Concentrations of trace elements in sea urchins and macroalgae commonly present in Sargassum *beds: implications for trophic transfer*

Pablo Hernández-Almaraz, Lía Méndez-Rodríguez, Tania Zenteno-Savín, Todd M. O'Hara, John R. Harley & Elisa Serviere-Zaragoza

Ecological Research

ISSN 0912-3814
Volume 31
Number 6

Ecol Res (2016) 31:785-798
DOI 10.1007/s11284-016-1390-7

Ecol Res (2016) 31: 785–798
DOI 10.1007/s11284-016-1390-7

ORIGINAL ARTICLE

Pablo Hernández-Almaraz · Lía Méndez-Rodríguez
Tania Zenteno-Savín · Todd M. O'Hara
John R. Harley · Elisa Serviere-Zaragoza

Concentrations of trace elements in sea urchins and macroalgae commonly present in *Sargassum* beds: implications for trophic transfer

Received: 5 January 2016 / Accepted: 19 August 2016 / Published online: 2 September 2016

Abstract The aim of the study was to evaluate the dynamic of cadmium, lead, copper, zinc, and iron among lower trophic levels, sea urchins and macroalgae. Diets and isotopic values were used in combination to explore trophic positions and potential transference of metals from primary producers to consumers. Concentrations of trace elements were measured in two species of sea urchin (*Tripneustes depressus* and *Eucidaris thouarsii*) and nine macroalgae that are usually used as food in four *Sargassum* beds, one of which is close to a phosphorite mine. Specimens were collected when *Sargassum* fronds were at their greatest (winter) and lowest (summer) abundance. Highest concentrations of Cd, and Cu in both urchin species were recorded in winter at the site near the phosphorite mine. Concentrations of Pb in *T. depressus* were below the detectable limit, whereas *E. thouarsii*, which in addition to a high concentration of Pb, had high amounts of Cu and Zn. Gut content analysis, indicates that the diet of both sea urchins at the four sites and two collection dates is mainly macroalgae. The δ^{15}N isotopic values in sea urchins in a typical *Sargassum* bed were in good agreement with a diet dominated by macroalgae, with *T. depressus* having herbivorous habits and *E. thouarsii* having omnivorous habits in this environment. We found macroalgae important in the dynamics of metals in food webs, potentially contributing to transferring Cd, Cu, and Zn to key invertebrate species, such as sea urchins, indicating connectivity of food webs and ecological structuring of marine environments.

Keywords Gut content · Macroalgae · Sea urchins · Stable isotopes · Metals

Introduction

Sea urchins (Phylum Echinodermata, Class Echinoidea) commonly form dense colonies in rocky and coral reefs; sandy areas with stone fragments; and areas with seagrass and macroalgae beds (Steneck 2013). In some ecosystems, they play a significant role by modifying the ecological structure of the communities. For example, an increase in sea urchin biomass was correlated with a 12-fold decrease in kelp density on Adak Island, AK, illustrating their ability to alter the physical structure of marine communities (Estes et al. 1998). The sea urchin *Tripneustes ventricosus* can consume about 3.6 % of daily seagrass production of *Thalassia testudinum* (Tertschnig 1988) and the sea urchins *T. gratilla* and *Salmacis sphaeroides* browse almost 100 % of the annual production of macrophytes in seagrass beds (Klumpp et al. 1993). *Paracentrotus lividus* and *Arbacia lixula* play a role in controlling macroalgal communities by reducing the coverage of foliose macroalgae, thus favoring the recruitment of corals and coralline crustose macroalgae (Ruitton et al. 2000). Sea urchins also serve as links in several food webs; they are prey for starfish, lobsters, crabs, snails, fish, birds, and some marine mammals, as well as humans (Tertschnig 1988; Agatsuma 2013; Steneck 2013).

The sea urchins *Tripneustes depressus* and *Eucidaris thouarsii* include macroalgae in their diet, but little is known about the items consumed by them (Lawrence and Agatsuma 2013; Lawrence and Jangoux 2013). Both species inhabit a wide range of reef habitats in the Gulf of California and are among the dominant macroinvertebrates along the southern coasts (Herrero-Pérezrul 2008; Herrero-Pérezrul et al. 2008; Ríos-Jara et al. 2008;

P. Hernández-Almaraz · L. Méndez-Rodríguez ·
T. Zenteno-Savín · E. Serviere-Zaragoza (✉)
Centro de Investigaciones Biológicas del Noroeste (CIBNOR),
Calle IPN #195, 23096 La Paz, B.C.S., Mexico
E-mail: serviere04@cibnor.mx
Tel.: (+52) 612-123-8484

T. M. O'Hara
Department of Veterinary Medicine, University of Alaska
Fairbanks, 901 Koyukuk Dr., Fairbanks, AK 99775-7750, USA

J. R. Harley
Department of Chemistry and Biochemistry, University of Alaska
Fairbanks, 900 Yukon Dr., Fairbanks, AK 99775-6160, USA

Solís-Marín et al. 2013, 2014). In the southwestern Gulf of California, both sea urchins dominate some rocky and coral reefs (Reyes-Bonilla and Calderón-Aguilera 1999; Holguín-Quiñones et al. 2000; Herrero-Pérezrul 2008; Solís-Marín et al. 2013), as well as communities associated with *Sargassum* beds (Foster et al. 2007; Suárez-Castillo 2008).

Sargassum beds are critical habitat for biological diversity and fisheries in coastal areas in the Gulf of California (Suárez-Castillo et al. 2013; Hinojosa-Arango et al. 2014). They exhibit an annual growth cycle; the fronds develop in winter and are senescent during summer (Hernández-Carmona et al. 1990; Núñez-López and Casas-Valdez 1996; Sánchez-Rodríguez and Cervantes-Duarte 1999). For example, fronds of *S. horridum* reach lengths up to 108 cm and growth rates of up to 60 cm month^{-1} in winter, but grow less than 10 cm and growth rates under 10 cm month^{-1} during the summer (Muñetón-Gómez and Hernández-Carmona 1993). The growing pattern of *Sargassum* is synchronized with the growth of other flora and fauna, reaching its fullness at the same time, with maximum richness and abundance as other species (Foster et al. 2007; Pacheco-Ruíz et al. 2008). During rapid development of the bed, the number of macroalgae species increases (Foster et al. 2007); associated fauna generally is dominated by a few species of animals (Suárez-Castillo 2008), including some macroinvertebrates that benefit from the abundance of macroalgae, using them as a food source (Brusca 1980; Pacheco-Ruíz et al. 2008; Suárez-Castillo et al. 2013).

Sargassum beds may cover only a few square meters to several kilometers along the coastline in the southwestern Gulf of California (Casas-Valdez et al. 1993). A few beds are located in areas with large phosphorite deposits (Riley and Chester 1971; Servicio Geológico Mexicano 2008). These kinds of deposits contain Cd, Pb, Cu, and Zn (Voulgaropoulos et al. 1991; Sabiha-Javied et al. 2009). Although phosphorite is not a common source of Fe, this element in the marine environment indicates re-suspension of sediments derived from the continental crust (Elrod et al. 2004). These five metals can be absorbed and accumulated by biota, including primary producers and their consumers (Ray 1984; Wang 2002; Ruelas-Inzunza and Páez-Osuna 2008; Eisler 2010), suggesting that *T. depressus* and *E. thouarsii* could absorb and accumulate metals through consumption of macroalgae in habitats dominated by *Sargassum*, as found in other sea urchins in habitats dominated by macroalgae (Bohn 1979; Ahn et al. 2009).

Sea urchins bioaccumulate toxic metals (Bohn 1979; Sadiq et al. 1996; Deheyn et al. 2005; Ahn et al. 2009) and serve as sentinels of pollution (Dafni 1980, 2009; Ablanedo et al. 1990; Aspholm and Hylland 1998). Their importance in transferring metals and other contaminants to organisms at higher trophic levels, such as fish, seals, and whales is also documented (Ahn et al. 2009). Studies of Arctic kelp forests (*Laminaria saccharina*, *L. digitata*, and *Alaria esculenta*) and habitats of the brown macroalgae *Fucus distichus* suggest that Cd, Pb, Cu, Zn, and Fe are accumulated in sea urchins after consumption of macroalgae (Bohn 1979; Ahn et al. 2009). Green macroalgae containing metals also demonstrated trophic transference to urchins (Bielmyer et al. 2012).

To study the dynamic of Cd, Pb, Cu, Zn, and Fe in the lower trophic levels, these metals in *T. depressus* and *E. thouarsii*, as well as nine macroalgae, were measured. Samples were collected from four reefs dominated by *Sargassum*, one of which is located near a coastal phosphorite deposit that is actively mined. Collections were made in winter and summer. Gut contents of all collected sea urchin were analyzed to identify the principal components of their diet. Additionally, δ^{15}N isotopic values were measured in samples of both sea urchins and the nine macroalgae species from a *Sargassum* bed with minimal human disturbance. Diets and isotopic values were used in combination to explore trophic positions and potential transference of metals from primary producers to consumers.

