13}\text{C}$ values ($F=2.0$, $d=1$, $p=0.16$). Similarly, $\delta^{15}\text{N}$ values varied between species’ class ($F=59.2$, $d=8$, $p<0.001$; 88.2% of explained variability), with values ranging from 6.0 ± 0.4 h for *Turbinaria decurrens* to 12.4 ± 0.1 h for the Echinoidea class (sea urchin *Echinothrix diadema*) (Fig. 2a). The reef type explained only 0.1% of the variability in $\delta^{15}\text{N}$ values ($F=0.3$, $d=1$, $p=0.59$).

Regarding FA profiles, species’ class and reef type explained 85.1% ($\chi^2 = 645.9$, $d=8$, $p < 0.001$) and 0.3% ($\chi^2 = 1.9$, $d=1$, $p = 0.17$) of the variability, respectively. PCA projection captured 39.5% the variability in FA profiles with 16:0, 18:0 DMA, 18:4n-1, 20:1n-11, 20:5n-3, and 22:5n-6 the six main discriminant FAs (Fig. 2b). PCA highlighted differences in FA profiles according to the phylogenetic groups: (i) the algae group (including Chlorophyta, Phaeophyceae, and Rhodophyta phylums; PCA’ bottom left panel) with the highest levels in 14:0, 16:0, 16:1n-7, and 18:3n-6; (ii) the Arthropod phylum (Malacostraca class; PCA’ top left panel), with the highest levels in 18:1n-7, 20:4n-6 and 20:5n-3; (iii) the Mollusc phylum (Bivalvia and Gastropoda classes; PCA’ right panels), with the highest levels in three nonmethylated interrupted (NMI) FAs (22:2 NMI (7,13), 22:2 NMI (7,15), and 22:3 NMI), 16:1n-7 DMA, 22:1n-11, 22:5n-3, and 22:4n-6; (iv) the Echinoderm phylum (Echinoidea class; PCA’ top right panel), with the highest levels in 18:0; and (v) the Cnidaria phylum (Anthozoa class), rich in 18:4n-1 (Fig. 2b; Table 2).

3.2. Variability in trophic tracers among spiny lobsters

Regarding carapace length, individuals ranged from 60.2–143.2 mm CL with *P. penicillatus* significantly larger than *P. longipes* and *P. versicolor* ($F=17.2$, $df=2$, $p < 0.001$; Fig. 3a). Regardless of species and due to unbalanced sampling design, individuals from both reef types were of similar carapace length (101.3 ± 27.1 and 88.8 ± 16.7 mm CL for carbonate and granite reefs, respectively; $F=3.6$, $df=1$, $p = 0.06$). *P. penicillatus* tended however to be larger in carbonate than in granite reefs (Table 1). Regarding $\delta^{13}\text{C}$ values, studied individuals ranged from -15.3 to -12.2 h with no difference observed among species, reef type, or interaction between species and carapace length ($F=2.2$, $df=2$, $p = 0.13$; $F=0.3$, $df=1$, $p = 0.58$; and $F=2.3$, $df=2$, $p = 0.11$, respectively). However, $\delta^{13}\text{C}$ values increased with carapace length (slope = 0.02; $R^2 = 0.25$; $F=12.6$, $df=1$, $p < 0.001$; 16.0% of explained variability), from -14.3 ± 0.6 h below 80 mm CL to -13.0 ± 0.3 h above 120 mm CL, especially for *P. penicillatus* (slope = 0.02, $R^2 = 0.20$; $F=8.6$, $d=1$, $p < 0.01$; Fig. 4).

Regarding trophic level, studied individuals ranged from 2.3 to 3.1, and only an inter-specific difference was detected ($F=32.1$, $d=2$, $p < 0.001$; 51.9% of explained variability),i having a significantly lower trophic level than the two other species (mean trophic level of 2.4 vs. 2.8; Fig. 3b). No influence of carapace length, reef type, or interaction between species and carapace length on the trophic level was detected ($F=0.1$, $d=1$, $p = 0.94$; $F=2.0$, $d=1$, $p = 0.17$; and $F=0.3$, $d=2$, $p = 0.75$, respectively). Regarding FA profiles, only the carapace length was found to be an influencing factor ($\chi^2 = 17.8$, $d=1$, $p < 0.001$; 7.2% of explained variability). No influence of species, reef type, or interaction between species and carapace length on FA profiles was detected ($\chi^2 = 4.1$, $d=2$, $p = 0.13$; $\chi^2 = 0.4$, $d=1$, $p = 0.55$; and $\chi^2 = 3.3$, $d=2$, $p = 0.19$, respectively).

3.3. Mixing model outputs

3.3.1. Model diagnostics

MCMC chains of the mixing model did converge to 1. However, a posteriori groupings were required due to negative correlations between Malacostraca and Phaeophyceae (Pearson's correlation coefficient r ranged from -0.57 to -0.92 according to lobster species and reef type), and to a lesser extent between Echinoidea and Malacostraca or Gastropoda. Despite the grouping of Chlorophyta, Ochrophyta, and Rhodophyta (under the 'Algae' group) and of Gastropoda and Bivalvia (under the 'Mollusc' group), a strong negative correlation remained for some groups (r of -0.87 between Malacostraca and Alga and of -0.69 between Echinoidea and Mollusc; Fig. S2), indicating the low discrimination of these groups in lobster diets and the possible overestimation of Malacostraca and Echinoidea proportions over Algae and Mollusc proportions in the spiny lobsters' diet.

3.3.2. Model outputs

Inter-specific comparisons. Similar trends were observed for the three spiny lobster species, with Malacostraca identified as their main prey (average proportion: 47%–63% of the diet), followed by Echinoidea (16%–32% of the diet), Algae (8%–18% of the diet), Mollusc (5%–17% of the diet), and Anthozoa (1%–2% of the diet) (Fig. 5a). Due to large distributions in model outputs, dietary proportions of most potential prey were similar among spiny lobsters (at $\alpha = 0.05$). For instance, dietary proportions for Malacostraca were as follows: *P. penicillatus* $63 \pm 14\%$, *P. longipes* $48 \pm 10\%$, and *P. versicolor* $47 \pm 12\%$, with 81.7% and 90.7% probability that *P. penicillatus* consumed more Malacostraca than *P. longipes* and *P. versicolor*, respectively. Noticeable differences mainly concerned *P. versicolor*: (i) 94.8% and 88.3% probability that *P. versicolor* consumed less Echinoidea than *P. longipes* and *P. penicillatus*, respectively; (ii) 78.6% and 83.6% probability that *P. versicolor* consumed more Mollusc than *P. longipes* and *P.*

penicillatus, respectively, and (iii) 50.2% and 66.2% probability that *P. versicolor* consumed more Algae than *P. longipes* and *P. penicillatus*, respectively (Fig. 5a).