Materials and methods

Concentration of metals

Study area

The study was conducted at four sites in the coastal area of the southwestern Gulf of California: El Coyote (24°41′12.7″N, 110°43′9.96″W), Las Ánimas (24°31′43.8″N, 110°44′1.50″W), San Juan de la Costa (24°21′58.2″N, 110°40′50.7″W), and El Sauzoso (24°18′38.6″N, 110°38′28.8″W) (Fig. 1). A phosphorite mine is located at San Juan de la Costa. El Coyote and Las Ánimas are located 32 and 14.1 km north of San Juan de la Costa, respectively, and El Sauzoso is located 10.3 km to the south. There are minimal anthropogenic disturbances evident at these three sites; access is by rural roads and trails. Population near each of these three sites is less than 40 people within a radius of 5 km (CONABIO 2014). There are no mining activities; the families depend mostly on small-scale fishing (Díaz-Uribe et al. 2007). All four sites are characterized by *Sargassum* beds (Hernández-Carmona et al. 1990; Muñetón-Gómez and Hernández-Carmona 1993) containing a highly diverse flora and fauna, as do other *Sargassum* beds in the region (Foster et al. 2007; Pacheco-Ruíz et al. 2008).

At each site, sampling was conducted on the *Sargassum* bed along transects, over a span of a few days, during March and September 2011; March represents winter conditions (highest productivity) and September represents summer, when the fronds are at their lowest productivity (Hernández-Carmona et al. 1990; Núñez-López and Casas-Valdez 1996; Sánchez-Rodríguez and Cervantes-Duarte 1999). On each visit, ten of the larger specimens of *Tripneustes depressus* and *Eucidaris*

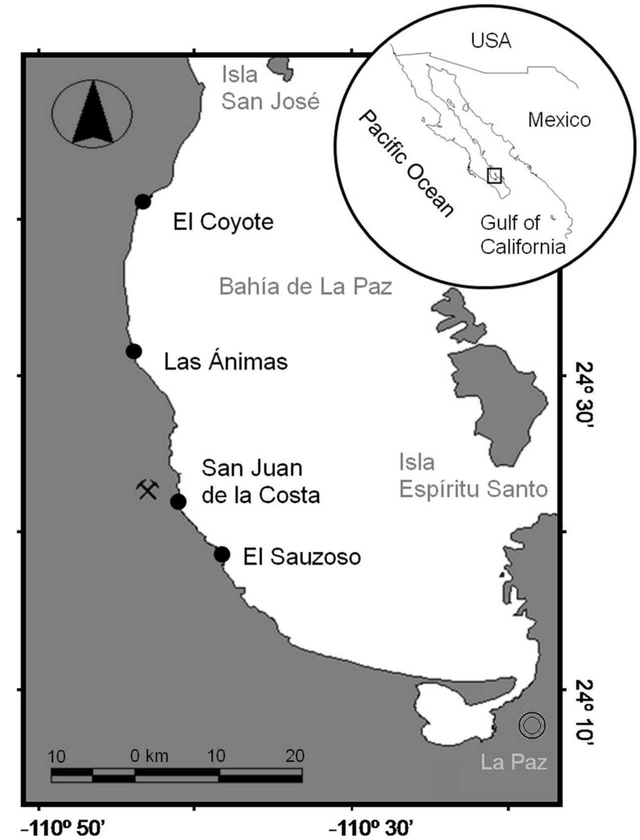

Fig. 1 Collection sites in the southwestern Gulf of California, Baja California Sur, Mexico. Circle, collection sites

thouarsii were collected, except at El Coyote where only two specimens of *T. depressus* could be found in winter and eight in summer. Overall, 70 *T. depressus* specimens (104.0 ± 14.7 mm diameter) and 84 *E. thouarsii* specimens (31.8 ± 8.2 mm diameter) were examined. Additionally, 18–30 healthy fronds of nine macroalgal species were detached at the basal holdfast by hand: three green (*Caulerpa sertularioides*, *Codium simulans*, and *Ulva lactuca*), two brown (*Dictyota flabellata* and *Sargassum sinicola*), and four red (*Gracilaria pachydermatica*, *Hypnea valentiae*, *Laurencia papillosa*, and *Polysiphonia pacifica*) macroalgae. These species are among the most abundant in this region, contributing >70 % of the seaweed biomass in *Sargassum*-dominated communities (Cruz-Ayala et al. 1998). All macroalgal species were considered potential food sources. Sea urchins and macroalgae were collected at a depth of 0.5–2.0 m along the beds.

Sample preparation

Sea urchin specimens were dissected to extract the gonads. For *T. depressus,* the gonads from each specimen were processed as separate samples; for *E. thouarsii*, the gonads from two or three specimens were pooled to obtain a composite sample of ~1.0 g DW, sufficient to carry out all analyses. Whole specimens of macroalgae were carefully cleaned by hand to remove sand and epiphytes. Thalli of each species were separated into three batches of 6–10 specimens. Three composite samples (n = 3) were prepared for each macroalgae species, site, and collection time.

Gonads and macroalgae samples were oven-dried at 75 °C for 24–72 h to constant weight and then ground in a steel ball mill to obtain a homogeneous mixture suitable for metal and isotope analyses. To avoid damage by humidity, all samples were stored under dry conditions until analysis.

Metal concentrations

Concentrations of Cd, Pb, Cu, Zn, and Fe were determined in gonads of both sea urchin species and from whole thalli of nine macroalgae species. Approximately 0.5 g of dried samples were digested with a 3:1 solution of nitric acid with hydrogen peroxide, 70 and 30 %, respectively (reagent grade, Avantor Performance Material, Central Valley, PA) in a microwave oven (Mars 5X, CEM, Matthews, NC). Concentrations of Cd, Pb, Cu, Zn, and Fe in each digested sample were quantified using air-acetylene flame atomic absorption spectrophotometry (Avanta, GBC Scientific Equipment, Braeside, VIC, Australia) (Méndez-Rodríguez et al. 2006; Hernández-Almaraz et al. 2014). The reference standards TORT-2, DORM-2, and ALGAE (National Research Council Canada, Institute for Marine Biosciences, Certified Reference Materials Programme, Halifax, NS, Canada) were used for validating the accuracy of the analytical method. Recovery of standard reference materials were 97–103 % for Cd, 95–100 % for Pb, 96–97 % for Cu, 94–105 % for Zn, and 95–101 % for Fe. Detection limits (μg g^{-1}; DW) for Cd, Pb, Cu, Zn, and Fe were 0.017, 0.07, 0.02, 0.02, and 0.07, respectively.

Statistics

Before each statistical analysis was run, Kolmogorov–Smirnov and Levene tests were performed to test dataset normality and homoscedasticity, respectively. Log transformation was performed to obtain normal distributions and homogeneous variances for data set of five metals on *E. thouarsii*, and log (x + 1) transformation on data sets of *T. depressus* and macroalgae (Zar 2010). Data were compared by two-way ANOVA to assess significant differences in sea urchins metal concentrations, followed by the post hoc Tukey test, among sites and seasons. All macroalgae were analyzed separately, but pooled to assess significant differences in metal concentrations among sites. All the analyses were performed using STATISTICA 8 software (Statsoft 2007). Data are presented as mean ± SE; all concentrations are expressed as μg g^{-1} DW, unless otherwise noted.

Results at $P < 0.05$ were considered statistically significant.

Because 67 of 70 samples of *T. depressus* had less than detectable amounts of Pb and the remaining three Pb samples were considered outliers (Gilbert 1987), comparative analysis was not made with this dataset. Moreover, because there were on two samples of detected elements in *T. depressus* collected in winter at El Coyote, these data were not used to compare between sites and dates. Metal concentrations reported in Hernández-Almaraz et al. (2014) for specimens of *Sargassum sinicola* and *Gracilaria pachydermatica* collected in winter at Las Ánimas, San Juan de la Costa, and El Sauzoso were included for comparison.

Macroalgae in the sea urchins' diet

Gut content components

To determine diet composition of the two sea urchin species at each site during each season, the gut contents of 70 *T. depressus* and 84 *E. thouarsii* specimens were extracted and placed in a 4 % formalin-seawater solution. Each sample was washed through a 700 µm mesh sieve. Approximately 1 g of gut contents and 5 mL of distilled water were placed in a Petri dish previously marked with randomly arranged points (Hyslop 1980). The number of points needed to evaluate each specimen's diet was chosen, based on a trophic diversity curve, in which the asymptotic relationship between the number of points examined and the number of new items found provided an estimate of the number of points needed to achieve precision and a reliable description of diet (Cortés 1997). For *T. depressus*, gut content fragments from 60 points were examined; for *E. thouarsii*, 30 points were examined. Each fragment was examined under a stereo microscope, and identified to the lowest possible taxonomic level, using taxonomic keys and descriptions (Setchell and Gardner 1924; Joly 1967; Abbott and Hollenberg 1976; Brusca 1980; Guiry and Guiry 2014).

Relative frequency of occurrence (RFO) of each component of the diet was based on the number of guts in which the component was found, expressed as a percent of the total number of guts examined. Relative abundance (RA) of each component in the diet was based on the number of fragments of each component, expressed as a percent of the total number of fragments. The index of relative importance ($\mathrm{IRI_n}$) of each component (n) was calculated as the product of the two percentages, RFO and RA, divided by 100. The relative importance index (IRI) of each component is expressed as a percent of the sum of the indices (Pinkas et al. 1971; Hyslop 1980; Mazariegos-Villarreal et al. 2013):

$$IRI(\%) = \left(IRI_n / \sum_{i=1}^{z} IRI_n \times 100 \right)$$

The IRI was developed to assess the relationship of the various food items in stomachs (Pinkas et al. 1971). Results are expressed as a percentage unit, it being possible to compare among datasets.