Reef habitats' comparison. Spiny lobsters caught from both carbonate reefs (n=7) and granite reefs (n=57) were found to mainly feed on Malacostraca (half of their diets; Fig. 5b). However, spiny lobsters from granite reefs had 83.9% and 92.4% probability of feeding more on Echinoidea and less on Molluscs than their congeners from carbonate reefs, respectively (Fig. 5b).

4. Discussion

Diet estimations from the mixing model were based on trophic tracers, and thus correspond to a diet assimilation over several days to months, based on turnover rates of SI and FA muscle and hepatopancreas (Antonio and Richoux, 2016; Waddington and MacArthur, 2008). According to the mixing model, diet profiles of the three spiny lobster species were similar, with Malacostraca: Anomura (hermit crabs) found to be their main prey. A similar finding has been observed from a reef lagoon of Puerto Morelos, Mexico, where hermit crabs and brachyurans were the main prey in the gut contents of *P. argus* juveniles (Briones-Fourzán et al., 2003). Preponderance of crustaceans in the diet of spiny lobsters has been observed in several *Panulirus* spp., usually in similar proportions to those of molluscs, depending on environmental factors such as reef type, season etc. (Table S1). In our study, molluscs (gastropods + bivalves) ranked third to fourth in main prey items of spiny lobsters, which was lower than expected based on data from the literature (first to third main prey; Table S1). Around Mahé Island, bivalves do not form dense settlements (Selin et al., 1992; Taylor, 1968) and represent less than 14% of molluscs, which are dominated by gastropods (Barnes et al., 2009). It may explain why few bivalves were found by divers at the sampled sites (only the oyster *Pinctada margaritifera*). Consequently, bivalves contributed to a low proportion of the Seychelles spiny lobster' diets (<10% of the diets whatever the species), while they can be an important food source of *Panulirus* spp. in other regions of the world (e.g. Gulf of Mexico, Baja

California; Table S1) and of other spiny lobsters e.g. *Jasus* spp. (Mayfield et al., 2000; Pollock, 1979). Echinoidea (sea urchins) were found to be an important diet component for the three species (16–32% of the diet). Our results are in accordance with observations from other regions, although Seychelles spiny lobsters seemed to rely more on Echinoidea compared to the 4–28% and 4–8% gut volume for *P. guttatus* and *P. argus* from Yucatan peninsula, Mexico, respectively (Briones-Fourzán et al., 2019). This relatively high consumption of sea urchin could be related to the high abundance of sea urchins in shallow areas around Mahé Island (Pittman, 1996) and has already been suggested based on the high $\delta^{15}\text{N}$ levels of both sea urchins and spiny lobsters in Seychelles (Sabino et al., 2020; Sardenne et al., 2017). However, spiny lobsters from granite reefs seemed to feed more on Echinoidea and less on molluscs than individuals from carbonate reefs, while sea urchins are mainly found in patchy reefs of Mahé Island, i.e. reefs with a sand, rock or rubble base (Jan-Claas, 2020). This suggests spiny lobsters might move between reef types, despite narrow home ranges being suspected for these species and similar to results from related species (650 m² per week based on 50% utilisation distribution of *P. interruptus* from California, USA; Withy-Allen and Hovel, 2013).

Interspecific differences in diet proportions were small, but *P. versicolor* consumed slightly more algae than the two other species, which is consistent with its lower trophic level (2.4 vs. 2.8). This dietary difference could help to reduce interspecific competition, as even a small difference in trophic level favoured co-occurrence between *Panulirus* species on Caribbean reefs (Segura-García et al., 2016). Overall, the trophic levels of Seychelles spiny lobsters (ranging from 2.3 to 3.1, n=64) were similar to those derived from $\delta^{15}\text{N}$ values for *P. cygnus* from Western Australia (1.9–2.2) (Waddington et al., 2008) and *Jasus edwardsii* from New Zealand (2.5–4.0) (Jack and Wing, 2011). Such large ranges highlight the lobster's diet plasticity and their opportunistic feeding behaviour (Table S1). Regardless of the species, no ontogenetic change in trophic level was observed (i.e. no correlation between $\delta^{15}\text{N}$ derived trophic level and carapace length). In contrast, $\delta^{13}\text{C}$ values increased with spiny lobster size, indicating that large individuals may feed closer to

the coast or more on prey with a strong benthic affinity (e.g. prey feeding on benthic organic matter with high $\delta^{13}\text{C}$ values) than their smaller/younger congeners. While limited information is available on lobster size distribution with bathymetry in shallow areas, ontogenetic changes in dietary preferences are known for other spiny lobster species: e.g. it was observed for *Jasus paulensis*, *J. lalandii* and *P. cygnus* (Blamey et al., 2019; Dumas et al., 2013; Haley et al., 2011; Mayfield et al., 2000). However, size related changes either in spatial distribution or in diet remain to be detangled. The size ranges of the three species were too narrow in the present study to define size classes to be included in the mixing model, but it would be interesting to explore this aspect further by including juveniles in the dataset.

4.2. Study caveats

Caveats regarding mixing model outputs should be highlighted, since uncertainties in the estimated dietary proportions of algae, Malacostraca, and Echinoidea remain strong despite a posteriori aggregations of specific prey classes, which could thus bias the results. These uncertainties have two main origins: (i) a low discrimination of some potential prey based on their SI compositions and FA profiles. Using additional trophic tracers such as sulphur or mercury stable isotopes might improve this discrimination (e.g. Higgs et al., 2016; Jack et al., 2009); and (ii) the influence of FF parameters, which were estimated from linear regressions based on one diet experiment only. Additional diet experiments would be required to accurately estimate FFs (for both SI and FAs) and thus increase robustness of the model setup as previously highlighted (Brett et al., 2016; Phillips et al., 2014).

Apart from the model parameters, more samples of spiny lobsters from carbonate reefs and of juveniles/small adults is needed. Due to loss of carbonate reefs in the region, samples of lobsters from this habitat were small and could prevent detection of any differences between reef types and

ontogenetic stages. Some potential prey were also probably missing, especially bivalve and decapod species, which have reduced contributions to lobster's diets.