Stable isotope analysis (δ^{15}N)

To assess the trophic level of primary producers and consumers, δ^{15}N isotopic values were measured for both sea urchin species and nine macroalgae in triplicate analyses. The same samples used for metal analysis from El Sauzoso collected at winter 2011 were selected to exemplify a representative *Sargassum* bed with no human disturbance and under conditions of maximum richness and abundance of species. To avoid altering isotope content, none of the samples were acid washed (Bunn et al. 1995; Carabel et al. 2006; Ng et al. 2007; Serrano et al. 2008; Schlacher and Connolly 2014).

Isotopic analysis was conducted, as described by Bentzen et al. (2007); 0.2–0.5 mg of each sample was placed in individual tin capsules (Costech Analytical Technologies, Valencia, CA). All samples were analyzed at the Alaska Stable Isotope Facility, University of Alaska Fairbanks, Fairbanks, Alaska, with a continuous flow, isotope ratio mass spectrometer (DeltaV Plus, Thermo Fisher Scientific, Waltham, MA). Empty capsules and capsules filled with peptone (Sigma-Aldrich, St. Louis, MO) as reference material were used in each run; mean and SD of the reference material was $\delta^{15}\mathrm{N} = 7.00 \pm 0.14$; recovery, 99.9 %, were used as a quality assurance measure. All samples were run in triplicate. Results are expressed as ratios in delta (δ) notation in parts per thousand (‰) relative to atmospheric nitrogen (N_2) as international standard (Peterson and Fry 1987; Coplen 2011):

$$\delta^{15}N = \frac{\left({}^{15}N/{}^{14}N\right)_{Sample}}{\left({}^{15}N/{}^{14}N\right)_{Standard}} - 1$$

The mean isotopic values of δ^{15}N and its SE were obtained for each species.

To analyze the relationship between trophic levels and metal concentrations, δ^{15}N content versus metal concentrations in sea urchins and macroalgae from El Sauzoso in winter 2011 were plotted.

Results

Concentrations of metals

Concentrations of Cd, Cu, Zn, and Fe were detected in *T. depressus* collected from the four *Sargassum* beds, but Pb concentrations were below detectable limits at all sites (Table 1). Concentrations of Cd, and Fe in sea urchins from San Juan de la Costa were statistically higher than in sea urchins collected at the other sites.

The means of Cd, Cu, and Fe were higher in sea urchins sampled in winter than those sampled in summer. Metal concentrations of Cd, Cu, and Fe changed according to the site and the season (Table 1).

Concentrations of the five metals were detected in *E. thouarsii* from the four sites (Table 2). The highest concentrations of Cd, Cu, and Zn were found in San Juan de la Costa, and were statistically different from those in the sea urchins sampled at the other sites. Pb concentrations decreased from north to south (El Coyote = Las Ánimas = San Juan de la Costa > El Sauzoso); concentrations at El Coyote were 2.5–3 times higher than in El Sauzoso. The Pb concentration was higher in sea urchins collected in summer; Cd and Cu concentrations were not. Concentrations of Cd, Pb, Cu, and Zn varied with the site and the season (Table 2).

In the macroalgae, concentrations of Cd, Cu, and Zn were statistically different among sites (Table 3). Concentrations of Cd in macroalgae from San Juan de la Costa were statistically higher than in macroalgae at the other sites. The means of Cu were statistically higher in macroalgae collected at Las Ánimas and were statistically different from those sampled at the other sites. The means of Zn were statistically higher in macroalgae collected at El Coyote and San Juan de la Costa, and were statistically different from those sampled at Las Ánimas and El Sauzoso. The concentrations of Pb and Fe were not different among sites (Table 3).

Concentrations of Cu, Zn, and Fe were detected in macroalgae collected from the four *Sargassum* beds, but Cd and Pb concentrations were below detectable limits in macroalgae on some sampling dates (Table 4). Almost 72 % of the macroalgae were collected during winter. The maximum Cd content was found in thalli of brown macroalgae *Dictyota flabellata* from San Juan de la Costa (13.2 ± 0.5 μg g^{-1}), and *Sargassum sinicola* from El Sauzoso (13.1 ± 0.7 μg g^{-1}) and Las Ánimas (11.7 ± 0.7 μg g^{-1}), all were collected in summer. Among the red macroalgae, *Gracilaria pachydermatica* from El Coyote contained the highest Cd concentration in winter (8.0 ± 0.6 μg g^{-1}). The green macroalgae *Codium simulans* contained the highest Pb (3.9 ± 0.9 μg g^{-1}) at Las Ánimas in winter. The mean concentrations of Cu were higher in the thalli of the red macroalgae, *Polysiphonia pacifica*, from El Coyote and Las Ánimas in winter (5.9 ± 0.0 and 5.0 ± 0.8 μg g^{-1}, respectively) and in thalli of *Sargassum sinicola* from Las Ánimas in summer (5.0 ± 0.9 μg g^{-1}). The highest concentrations of Zn occurred in macroalgae from El Coyote: red (*Polysiphonia pacifica*; 47.3 ± 0.0 μg g^{-1} in winter), brown (*Dictyota flabellata*; 44.0 ± 0.8 μg g^{-1} in winter), and green (*Caulerpa sertularioides*; 41.9 ± 0.7 μg g^{-1} in summer). The highest concentrations of Fe were in red macroalgae *Laurencia papillosa* from Las Ánimas (1920.1 ± 187.3 μg g^{-1}) and *Dictyota flabellata* from El Coyote (1518.5 ± 137.3 μg g^{-1}), both in winter.

Table 1 Concentration of cadmium, lead, copper, zinc, and iron (μg g^{-1} (DW)) in gonads of the sea urchin *Tripneustes depressus* from *Sargassum* beds at four sites in the Gulf of California

	n	Cd	Pb	Cu	Zn	Fe
Site						
El Coyote	10	7.3 ± 1.6^a	< 0.07	1.0 ± 0.3	24.8 ± 5.0	52.4 ± 13.6^a
Las Ánimas	20	7.7 ± 1.0^a	< 0.07	1.1 ± 0.2	23.7 ± 3.7	42.5 ± 9.2^a
San Juan de la Costa	20	30.9 ± 5.5^b	< 0.07	1.7 ± 0.3	60.6 ± 12.8	165.3 ± 27.9^b
El Sauzoso	20	9.2 ± 1.0^a	< 0.07	1.3 ± 0.3	31.6 ± 7.0	40.0 ± 5.4^a
		$P < 0.0001$		$P = 0.39$	$P = 0.11$	$P < 0.0001$
Season						
Winter	32	20.5 ± 4.0^b	< 0.07	1.8 ± 0.2^b	34.8 ± 5.8	106.3 ± 21.7^b
Summer	38	9.8 ± 1.2^a	< 0.07	0.9 ± 0.2^a	38.2 ± 7.1	54.7 ± 6.4^a
		$P < 0.0001$		$P < 0.05$	$P = 0.83$	$P < 0.05$
Site × season						
El Coyote						
Winter*	2	10.4 ± 0.0	< 0.07	1.0 ± 1.0	23.0 ± 13.1	41.9 ± 5.5
Summer	8	6.5 ± 1.9^b	< 0.07	1.1 ± 0.3^{ab}	25.3 ± 5.8	55.1 ± 17.0^a
Las Ánimas						
Winter	10	10.6 ± 1.0^b	< 0.07	1.4 ± 0.3^{ab}	23.1 ± 2.7	53.4 ± 17.8^a
Summer	10	4.7 ± 1.0^a	< 0.07	0.8 ± 0.3^a	24.3 ± 7.1	31.6 ± 3.6^a
San Juan de la Costa						
Winter	10	42.6 ± 9.6^d	< 0.07	2.5 ± 0.4^b	58.3 ± 14.7	239.1 ± 44.4^b
Summer	10	19.2 ± 2.2^c	< 0.07	0.9 ± 0.3^a	62.9 ± 21.9	91.5 ± 10.6^b
El Sauzoso						
Winter	10	10.5 ± 1.7^b	< 0.07	1.6 ± 0.4^{ab}	25.4 ± 7.6	39.4 ± 4.4^a
Summer	10	7.9 ± 1.1^b	< 0.07	0.9 ± 0.4^a	37.8 ± 11.8	40.7 ± 10.1^a
		$P < 0.05$		$P < 0.05$	$P = 0.66$	$P < 0.05$

Samples were collected in winter (March) and summer (September) 2011. Data are shown as mean ± SE; lowercase letters indicate significant differences by two-way ANOVA, followed by post hoc Tukey test, $P < 0.05$

* Not considered in statistical comparisons

Table 2 Concentration of cadmium, lead, copper, zinc, and iron (μg g^{-1} (DW)) in gonads of the sea urchin *Eucidaris thouarsii* from *Sargassum* beds at four sites in the Gulf of California

	n	Cd	Pb	Cu	Zn	Fe
Site						
El Coyote	10	5.6 ± 0.4^{b}	36.4 ± 2.2^{b}	6.9 ± 0.3^{a}	87.6 ± 13.9^{a}	202.6 ± 25.2^{b}
Las Ánimas	9	6.1 ± 0.6^{b}	38.6 ± 4.2^{b}	6.6 ± 0.7^{a}	167.9 ± 22.6^{b}	142.6 ± 24.0^{ab}
San Juan de la Costa	9	29.0 ± 3.0^{c}	27.4 ± 2.3^{b}	8.7 ± 0.8^{b}	327.0 ± 51.9^{c}	155.7 ± 37.9^{ab}
El Sauzoso	10	3.3 ± 0.2^{a}	12.8 ± 1.7^{a}	6.1 ± 0.4^{a}	197.8 ± 35.9^{b}	105.5 ± 17.8^{a}
		$P < 0.0001$	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$	$P < 0.05$
Season						
Winter	18	10.1 ± 3.1^{b}	24.9 ± 3.7^{a}	8.0 ± 0.5^{b}	203.4 ± 37.4	179.4 ± 27.8
Summer	20	9.5 ± 1.7^{a}	33.6 ± 2.3^{b}	6.1 ± 0.3^{a}	176.6 ± 23.3	140.3 ± 14.8
		$P < 0.05$	$P < 0.0001$	$P < 0.0001$	$P = 0.33$	$P = 0.20$
Site × season						
El Coyote						
Winter	5	5.1 ± 0.8^{ab}	31.8 ± 3.7^{b}	7.8 ± 0.4^{b}	112.7 ± 15.3^{a}	214.6 ± 43.0
Summer	5	6.1 ± 0.3^{b}	39.7 ± 1.5^{b}	6.1 ± 0.3^{ab}	62.6 ± 13.7^{a}	192.7 ± 23.5
Las Ánimas						
Winter	4	5.8 ± 2.4^{ab}	37.7 ± 17.6^{b}	8.8 ± 1.4^{bc}	209.4 ± 18.0^{bc}	120.6 ± 43.0
Summer	5	6.2 ± 0.5^{b}	38.9 ± 3.5^{b}	5.4 ± 0.2^{a}	154.1 ± 27.9^{b}	149.9 ± 30.2
San Juan de la Costa						
Winter	4	38.3 ± 2.3^{c}	24.1 ± 2.1^{b}	10.8 ± 0.9^{c}	425.5 ± 82.7^{c}	259.8 ± 61.2
Summer	5	23.4 ± 1.8^{c}	29.4 ± 3.3^{b}	7.1 ± 0.8^{ab}	228.5 ± 27.9^{bc}	93.2 ± 17.0
El Sauzoso						
Winter	5	3.4 ± 0.4^{a}	10.6 ± 1.5^{a}	6.2 ± 0.5^{ab}	129.0 ± 39.7^{ab}	104.6 ± 23.3
Summer	5	3.1 ± 0.2^{a}	15.7 ± 2.8^{a}	6.0 ± 0.8^{ab}	301.0 ± 25.1^{c}	106.3 ± 32.7
		$P < 0.001$	$P < 0.05$	$P < 0.05$	$P < 0.0001$	$P = 0.10$

Samples were collected in winter (March) and summer (September). Data are shown as mean ± SE; lowercase letters indicate significant differences by two-way ANOVA, followed by post hoc Tukey test, $P < 0.05$

Table 3 Concentration of cadmium, lead, copper, zinc, and iron (μg g^{-1} (DW)) in macroalgae group sampled at four *Sargassum* beds at four sites in the Gulf of California collected in winter (March) and summer (September)

	Cd	Pb	Cu	Zn	Fe
El Coyote	2.4 ± 0.4^{a}	0.3 ± 0.1	2.3 ± 0.3^{a}	27.4 ± 2.3^{b}	510.5 ± 71.3
Las Ánimas	3.6 ± 0.9^{a}	0.9 ± 0.4	3.7 ± 0.4^{b}	17.0 ± 1.0^{a}	456.5 ± 123.8
San Juan de la Costa	5.9 ± 0.8^{b}	0.5 ± 0.2	2.2 ± 0.3^{a}	25.1 ± 1.3^{b}	449.6 ± 44.2
El Sauzoso	4.1 ± 0.9^{a}	0.7 ± 0.3	1.6 ± 0.2^{a}	14.4 ± 0.9^{a}	408.2 ± 55.9
	$P < 0.01$	$P = 0.32$	$P < 0.0001$	$P < 0.0001$	$P = 0.401$

Data are shown as mean ± SE; lowercase letters indicate significant differences by one-way ANOVA, followed by post hoc Tukey test, $P < 0.05$

Macroalgae in sea urchin diets

Gut content

The diet of both sea urchin species was mostly macroalgae (>99 %) at all four sites during both seasons. Some cyanoprokaryotes and invertebrates were also identified, but these contributed less than 1 % to the diet and are components that were probably ingested with macroalgae (Tables 5, 6).

The relative importance index (IRI) indicates that macroalgae species in the diet varied with the season and site. In *T. depressus*, the most common types were brown (1.3–76.2 %) and red macroalgae (11.3–95.0 %) (Table 5). *Sargassum*, *Gracilaria*, *Laurencia*, *Amphiroa*, and *Hypnea* were the most important components. *Gracilaria* fragments were the primary component (62 %) in winter at El Coyote, while more *Sargassum* fragments were the primary component at Las Ánimas (75 %) and El Sauzoso (76 %). At the San Juan de la Costa, IRI was 20–30 % for *Laurencia*, *Ulva*, and *Sargassum* fragments. In summer, *Sargassum* fragments were an important component at El Coyote (56 %) and El Sauzoso (64 %), while *Sargassum* and *Dictyota* fragments accounted for 61 % at Las Ánimas. At the San Juan de la Costa, articulated coralline *Amphiroa* represented 54 % IRI (Table 5).

Red macroalgae are the main food for *E. thouarsii*, reaching as much as 99 % (Table 6). *Lithophyllum*, *Hypnea*, *Polysiphonia*, *Ceramium*, and *Gracilaria* were the most important components. Fragments of the crustose algae *Lithophyllum* were consumed at all sites, with the highest IRI at Las Ánimas in winter (72 %) and summer (90 %). The filamentous algae *Ceramium* were

Table 4 Concentration of cadmium, lead, copper, zinc, and iron (μg g^{-1} (DW)) in macroalgae sampled at four *Sargassum* beds from the Gulf of California

Species	Site	Season	Cd	Pb	Cu	Zn	Fe
Chlorophyta							
Caulerpa sertularioides	EC	W	< 0.017	< 0.07	1.9 ± 0.0	26.7 ± 0.0	263.1 ± 0.0
	EC	S	< 0.017	< 0.07	1.4 ± 0.3	41.9 ± 0.7	317.1 ± 16.4
	ES	S	< 0.017	< 0.07	1.0 ± 0.2	12.1 ± 1.3	424.1 ± 23.2
Codium simulans	EC	W	1.3 ± 0.3	< 0.07	1.6 ± 0.6	18.8 ± 0.7	647.4 ± 143
	LA	W	0.7 ± 0.2	3.9 ± 1.7	3.2 ± 0.5	16.0 ± 1.3	333.0 ± 82.4
	SJ	W	2.8 ± 0.9	< 0.07	1.3 ± 0.7	19.9 ± 1.8	378.3 ± 50.0
	ES	W	1.7 ± 0.2	< 0.07	1.7 ± 0.2	16.6 ± 0.6	565.1 ± 171
Ulva lactuca	SJ	W	< 0.017	< 0.07	1.1 ± 0.2	31.9 ± 2.5	351.5 ± 32.8
	ES	W	0.1 ± 0.0	< 0.07	2.2 ± 0.2	11.9 ± 0.0	208.0 ± 0.8
Ochrophyta							
Dictyota flabellata	EC	W	2.7 ± 0.2	1.4 ± 0.3	2.4 ± 0.3	44.0 ± 0.8	1518.5 ± 137.3
	EC	S	0.6 ± 0.0	0.1 ± 0.0	2.9 ± 0.3	26.6 ± 4.4	774.0 ± 43.8
	LA	S	0.9 ± 0.1	< 0.07	2.3 ± 0.2	22.3 ± 1.3	591.1 ± 77.8
	SJ	W	9.2 ± 0.2	0.2 ± 0.1	1.4 ± 0.2	26.4 ± 0.8	526.0 ± 42.5
	SJ	S	13.2 ± 0.5	0.8 ± 0.5	3.4 ± 0.3	38.7 ± 2.5	789.3 ± 33.5
	ES	W	2.8 ± 0.4	< 0.07	1.9 ± 0.0	19.5 ± 0.4	809.9 ± 144.0
Sargassum sinicola	EC	W	5.5 ± 0.2	< 0.07	3.1 ± 1.5	17.5 ± 1.3	140.8 ± 28.2
	EC	S	3.2 ± 0.3	0.3 ± 0.3	3.2 ± 0.5	16.9 ± 0.7	263.5 ± 28.8
	LA	W*	5.2 ± 0.6	< 0.07	3.3 ± 0.4	17.1 ± 1.4	116.2 ± 8.0
	LA	S	11.7 ± 0.7	< 0.07	5.0 ± 0.9	23.8 ± 0.9	224.5 ± 39.3
	SJ	W*	9.6 ± 0.4	2.8 ± 1.1	4.5 ± 0.9	23.0 ± 1.6	134.9 ± 15.3
	ES	W*	6.7 ± 0.3	< 0.07	1.5 ± 0.2	15.3 ± 0.2	123.1 ± 3.1
	ES	S	13.1 ± 0.7	3.2 ± 0.4	1.6 ± 0.5	19.8 ± 0.6	490.9 ± 3.0
Rhodophyta							
Gracilaria pachydermatica	EC	W	8.0 ± 0.6	0.2 ± 0.1	< 0.02	10.7 ± 1.0	64.1 ± 8.6
	EC	S	1.5 ± 0.3	0.8 ± 0.4	1.3 ± 0.3	8.8 ± 0.6	130.5 ± 15.1
	LA	W*	5.0 ± 0.3	2.4 ± 0.4	5.1 ± 1.2	11.1 ± 0.7	66.4 ± 4.2
	SJ	W*	6.0 ± 0.5	< 0.07	3.8 ± 0.5	18.7 ± 1.0	197.0 ± 34.0
	ES	W*	5.4 ± 0.3	2.2 ± 0.7	1.6 ± 1.1	9.2 ± 1.4	98.1 ± 4.3
Hypnea valentiae	EC	W	1.0 ± 0.1	< 0.07	2.2 ± 0.2	45.7 ± 2.9	346.5 ± 88.3
	SJ	W	5.8 ± 0.0	< 0.07	1.6 ± 0.0	24.3 ± 0.0	565.9 ± 0.0
Laurencia papillosa var. *pacifica*	EC	S	0.9 ± 0.1	0.1 ± 0.0	1.5 ± 0.1	26.8 ± 2.6	464.5 ± 91.0
	LA	W	0.6 ± 0.0	< 0.07	1.7 ± 0.5	14.7 ± 0.9	1920.1 ± 187.3
	SJ	W	2.9 ± 0.2	< 0.07	1.5 ± 0.1	21.8 ± 0.3	701.4 ± 106.5
	ES	W	1.3 ± 0.2	< 0.07	1.3 ± 0.2	9.7 ± 0.7	479.4 ± 107.7
Polysiphonia pacifica	EC	W	1.7 ± 0.0	0.3 ± 0.0	5.9 ± 0.0	47.3 ± 0.0	1025.9 ± 0.0
	LA	W	1.0 ± 0.3	< 0.07	5.0 ± 0.8	14.2 ± 0.3	123.1 ± 3.0