4.3. Future directions

Several integrated trophic indicators are particularly suitable for the implementation of an ecosystem-based fisheries management, including the monitoring of trophic levels of catches and the mean number of trophic links per species (Tam et al., 2017). The present study will provide a baseline for trophic level of spiny lobsters from the Seychelles and might initiate monitoring. For instance, the seasonal monitoring of trophic level would help to detect diet shifts in the past few months (e.g. following coral bleaching or after the fishing season). For the sampling to be nonlethal, muscle tissue can be sampled from the last segment of the fourth walking leg (ca. 0.5 g of wet tissue is required), which will start to grow again at the next molt, as after natural autotomy. Monitoring may also allow trophic links per spiny lobsters species to be identified, especially regarding (i) potential shift in the diets of spiny lobsters before and after coral bleaching on carbonate reefs, as the dietary competition among spiny lobsters has increased after the coral bleaching event of 2016 (Sabino et al., 2020); and (ii) the influence of predator abundance, by comparing diets in heavily fished and protected regions throughout the ontogeny of spiny lobsters. Spiny lobsters have several predators especially at juvenile stage, such as octopus, small sharks, groupers, and snappers, which influence their sheltering and foraging behaviours (Ellis, 2018; Smith and Herrkind, 1992).

5. Conclusion

Spiny lobsters from the Seychelles are generalist feeders that appear to mainly feed on crustaceans, sea urchins and algae. Dietary differences related to species, reef type and ontogeny were weak, but *P. versicolor* had a lower trophic level than the two other species. Lobsters from

carbonate reefs fed more on Molluscs than those from granite reefs, and large lobsters probably fed closer to the coast or more on detritus feeders than the small ones. These results should however be refined by including more individuals (including size ranges) and prey types, trophic tracers, and parameters from diet experiments.

Authors' contribution

Conceptualization: NB, LBa, LBl, DR; Funding acquisition: DR, NB, RG; Sampling: NB, LBa, LBl, KC, RM; Laboratory analyses: FS, SB, AB, JMR, FLL, FLG; Statistical analysis: FS; Writing – 1st draft: FS; Review & editing: All co-authors

Acknowledgments

This work is a contribution to the project SEYCCAT-BGF1/L/N6 “*Science based restoration of commercially important spiny lobster habitats to help develop a sustainable fishery*” led by the Marine Conservation Society Seychelles (MCSS); and to the Seychelles Participatory Lobster Monitoring Programme (PLMP) led by the Seychelles Fishing Authority (SFA). The project benefited from the financial support of the Seychelles' Conservation and Climate Adaptation Trust (SEYCCAT), the Seychelles Gouvernement, the European Fisheries Partnership Agreement (EU-FPA), and the Institute for Research and Development (IRD). The authors would like to thank the lobster fishermen and the R.V. L’Amitie crew who assisted with sampling. The authors are also grateful to G. Guillou from the Plateforme Analyses Isotopiques of LIENSs, (University of La Rochelle, France) for his support in the lobster stable isotope analysis. Thanks are due to the CPER (Contrat de Projet Etat-Région) and the FEDER (Fonds Européen de Développement Régional) for funding the IRMS of LIENSs laboratory. The IUF (Institut Universitaire de France) is also acknowledged for its support to PB as a senior member.

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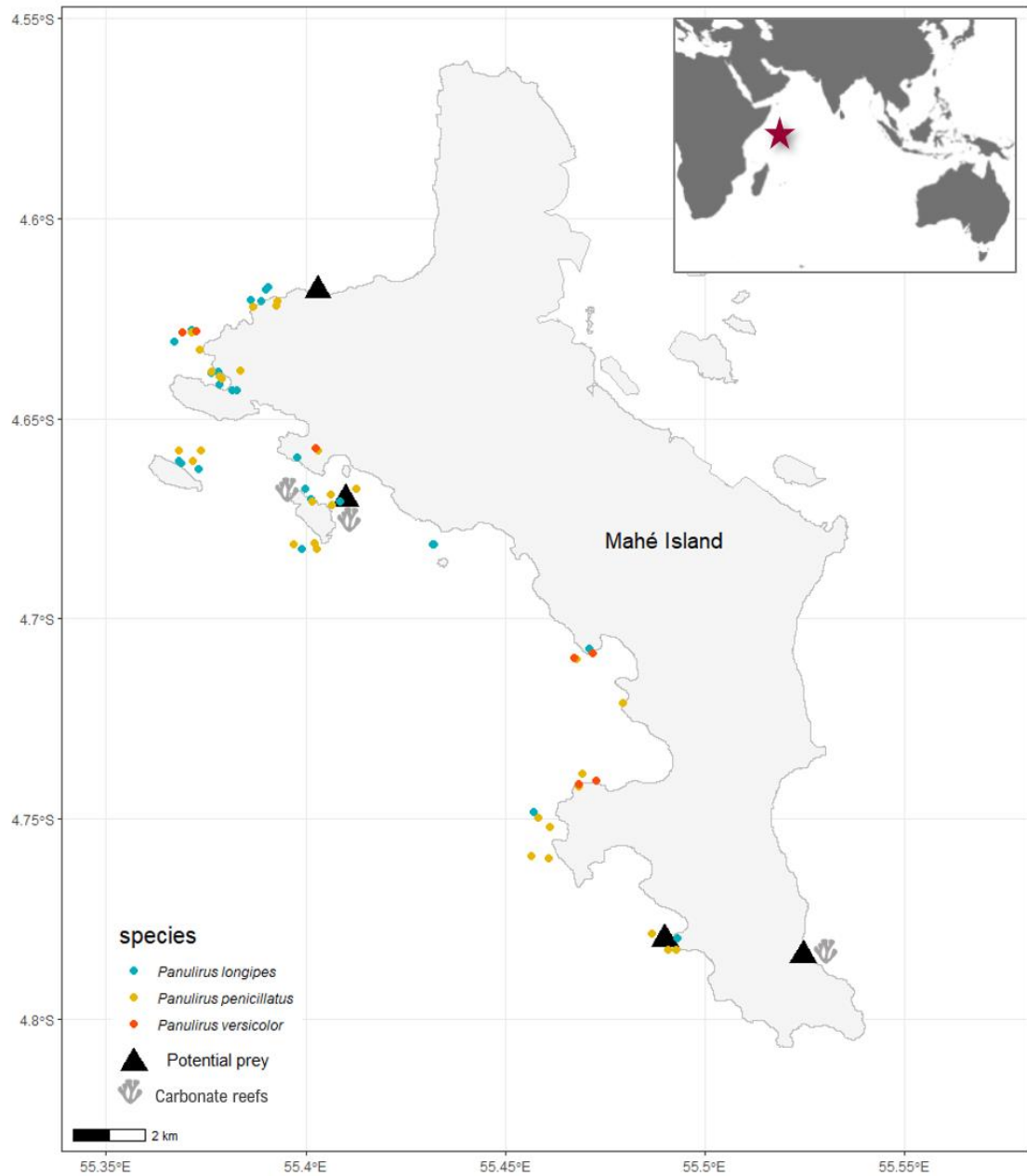


Figure 1. Sampling sites of spiny lobsters *Panulirus longipes*, *P. penicillatus* and *P. versicolor* and their potential prey collected from the coastal shallow waters around Mahé Island, Seychelles, located in the Western Indian Ocean (red star on the top right panel). Sites correspond to granitic reefs unless specified (three carbonate reef sites).