Site: El Coyote (EC), Las Ánimas (LA), San Juan de la Costa (SJ), El Sauzoso (ES); Collection Date: winter (W, March) and summer (S, September) 2011. Three composite samples (n = 3) were analyzed for each macroalgae species. Data are shown as mean ± SE

* From Hernández-Almaraz et al. (2014)

an important component at San Juan de la Costa in winter (58 %). Fragments of *Hypnea* (26 %) and *Laurencia* (22 %) were highest in sea urchins at El Coyote in winter and summer, respectively (Table 6).

Stable isotopes

At El Sauzoso during winter, δ^{15}N content in gonad tissue of sea urchins ranged from 10.5 to 12.0 ‰ in *T. depressus* and 13.5–14.8 ‰ in *E. thouarsii*; a difference of 3.2 ± 0.4 ‰ between species was calculated. In macroalgae, the δ^{15}N content ranged from 7.2 to 13.0 ‰ (Table 7). Plots of isotope content *versus* concentration of each metal showed that *T. depressus* sea urchins with the lowest δ^{15}N content had high Cd concentrations with respect to *E. thouarsii* sea urchins and macroalgae (Fig. 2a). The sea urchin species with the highest δ^{15}N, *E. thouarsii*, had high concentrations of Pb, Cu, and Zn, compared to the other species (Fig. 2b shows this pattern, as an example, using only Pb). No patterns for Fe concentrations were identified.

Discussion

Metals

The maximum concentrations of Cd in gonads of *T. depressus* (42.6 ± 9.6 μg g^{-1}) and in *E. thouarsii* (38.3 ± 2.3 μg g^{-1}) are higher than concentrations in of sea urchins *Strongylocentrotus* spp. (Bohn 1979; Ahn et al. 2009), *Sterechinus neumayeri* (Majer et al. 2014), and *Echinometra mathaei* from sites in the Persian Gulf (Sadiq et al. 1996). The maximum concentration measured in specimens of the sea urchin *Strongylocentrotus*

Table 5 The index of relative importance (IRI) of macroalgae found in gut contents of the sea urchin *Tripneustes depressus* from *Sargassum* beds at four sites in the Gulf of California

Taxonomic group	Winter				Summer			
	EC	LA	SJ	ES	EC	LA	SJ	ES
Chlorophyta								
Caulerpa	0.0	0.0	0.0	0.1	0.1	6.6	0.9	20.7
Ulva	0.0	0.0	29.6	0.0	0.0	0.0	0.2	0.0
Subtotal	*0.0*	*0.0*	*29.6*	*0.1*	*0.1*	*6.6*	*1.1*	*20.7*
Ochrophyta								
Dictyota	0.0	1.1	0.6	0.2	0.5	23.0	1.2	4.2
Sargassum	1.3	75.1	21.5	76.0	56.5	38.5	5.8	63.6
Subtotal	*1.3*	*76.2*	*22.1*	*76.2*	*57.0*	*61.5*	*7.0*	*67.8*
Rhodophyta								
Amphiroa	0.0	0.0	6.4	6.8	0.3	2.4	54.1	0.6
Gelidiella	0.0	0.0	0.0	1.1	0.0	1.9	11.0	0.6
Gracilaria	61.9	15.0	4.2	9.8	13.9	10.7	8.9	3.4
Hypnea	10.6	0.6	3.8	0.7	5.3	4.3	6.6	3.5
Laurencia	21.2	2.5	29.9	5.2	14.2	12.1	2.7	3.1
Polysiphonia	0.9	5.2	1.9	0.0	0.0	0.0	0.2	0.0
Spyridia	0.4	0.0	0.0	0.0	8.1	0.0	0.0	0.1
Subtotal	*95.0*	*23.3*	*46.2*	*23.6*	*41.8*	*31.4*	*83.5*	*11.3*
Cyanoprokaryota	0.0	0.0	0.0	0.0	0.0	0.0	5.5	0.0
Invertebrates	0.4	0.2	0.0	0.0	0.0	0.2	0.7	0.0
Subtotal	*0.4*	*0.2*	*0.0*	*0.0*	*0.0*	*0.2*	*5.2*	*0.0*

The sea urchin specimens were collected in winter (March) and summer (September). Data are expressed as relative percentages (%). The subtotal IRI for each taxonomic group is shown in italic

EC El Coyote, *LA* Las Ánimas, *SJ* San Juan de la Costa, *ES* El Sauzoso

Table 6 The index of relative importance (IRI) of macroalgae found in gut contents of the sea urchin *Eucidaris thouarsii* from *Sargassum* beds at four sites in the Gulf of California

Taxonomic group	Winter				Summer			
	EC	LA	SJ	ES	EC	LA	SJ	ES
Chlorophyta								
Caulerpa	0.0	0.0	0.0	0.1	4.6	0.0	0.0	3.0
Ulva	0.0	0.0	19.2	0.0	0.0	0.0	0.0	0.0
Subtotal	*0.0*	*0.0*	*19.2*	*0.1*	*4.6*	*0.0*	*0.0*	*3.0*
Ochrophyta								
Dictyota	0.0	0.0	0.1	0.0	0.0	4.4	0.0	0.2
Sargassum	0.0	0.0	0.9	10.0	0.0	0.0	0.0	0.0
Subtotal	*0.0*	*0.0*	*1.0*	*10.0*	*0.0*	*4.4*	*0.0*	*0.2*
Rhodophyta								
Amphiroa	1.3	0.2	0.0	5.3	0.2	0.3	0.0	0.0
Ceramium	0.0	0.0	58.3	5.1	0.0	0.0	0.0	0.0
Gracilaria	0.1	0.5	0.2	5.1	1.0	0.5	17.8	0.0
Hypnea	26.1	2.4	0.4	0.7	8.2	4.1	29.4	66.1
Laurencia	0.2	0.0	3.0	0.1	22.2	0.0	0.0	0.0
Lithophyllum	29.4	72.2	17.4	52.7	42.2	90.0	51.9	28.7
Polysiphonia	36.8	23.1	0.5	17.8	0.0	0.0	0.0	0.0
Spyridia	5.4	0.0	0.0	0.0	21.2	0.0	0.0	1.7
Subtotal	*99.3*	*98.4*	*79.8*	*86.8*	*95*	*94.9*	*99.1*	*96.5*
Invertebrates	0.3	0.2	0.0	0.3	0.3	0.0	0.0	0.0

The sea urchin specimens were collected in winter (March) and summer (September). Data are expressed as relative percentages (%). The subtotal IRI for each taxonomic group is shown in italic

EC El Coyote, *LA* Las Ánimas, *SJ* San Juan de la Costa, *ES* El Sauzoso

spp. from Arctic environments near mine tailings was 1.6 μg Cd g^{-1} DW (Bohn 1979; Ahn et al. 2009); specimens of *S. neumayeri* from Antarctic environments nearby to an area affected by fire had a maximum concentration of 0.98 μg Cd g^{-1} DW (Majer et al. 2014); the maximum concentration in specimens of *E. mathaei* from an area impacted principally by an oil spill in the Persian Gulf was 5.49 μg Cd g^{-1} WW (Sadiq et al. 1996) (~0.7 μg Cd g^{-1} DW, assuming a ratio of wet to dry weight of 8:1 in the gonads; Garmendia et al. 2010).