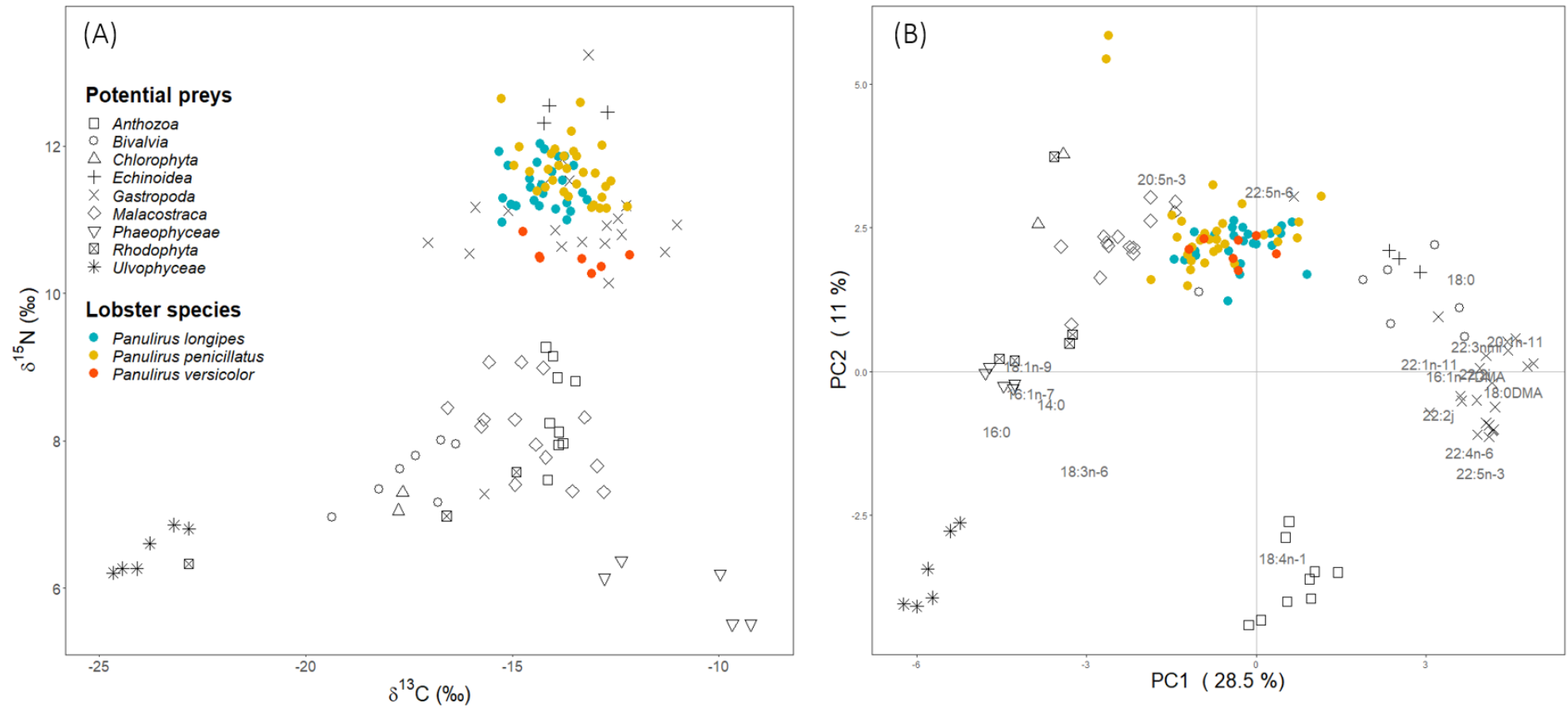
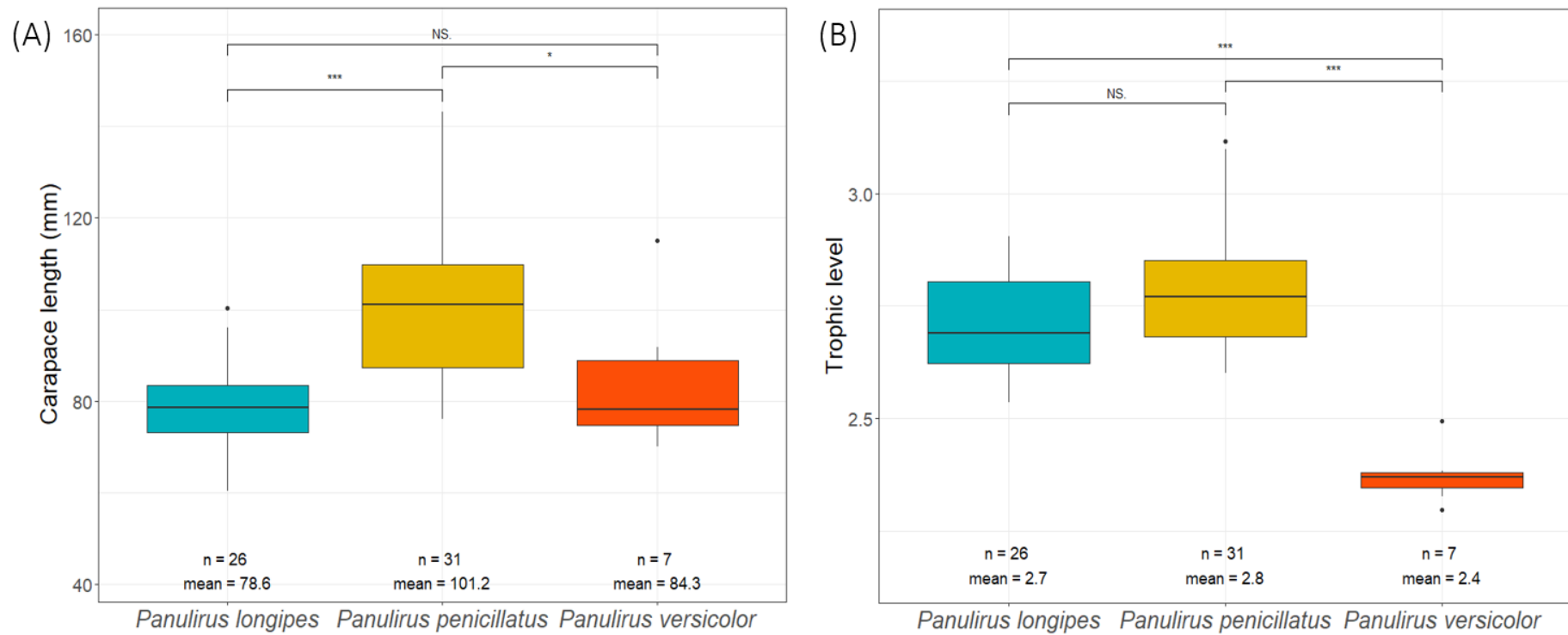


Figure 2. Trophic tracers of spiny lobsters and their potential prey presented with (A) a biplot of nitrogen ($\delta^{15}\text{N}$) and carbon ($\delta^{13}\text{C}$) isotopic values, and (B) a principal component analysis based on 44 fatty acids with spiny lobsters' observations superimposed (only FA contributing the most to the discrimination are indicated in grey).



Figure

3. Boxplots of (A) carapace length, and (B) trophic level, for three spiny lobster species of Mahé Island, Seychelles. Trophic level was estimated based on $\delta^{15}\text{N}$ values of the lobster tail muscle relative to the $\delta^{15}\text{N}$ values of macroalgae (red, green and brown macroalgae) (see material and methods for details). Stars denotes significant differences between species (Tukey HSD test; NS = $p > 0.05$, * = $p < 0.05$, ** = $p < 0.01$, and *** = $p < 0.001$).

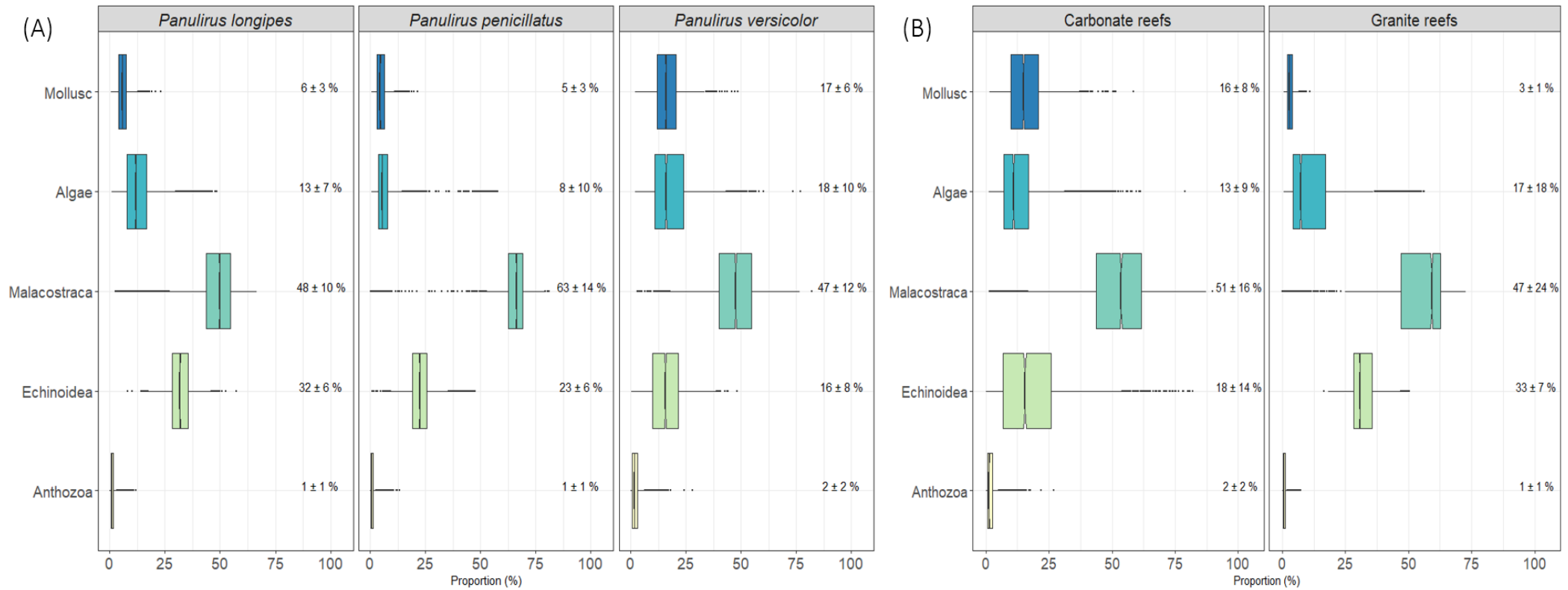


Figure 4. Outputs of the Bayesian mixing model estimating the prey proportions in the diet of spiny lobsters (A) among species, and (B) between habitat types, based on isotopic values and fatty acid profiles. To improve model diagnostics, bivalves and gastropods were grouped *a posteriori* under the ‘Mollusc’ group, and brown, green and red algae were grouped under the ‘Algae’ group. Proportions’ summary (Mean±SD) is indicated for each group.

Table 1. Details on the sampling design of spiny lobsters from reefs of Mahé Island, Seychelles.

Species	Habitat type	N	Carapace length (mm)
<i>Panulirus longipes</i>	Carbonate reefs	3	77.6 ± 5.1
<i>Panulirus longipes</i>	Granitic reefs	23	78.7 ± 10.6
<i>Panulirus penicillatus</i>	Carbonate reefs	4	119.1 ± 21.7
<i>Panulirus penicillatus</i>	Granitic reefs	27	98.5 ± 16.0
<i>Panulirus versicolor</i>	Granitic reefs	7	84.3 ± 15.5

Table 2. Mean \pm standard deviation for isotopic values ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, in ‰), total lipid content (TLC, in $\mu\text{g}\cdot\text{mg}^{-1}$ in dry weight), and proportions of fatty acids contributing the most to species discrimination (in % of total fatty acids) for 13 potential prey species and three spiny lobster species caught from reefs around Mahé Island, Seychelles. See material and methods for details on analysis.