Table 7 Mean and range of δ^{15}N content in gonads of two sea urchin species and their potential dietary items (nine macroalgae species) in a *Sargassum* bed in the Gulf of California

Taxonomic group	Species	δ^{15}N/‰	(Min–Max)
Invertebrates			
Echinodermata	*Tripneustes depressus*	11.2 ± 0.6	(10.5–12.0)
	Eucidaris thouarsii	14.3 ± 0.5	(13.5–14.8)
Macroalgae			
Chlorophyta	*Caulerpa sertularioides*	9.6 ± 0.2	(9.2–10.1)
	Codium simulans	12.3 ± 0.2	(11.9–12.7)
	Ulva lactuca	8.2 ± 0.3	(7.2–8.8)
Ochrophyta	*Dictyota flabellata*	10.0 ± 0.6	(7.8–10.7)
	Sargassum sinicola	12.5 ± 0.2	(12.1–13.0)
Rhodophyta	*Gracilaria pachydermatica*	12.2 ± 0.6	(11.1–12.9)
	Hypnea valentiae	11.5 ± 0.2	(10.9–11.9)
	Laurencia papillosa var. *pacifica*	10.4 ± 0.1	(9.8–10.9)
	Polysiphonia pacifica	10.0 ± 0.2	(9.8–10.1)

Specimens were collected at a representative site with minimal human disturbance and under conditions of maximum richness and abundance of species (El Sauzoso in winter-March 2011). Three composite samples (n = 3) were analyzed for each species. Data are shown as mean ± SE (Minimum–Maximum)

Concentrations of Pb in *T. depressus* were below the detectable limit (<0.07 μg g^{-1}), which is in agreement with the low concentrations in *Strongylocentrotus* spp. (0.03–0.08 μg g^{-1}, Ahn et al. 2009). By contrast, concentrations up to 39.7 ± 1.5 μg Pb g^{-1} were found in *E. thouarsii*, suggesting that either this species is exposed to a specific source of Pb from crustose (*Lithophyllum*) or articulated (*Amphiroa*) coralline algae and/or other organisms (mollusks, barnacles, filamentous, or turf-forming algae) attached to the macroalgae or rocks (Reyes-Bonilla and Calderón-Aguilera 1999). Previously, we measured a concentration of Pb of 32.5 μg g^{-1} in *Amphiroa beauvoisii* collected in winter 2011 at El Sauzoso (unpublished data); this amount, along with up to 9.7 μg Pb g^{-1} in *A. rigida* near a lead–zinc smelter (Schintu et al. 2010) indicates that these algae, containing calcified cell walls, can trap Pb (Davis et al. 2003). This suggests a route of exposure for *E. thouarsii* to appreciable concentrations of Pb through its diet.

Compared with concentrations of Cu (1.2–3.3 μg g^{-1}), Zn (20–134 μg g^{-1}), and Fe (32–133 μg g^{-1}), contents in *Strongylocentrotus* spp. (Ahn et al. 2009), the concentrations in *T. depressus* are consistent, while concentrations in *E. thouarsii* are higher. Such variations could be related to species-specific biological characteristics, because these three elements are essential for metabolism; their concentrations are therefore subject to homeostatic cellular regulation (Warnau et al. 1995a; Bielmyer et al. 2012; Watts et al. 2013). Seasonable differences could be related to physiological changes during the life cycle (Warnau et al. 1995a, b, c; Walker et al. 2013). In general, both species of sea urchins tend to display peaks of Cu, Zn, and Fe concentrations in winter, when the *Sargassum* bed reached maximum development, with a highest abundance of macroalgae.

However the high concentrations of Cd, Cu, Zn, and Fe in *T. depressus* and *E. thouarsii* at San Juan de la Costa are probably related to the presence of phosphorite deposits in the area. Marine upwelling, rain runoff, sewage discharge, and mining wastes can transport these metals to offshore areas and eventually increase their concentration in marine organisms (Neff 2002; Barwick and Maher 2003; Manahan 2007; Chojnacka 2009). Investigations at the same site on the chocolate clam *Megapitaria squalida* (Méndez-Rodríguez et al. 2006) and the sea slug *Elysia diomedea* (Hernández-Almaraz et al. 2014) show that the origin of these elements is the phosphate deposits that erode by natural or anthropogenic means, releasing these elements into the marine environment, which in turn accumulate in resident species of plants and animals. As generalist consumers of macroalgae and invertebrates, *T. thouarsii* and *E. depressus* can act as bioindicators of contaminants (Bohn 1979; Ablanedo et al. 1990; Warnau et al. 1995a; Sadiq et al. 1996; Storelli et al. 2001; Deheyn et al. 2005, Ahn et al. 2009).

Macroalgae in the sea urchins' diet

T. depressus and *E. thouarsii* are common in reefs located in the southwestern Gulf of California (Reyes-Bonilla and Calderón-Aguilera 1999; Herrero-Pérezrul 2008; Herrero-Pérezrul et al. 2008; Ríos-Jara et al. 2008; Solís-Marín et al. 2013, 2014). We found that, in *Sargassum* habitats, *T. depressus* feeds on brown and red macroalgae during the highest (winter) and lowest (summer) of algal biomass. The gut contents were mainly macroalgae of the genera *Sargassum*, *Gracilaria*, and *Laurencia*. In tropical and subtropical rocky reefs, sea urchins of the genus *Tripneustes* (*T. gratilla*, *T. ventricosus*, and *T. depressus*) are opportunistic consumers; however, a preference for macroalgae and even preference for a particular species has been reported for *T. gratilla* (Dy et al. 2002; Stimson et al. 2007; Lawrence and Agatsuma 2013). Experimentally, *Sargassum*, *Hyp-*

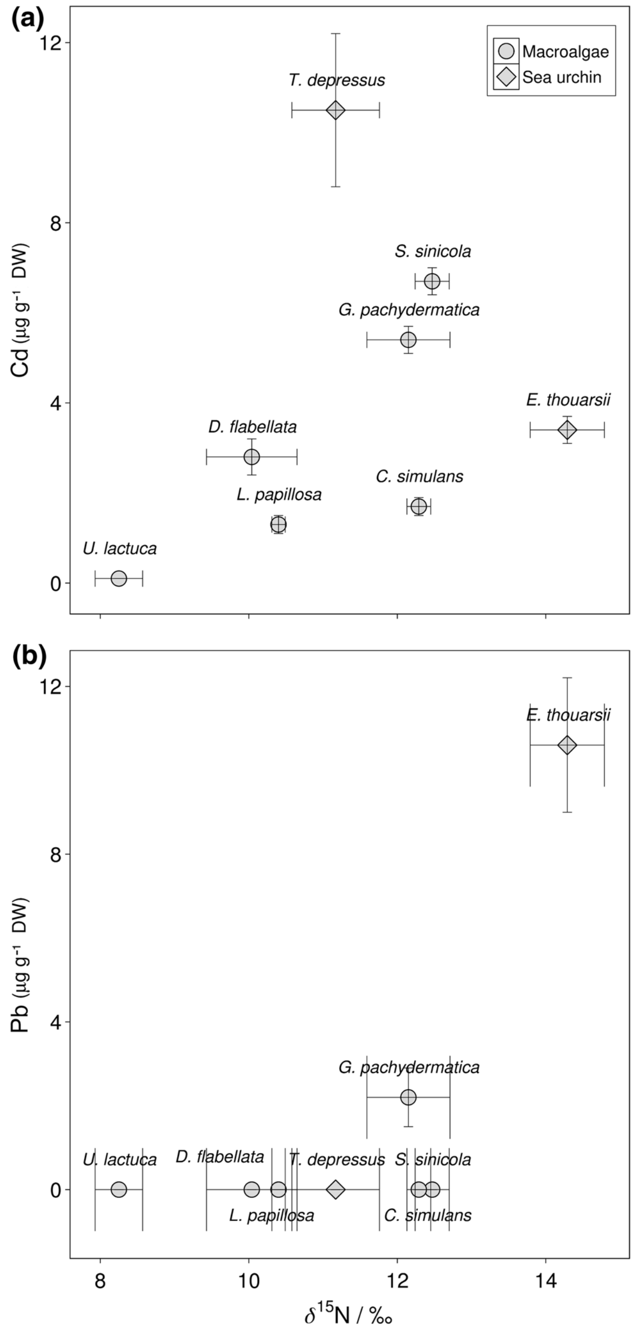

Fig. 2 δ^{15}N values and concentrations of cadmium (**a**) and lead (**b**) in gonads of two sea urchin species (*Tripneustes depressus* and *Eucidaris thouarsii*) and their potential dietary items (macroalgae species, *Codium simulans, Dictyota flabellata, Gracilaria pachydermatica, Sargassum sinicola, Ulva lactuca*) from a *Sargassum* spp. bed in the Gulf of California, El Sauzoso in winter 2011. *Diamonds* sea urchins; *Circles* macroalgae species. Data are shown as mean ± SD, (µg g^{-1} DW)

nea, and *Laurencia* are among the preferred macroalgae of *T. gratilla* and consumption of these taxa is related to higher growth rates in sea urchins (Dworjanyn et al. 2007; Seymour et al. 2013).

E. thouarsii has been described as an avid coral consumer in coral reefs or as a generalist omnivore that grazes on organisms attached to rocks, such as polychaetes, bryozoans, foraminifera, mollusks, barnacles, and filamentous and crustose macroalgae (Bautista-Romero et al. 1994; Reaka-Kudla et al. 1996; Reyes-Bonilla and Calderón-Aguilera 1999). Our analysis of gut content suggest that, in *Sargassum* beds, *E. thouarsii* likely feeds on red algae, including articulated (*Amphiroa*) and non-articulated (*Lithophyllum*) coralline red algae, as well as the filamentous macroalgae (*Spyridia*, *Polysiphonia*, and *Ceramium*) and fleshy macroalgae (*Gracilaria*, *Laurencia* and *Hypnea*). Green and brown macroalgae were present in large amounts in only a few sea urchins, although diet preference might be related to variations in local availability rather than preference for a specific species. McPherson (1968a) found that a closely related species, *Eucidaris tribuloides*, preferred sponges (*Cliona lampa*), followed by seagrass (*Thalassia testudinum*) and macroalgae (*Dictyota* sp. and *Liagora* sp.).

Prevalence of crustose (non-articulated) macroalgae in the gut of *E. thouarsii* may be explained by their foraging behavior, this sea urchin browses the rocks where the macroalgae grow, consuming the crustose algae and associated small organisms. Calcareous fragments are the major component of gut contents of *E. tribuloides* (McPherson 1968a), suggesting that this species obtains nutrients from microorganisms attached to calcareous fragments, as well as the macrophytes and sponges (McPherson 1968b; Santos et al. 2002). The sea urchins *Diadema antillarum* and *Toxopneustes roseus* primarily consume crustose coralline macroalgae (including *Lithophyllum* species) are their main food source for these urchins (Hawkins 1981; James 2000). Abundance of crustose coralline macroalgae and their relatively high nutritional value favors recruitment and survival of the sea urchin *E. tribuloides* (Reaka-Kudla et al. 1996).

Field and laboratory studies have shown that consumption of macroalgae provides nutrients and better gonadal growth in sea urchins (Dafni 1992; Schlosser et al. 2005; Dworjanyn et al. 2007; Sonnenholzner et al. 2011; Seymour et al. 2013). Gut content analysis and stable isotope analysis were used to show food sources and trophic position of sea urchins in subtidal rocky reefs (Vanderklift et al. 2006). The stable isotope composition of prey is reflected in the prey's isotope signature (Peterson and Fry 1987; Boecklen et al. 2011). Isotope composition increases from one trophic level to the next, particularly from 3 to 5 ‰ for δ^{15}N (Peterson and Fry 1987). We found that the increase in δ^{15}N value in sea urchins was 3.2 ± 0.4 ‰, which falls within the suggested enrichment. The trophic level of *T. depressus*, as inferred from its δ^{15}N values, corresponds to a diet based primarily on macroalgae, similar to other herbivorous sea urchins, based on a study of isotope and gut contents analysis of *Paracentrotus lividus* and *Sphaerechinus granularis* (Pinnegar and Polunin 2000). Our results are also similar to δ^{15}N values of the herbivorous gastropod that feeds on macroalgae, *Megas-*

traea undosa from Pacific coast of the Baja California Peninsula (δ^{15}N = 12.4 ± 0.6 ‰; Gómez-Valdez 2010) and *Elysia diomedea* from the *Sargassum* bed at El Sauzoso, one of the locations in our study (δ^{15}N = 14.3 ± 0.5 ‰; Hernández-Almaraz 2015).

The δ^{15}N value of *E. thouarsii* is consistent with the results of the diet analysis. In addition to feeding on macroalgae, *E. thouarsii* preys on invertebrates attached to the rocky substrate, as well as on invertebrates associated with the browsed macroalgae browsed, similar to *E. tribuloides* (McPherson 1968b; Reaka-Kudla et al. 1996). The contribution of nitrogen from epiphytic macrophytes has been observed in the sea urchin *Paracentrotus lividus* (Tomas et al. 2006). It follows that consumption of sponges, bryozoans, and other invertebrates contributes to higher δ^{15}N value. Hence, the δ^{15}N value of *E. thouarsii* should be greater than in *T. depressus*. The designation of *E. thouarsii* as an omnivore is thus the same *Megathura crenulata*, a gastropod that feeds on macroalgae and invertebrates (δ^{15}N = 15.5 ± 0.7 ‰; Gómez-Valdez 2010). The difference in feeding habits and the difference in trophic level of *T. depressus* and *E. thouarsii* has also been found in other sea urchins. This was examined by comparing the diet and isotopic data of species coexisting in patches of foliose and crustose coralline macroalgae, the herbivorous sea urchin *Heliocidaris erythrogramma* and carnivorous sea urchins *Phyllacanthus irregularis* and *Centrostephanus tenuispinus* (Vanderklift et al. 2006) and the herbivorous *Paracentrotus lividus* and carnivorous *Arbacia lixula* (Agnetta et al. 2013).

Fluctuations in isotope content of macroalgae are frequently reported, even in species belonging to the same taxonomic group (Wang and Yeh 2003; Lin et al. 2007; Hanson et al. 2010; Dethier et al. 2013). The cause for variations are complex, seemingly related to physical and chemical factors in the marine environment (Lin et al. 2007; Stephens and Hepburn 2014), as well as metabolic differences between macroalgae groups and presence of epibionts and/or storage of substances (Stephenson et al. 1984; Wang and Yeh 2003; Marconi et al. 2011). The δ^{15}N value in macroalgae could indicate differences in nitrogen availability, as occurs in other macrophytes (Stephens and Hepburn 2014). Variations in isotope values and consumer–resources discrimination have been widely documented and are currently of great interest in trophic ecological studies (Tomas et al. 2006; Boecklen et al. 2011; Dethier et al. 2013).

Diet as a potential route of transfer

Diet is the major route for transferring Cd, Pb, Cu, Zn, and Fe, among other elements, in marine food webs (Wang 2002; Barwick and Maher 2003; Jara-Marini et al. 2009). Evaluations of metal in food chains that begin by eating macrophytes suggest that macroalgae are responsible for transferring Cd, Pb, Cu, Zn, and Fe, among other elements, to herbivores and omnivores (Barwick and Maher 2003; Jara-Marini et al. 2009). Concentrations of these five elements in the sea urchin *Paracentrotus lividus* and three macroalgae species that it consumes, *Ulva lactuca*, *U. prolifera* (= *Enteromorpha prolifera*), and *Codium vermilara*, showed that concentrations of Cd, Pb, and Fe in the sea urchin gonads mirror their concentrations in macroalgae (Storelli et al. 2001). Concentrations of Cd and Cu in soft structures (gonads and gut) of sea urchin *E. mathaei*, were also related to feeding habits (Sadiq et al. 1996).

Studies of pollutant metals transfer across food chains, used isotopic values (δ^{15}N) to suggest that Cd, Cu, and Zn are accumulated and transferred from primary producers to primary and secondary consumers (Ruelas-Inzunza and Páez-Osuna 2008; Jara-Marini et al. 2009), unlike the Pb that is transferred mainly into higher consumers, i.e., omnivores, carnivores and predators (Ruelas-Inzunza and Páez-Osuna 2008). From gut content analysis, isotopic values, and metal concentrations in *T. depressus* and *E. thouarsii*, we suggest uptake of Cd, Cu, and Zn result from consumption of common macroalgae species that are present in the *Sargassum* beds; Cd is accumulated in macroalgae and transferred to the sea urchin *T. depressus* and *E. thouarsii*, but to a lesser extent (Fig. 2a). While *E. thouarsii* diet is more diverse, feeding on macroalgae and other items, which contributes to the transfer of Pb (Fig. 2b). Differences in the accumulation of metals in organisms of the same taxonomic group have been observed in other food chains of marine environments (Barwick and Maher 2003; Jara-Marini et al. 2009). The recognition of these kind of differences is important to understanding the dynamics in these elements in the basal levels of the food chain and its potential impact at higher levels, mainly because the macroalgae are basis for multiple food chains, and because sea urchins are species that contributes to structure of diverse marine environments.