	Phylum	Class	Species	N	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)	TLC ($\mu\text{g}\cdot\text{mg}^{-1}$)	16:0 (%)	18:0DMA (%)	18:1n-9 (%)	18:4n-1 (%)	20:1n-11 (%)	20:4n-6 (%)	20:5n-3 (%)	22:6n-3 (%)
Potential prey	Chlorophyta	Chlorophyta	<i>Chlorophyta spp</i>	2	-17.7 \pm 0.1	7.2 \pm 0.2	0.7 \pm 0.1	23.0 \pm 0.3	0.0 \pm 0.0	5.1 \pm 0.1	0.0 \pm 0.0	3.3 \pm 0.1	4.1 \pm 0.0	5.5 \pm 0.0	8.0 \pm 0.1
		Ulvophyceae	<i>Chlorodesmis spp</i>	6	-23.8 \pm 0.7	6.5 \pm 0.3	3.2 \pm 0.6	41.6 \pm 2.6	0.0 \pm 0.0	9.1 \pm 0.8	0.0 \pm 0.0	0.0 \pm 0.0	1.6 \pm 0.3	1.9 \pm 0.3	0.4 \pm 0.1
	Ochrophyta	Phaeophyceae	<i>Turbinaria decurrens</i>	5	-10.8 \pm 1.6	6.0 \pm 0.4	2.5 \pm 0.4	35.6 \pm 3.4	0.0 \pm 0.0	13.8 \pm 0.9	0.0 \pm 0.0	0.0 \pm 0.1	13.5 \pm 0.8	1.7 \pm 0.4	0.0 \pm 0.1
	Rhodophyta	Rhodophyta	<i>Rhodophyta sp1</i>	2	-22.8 \pm 0.0	6.3 \pm 0.0	0.3 \pm 0.0	51.3 \pm 0.5	0.0 \pm 0.0	11.9 \pm 0.1	0.0 \pm 0.0	0.0 \pm 0.0	8.6 \pm 0.0	5.0 \pm 0.0	0.0 \pm 0.0
		Rhodophyta	<i>Rhodophyta sp2</i>	3	-16.0 \pm 1.0	7.2 \pm 0.3	0.3 \pm 0.1	45.0 \pm 4.7	0.0 \pm 0.0	5.5 \pm 1.9	0.1 \pm 0.1	0.4 \pm 0.2	12.2 \pm 7.1	1.8 \pm 0.2	1.0 \pm 0.2
	Arthropod	Malacostraca	<i>Calcinus laevimanus</i>	10	-14.3 \pm 1.3	7.8 \pm 0.4	2.9 \pm 0.8	22.6 \pm 5.6	3.1 \pm 1.0	5.8 \pm 0.7	0.0 \pm 0.0	0.3 \pm 0.1	14.3 \pm 2.6	11.1 \pm 1.7	3.7 \pm 1.3
		Malacostraca	<i>Dardanus lagopodes</i>	5	-15.0 \pm 0.6	8.7 \pm 0.4	2.0 \pm 0.4	20.9 \pm 5.6	3.3 \pm 2.3	6.3 \pm 1.0	0.0 \pm 0.0	0.3 \pm 0.1	11.8 \pm 2.5	11.5 \pm 2.8	5.5 \pm 2.5
	Cnidaria	Anthozoa	<i>Palythoa natalensis</i>	9	-13.9 \pm 0.2	8.4 \pm 0.6	0.7 \pm 0.2	24.3 \pm 2.3	5.4 \pm 1.2	2.3 \pm 0.2	4.5 \pm 2.3	0.7 \pm 0.3	7.8 \pm 1.4	2.4 \pm 0.6	3.9 \pm 0.6
	Echinoderm	Echinoidea	<i>Echinothrix diadema</i>	3	-13.7 \pm 0.9	12.4 \pm 0.1	3.5 \pm 0.1	10.7 \pm 0.1	6.7 \pm 0.9	0.4 \pm 0.1	0.1 \pm 0.0	8.7 \pm 0.6	26.7 \pm 0.4	7.4 \pm 1.7	0.7 \pm 0.1
	Mollusc	Bivalvia	<i>Pinctada margaritifera</i>	7	-17.5 \pm 1.0	7.6 \pm 0.4	5.1 \pm 2.4	12.5 \pm 5.1	7.4 \pm 4.1	4.0 \pm 0.8	0.1 \pm 0.1	5.4 \pm 1.7	6.1 \pm 1.4	4.1 \pm 2.4	12.6 \pm 3.4
		Gastropoda	<i>Conus spp</i>	7	-13.7 \pm 2.0	10.8 \pm 1.8	3.8 \pm 1.4	12.0 \pm 7.5	8.5 \pm 2.4	2.7 \pm 0.7	0.0 \pm 0.0	5.5 \pm 1.2	11.3 \pm 3.2	3.0 \pm 1.8	0.8 \pm 0.7
		Gastropoda	<i>Latirolagena smaragdulus</i>	8	-13.6 \pm 1.5	10.7 \pm 0.3	3.1 \pm 0.2	5.9 \pm 0.3	8.5 \pm 1.7	2.5 \pm 0.2	0.2 \pm 0.1	8.1 \pm 0.5	14.3 \pm 0.5	1.3 \pm 0.2	0.2 \pm 0.0
		Gastropoda	<i>Vasum turbinellus</i>	7	-13.7 \pm 1.5	11.2 \pm 0.2	3.0 \pm 1.0	5.7 \pm 1.4	7.7 \pm 3.4	1.6 \pm 0.4	0.1 \pm 0.0	6.6 \pm 1.1	15.3 \pm 1.5	1.9 \pm 0.2	1.5 \pm 1.9
Lobsters	Arthropod	Malacostraca	<i>Panulirus longipes</i>	26	-14.3 \pm 0.6	11.5 \pm 0.3	17.8 \pm 6.4	17.1 \pm 2.1	2.4 \pm 0.6	7.4 \pm 1.4	0.0 \pm 0.0	2.9 \pm 0.6	10.8 \pm 1.3	7.7 \pm 1.3	4.0 \pm 1.0
		Malacostraca	<i>Panulirus penicillatus</i>	31	-13.6 \pm 0.7	11.7 \pm 0.4	16.9 \pm 6.4	17.4 \pm 2.0	2.3 \pm 0.8	10.5 \pm 3.6	0.0 \pm 0.0	2.8 \pm 0.9	8.5 \pm 1.9	7.1 \pm 2.0	4.3 \pm 2.2
		Malacostraca	<i>Panulirus versicolor</i>	7	-13.5 \pm 0.9	10.5 \pm 0.2	14.2 \pm 7.3	17.6 \pm 2.1	2.5 \pm 0.7	7.6 \pm 2.0	0.0 \pm 0.0	2.4 \pm 0.7	11.3 \pm 2.3	7.8 \pm 1.3	4.1 \pm 1.2

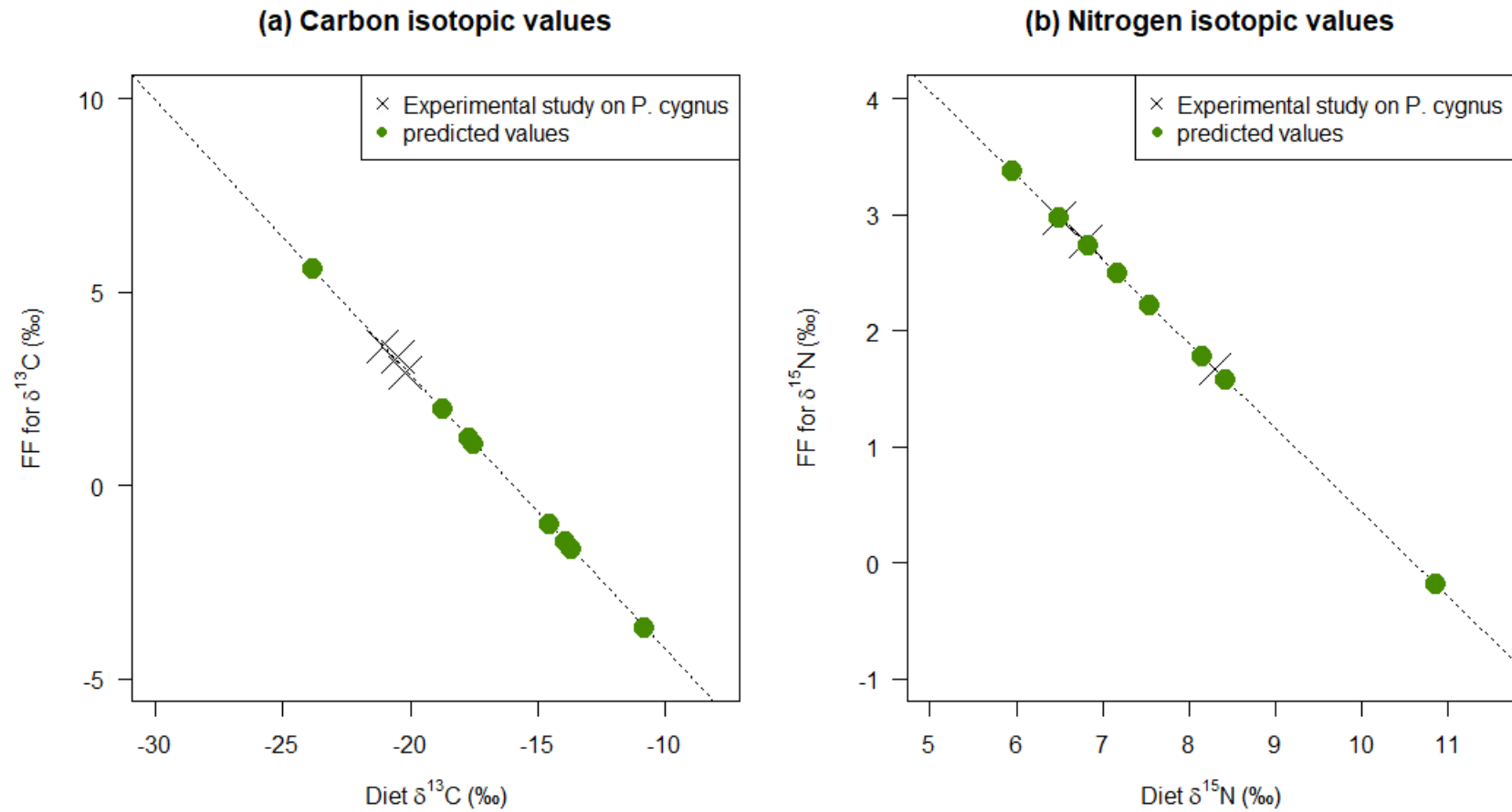


Figure S1. Isotopic fractionation factors (FFs; y-axis) for potential prey (n=9, green dots), predicted from a diet experimental study on *Panulirus cygnus* (tail muscle) (Waddington and MacArthur, 2008) and the isotopic values of potential prey (x-axis).

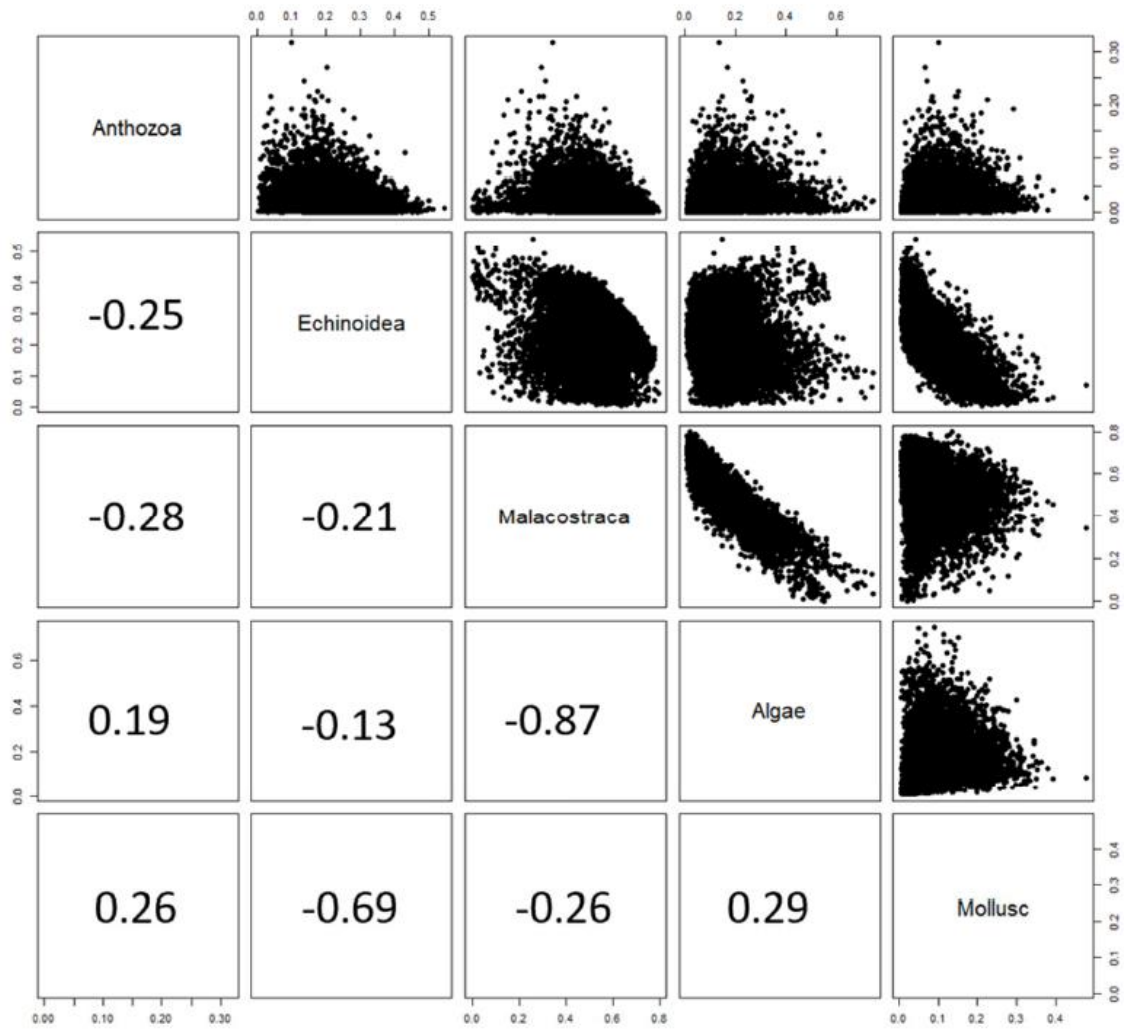


Figure S2. Correlation in mixing model outputs. Strong negative correlations (i.e. between Malacostraca and Algae, and between Echinoidea and Mollusc) indicate poor discrimination among these food sources.

Table S1. Review of gut content analysis of *Panulirus* species around the world. Prey species are grouped by class. Since not all studies used the same metrics, only prey ranks of importance were recorded: A-rank for the prey class contributing the most (first) to the *Panulirus* diet, B-rank for prey class contributing the second, etc.

Species	Region	Site	Habitat	Year	Season	CL (mm)	N	Crustacea	Eumollusca	Algae	Plant	Fish	Echinodermata	Annelida	Ectoprocta	Cnidaria	References
<i>Panulirus cygnus</i>	Western Australia	Seven Mile Beach	Coral reef	1977-1978	all	~ 28-70	372	D	C	A	B			E			Joll & Phillips 1984
		Cliff Head	Coral reef		all	~ 30-50	209	C	A	B	D			E			
<i>Panulirus argus</i>	Florida, USA	Biscayne & Dry tortugas national parks	Offshore reef	1988-1989	all	27-135	75	B	A		C		D				Cox et al 1997
<i>Panulirus cygnus</i>	Western Australia	Seven Mile Beach	Coral reef	1985-1986	all	25-85	73	D	A	C	E			B			Edgar 1990
		Cliff Head	Coral reef		all	25-90	48	D	A	E	B			C			
<i>Panulirus interruptus</i>	Baja California, Mexico		Rocky, sand with boulders	1986-1987	summer	57-132.2	16	B	A	D	E	C				F	Diaz-Arredondo & Guzman-del-Proo 1995
					autumn		21	C	A	D	E	B			F		
					winter		19	B	A	F	E	C			D		
					spring		18	E	A	C	B	D					
<i>Panulirus argus</i>	Yucatan Peninsula, Mexico	Puerto Morelos	Coral reef with sand and seagrass	1995	winter	11.1-80.0	34	A	B	D	C	E	F				Briones-Fourzan et al. 2003
					spring		41	A	B	E	C			D	F		
					summer		37	A	B	H	C	G	E	D		F	
					autumn		39	A	B	F	E	H	C	D		G	
<i>Panulirus interruptus</i>	Baja California, Mexico	Arvin	Bay with seagrass and macroalgae	2001-2002	autumn	56.7	28	A	E	B	C				D		Castañeda-Fernandez-de-Lara et al. 2005
					winter	55.4	22	B	E	C	A			D			
					spring	54	30	A	B	D	C			E			
					summer	55.9	29	B	D	C	A			E			
		Queen	Coastline with macroalgae		autumn	56.1	27	A	B	D	C				E		
					winter	53.8	34	A	C	D	B		E		E		
					spring	57	25	C	B	A	D				E		
					summer	55.8	21	B	D	E	A				C		
<i>Panulirus cygnus</i>	Western Australia	Lancelin	Reef	2006	spring	54-115	5	A	D	C		B				Waddington et al 2008	
		Jurien Bay				54-82	19	A	C	D		B					
		Dongara				64-145	6	A	C			B					
<i>Panulirus echinatus</i>	Brazil	São Pedro & São Paulo archipelago	Rocky	2002-2003	all	~40-50	68	B	D	C		A				Goes & Lins-Oliveira 2009	
<i>Panulirus homarus</i>	Iran	Ramin		1999	winter	~71.4	89	A	B	C		E		D			Mashaii et al. 2011
					spring		46	B	A	C		E		D			
					summer		37	A	B	C		F		D		E	
					autumn		88	A	B	C						D	
<i>Panilurus argus</i>	Gulf of Batabanó, Cuba			2014	spring	~ 76	30	A	B	C		D	E			Coello et al 2015	
<i>Panulirus homarus</i>	coast of Kerala, India	Vizhinjam harbour			46-61	13	C	A	B		D	E			F	Alka, 2016 (BSc)	
<i>Panulirus guttatus</i>	Florida, USA	Florida Keys	Reef	2006	summer	27-63	66	A	B	E		C	D			Butler & Kintzing 2016	
<i>Panulirus guttatus</i>	Yucatan Peninsula, Mexico	Limones	Coral reef with macroalgae	2015	autumn	~62.1	15	B	A				C	D			Briones-Fourzan et al. 2019
		Bonanza	macroalgae		autumn		15	B	A				D	C			
<i>Panulirus argus</i>	Yucatan Peninsula, Mexico	Limones	Coral reef with macroalgae		autumn	~56.2	15	B	A				D	C			
		Bonanza	macroalgae		autumn		15	A	B				D	C			