Acknowledgments The authors thank Alejandra Mazariegos Villarreal, Alejandra Piñón Gimate, Baudilio Acosta Vargas, Griselda Peña Armenta, Horacio Bervera León, and Mario Jaime Rivera at CIBNOR, and Lorrie D. Rea, J. Margaret Castellini, and Carla Willeto at the University of Alaska Fairbanks for assistance in sample collecting, processing, and comments on the manuscript. Ira Fogel at CIBNOR provided editorial services. Funding was provided by CIBNOR (PC 0.5) and the Consejo Nacional de Ciencia y Tecnología (CONACYT CB2012 179327). P.H.A. is a recipient of a joint doctoral fellowship and scholarship Grant (Beca Mixta CONACYT 48345). Sample were collected under Permit #PPF/DGOPA-010/14.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

Abbott IA, Hollenberg GJ (1976) Marine algae of California. Stanford University Press, Stanford

Ablanedo N, González H, Ramírez M, Torres I (1990) Evaluación del erizo de mar *Echinometra lucunter* como indicador de contaminación por metales pesados, Cuba. Aquat Living Resour 3:113–120

Agatsuma Y (2013) Stock enhancement. In: Lawrence JM (ed) Sea urchins: biology and ecology, developments in aquaculture and fisheries science, vol 38. Elsevier Science, Oxford

Agnetta D, Bonaviri C, Badalamenti F, Scianna C, Vizzini S, Gianguzza P (2013) Functional traits of two co-occurring sea urchins across a barren/forest patch system. J Sea Res 76:170–177

Ahn I-Y, Ji J, Park H (2009) Metal accumulation in sea urchins and their kelp diet in an Arctic fjord (Kongsfjorden, Svalbard). Mar Pollut Bull 58:1571–1577

Aspholm OØ, Hylland K (1998) Metallothionein in green sea urchins (*Strongylocentrotus droebachiensis*) as a biomarker for metal exposure. Mar Environ Res 46:537–540

Barwick M, Maher W (2003) Biotransference and biomagnification of selenium, copper, cadmium, zinc, arsenic and lead in a temperate seagrass ecosystem from Lake Macquarie Estuary, NSW, Australia. Mar Environ Res 56:471–502

Bautista-Romero J, Reyes-Bonilla H, Lluch-Cota D, Lluch-Cota S (1994) Aspectos generales de la fauna marina. In: Ortega AR, Castellanos AV (eds) La isla Socorro, Reserva de la Biosfera Archipiélago de Revillagigedo. Centro de Investigaciones Biológicas del Noroeste (CIBNOR), La Paz, pp 243–275

Bentzen TW, Follmann EH, Amstrup SC, York GS, Wooller MJ, O'Hara TM (2007) Variation in winter diet of southern Beaufort Sea polar bears inferred from stable isotope analysis. Can J Zool 85:596–608

Bielmyer GK, Jarvis TA, Harper BT, Butler B, Rice L, Ryan S, McLoughlin P (2012) Metal accumulation from dietary exposure in the sea urchin, *Strongylocentrotus droebachiensis*. Arch Environ Contam Toxicol 63:86–94

Boecklen WJ, Yarnes CT, Cook BA, James AC (2011) On the use of stable isotopes in trophic ecology. Annu Rev Ecol Evol S 42:411–440

Bohn A (1979) Trace metals in fucoid algae and purple sea urchins near a high Arctic lead/zinc ore deposit. Mar Pollut Bull 10:325–327

Brusca C (1980) Common intertidal invertebrates of the Gulf of California. University of Arizona Press, Tucson

Bunn SE, Loneragan NR, Kempster MA (1995) Effects of acid washing on stable isotope ratios of C and N in penaeid shrimp and seagrass: implications for food-web studies using multiple stable isotopes. Limnol Oceanogr 40:622–625

Carabel S, Godínez-Domínguez E, Verísimo P, Fernández L, Freire J (2006) An assessment of sample processing methods for stable isotope analyses of marine food webs. J Exp Mar Biol Ecol 336:254–261

Chojnacka K (2009) Biosorption and bioaccumulation in practice. Novinka, Nova Science Publishers, New York

CONABIO (2014) Población por localidad, 2010. Comisión Nacional para el Conocimiento y Uso de la Biodiversidad, México, Scale 1:1

Coplen TB (2011) Guidelines and recommended terms for expression of stable-isotope-ratio and gas-ratio measurement results. Rapid Commun Mass Spectrom 25:2538–2560

Cortés E (1997) A critical review of methods of studying fish feeding based on analysis of stomach contents: application to elasmobranch fishes. Can J Fish Aquat Sci 54:726–738

Cruz-Ayala MB, Casas-Valdez M, Ortega-García S (1998) Temporal and spatial variation of frondose benthic seaweeds in La Paz Bay, BCS, Mexico. Bot Mar 41:191–198

Dafni J (1980) Abnormal growth patterns in the sea urchin *Tripneustes* cf. *gratilla* (L.) under pollution (Echinodermata, Echinoidea). J Exp Mar Biol Ecol 47:259–279

Dafni J (1992) Growth rate of the sea urchin *Tripneustes gratilla elatensis*. Isr J Zool 38:25–33

Dafni J (2009) Pollution induced mass-deformities in *Tripneustes*: biomechanical aspects. Chapter 89. In: Harris LG, Böttger SA, Walker CW, Lesser MP (eds) Echinoderms: Durham 2006. Proceedings of the 12th international echinoderm conference, 7–11 August 2006, Durham, pp 601–607

Davis TA, Volesky B, Mucci A (2003) A review of the biochemistry of heavy metal biosorption by brown algae. Water Res 37:4311–4330

Deheyn DD, Gendreau P, Baldwin RJ, Latz MI (2005) Evidence for enhanced bioavailability of trace elements in the marine ecosystem of Deception Island, a volcano in Antarctica. Mar Environ Res 60:1–33

Dethier MN, Sosik E, Galloway AW, Duggins DO, Simenstad CA (2013) Addressing assumptions: variation in stable isotopes and fatty acids of marine macrophytes can confound conclusions of food web studies. Mar Ecol Prog Ser 478:1–14

Díaz-Uribe JG, Arreguín-Sánchez F, Cisneros-Mata MA (2007) Multispecies perspective for small-scale fisheries management: a trophic analysis of La Paz Bay in the Gulf of California, Mexico. Ecol Model 201:205–222

Dworjanyn SA, Pirozzi I, Liu W (2007) The effect of the addition of algae feeding stimulants to artificial diets for the sea urchin *Tripneustes gratilla*. Aquaculture 273:624–633

Dy DT, Uy FA, Coralles CM (2002) Feeding, respiration, and excretion by the tropical sea urchin *Tripneustes gratilla* (Echinodermata: echinoidea) from the Philippines. J Mar Biol Assoc UK 82:299–302

Eisler R (2010) Compendium of trace metals and marine biota, plants and invertebrates, vol 1. Elsevier Science, Oxford

Elrod VA, Berelson WM, Coale KH, Johnson KS (2004) The flux of iron from continental shelf sediments: a missing source for global budgets. Geophys Res Lett. doi:10.1029/2004GL020216

Estes JA, Tinker MT, Williams TM, Doak DF (1998) Killer whale predation on sea otters linking oceanic and near shore ecosystems. Science 282:473–476

Foster MS, McConnico LM, Lundsten L, Wadsworth T, Kimball T, Brooks LB, Medina-López M, Riosmena-Rodríguez R, Hernández-Carmona G, Vásquez-Elizondo RM, Johnson S, Steller DL (2007) Diversity and natural history of a *Lithothamnion muelleri–Sargassum horridum* community in the Gulf of California. Cienc Mar 33:367–384

Garmendia JM, Menchaca I, Belzunce MJ, Franco J, Revilla M (2010) Seasonal variability in gonad development in the sea urchin (*Paracentrotus lividus*) on the Basque coast (Southeastern Bay of Biscay). Mar Pollut Bull 61:259–266

Gilbert RO (1987) Statistical methods for environmental pollution monitoring. Van Nostrand Reinhold, New York

Gómez-Valdez M (2010) Análisis de la alimentación natural de *Megastraea undosa* y *Megathura crenulata* mediante isótopos estables de $\delta^{13}C$ y $\delta^{15}N$. Bachelor Thesis, Universidad Autónoma de Baja California Sur, La Paz

Guiry MD, Guiry GM (2014) AlgaeBase. Worldwide electronic publication, National University of Ireland, Galway. http://www.algaebase.org. Accessed 30 June 2014

Hanson CE, Hyndes GA, Wang SF (2010) Differentiation of benthic marine primary producers using stable isotopes and fatty acids: implications to food web studies. Aquat Bot 93:114–122

Hawkins CM (1981) Efficiency of organic matter absorption by the tropical echinoid *Diadema antillarum* Philippi fed non-macrophytic algae. J Exp Mar Biol Ecol 49:245–253

Hernández-Almaraz P (2015) Bioacumulación de Cd, Pb, Cu, Zn y Fe en un gasterópodo y dos especies de erizo por consumo de macroalgas asociadas a mantos de *Sargassum* spp. en Bahía de La Paz, Baja California Sur, México. Doctoral thesis, Centro de Investigaciones Biológicas del Noroeste, La Paz

Hernández-Almaraz P, Méndez-Rodríguez L, Zenteno-Savín T, García-Domínguez F, Vázquez-Botello A, Serviere-Zaragoza E (2014) Metal mobility and bioaccumulation differences at lower trophic levels in marine ecosystems dominated by *Sargassum* species. J Mar Biol Assoc UK 94:435–442

Hernández-Carmona G, Casas-Valdez M, Fajardo-León C, Sanchez-Rodríguez I, Rodríguez-Montesinos E (1990) Evaluación de *Sargassum* spp. en la Bahía de La Paz, BCS, México. Investigaciones Marinas CICIMAR-IPN, vol 5 pp 11–18

Herrero-Pérezrul MD (2008) Diversity and abundance of reef macro invertebrates (Mollusca; Echinodermata) in the southern Gulf of California, Mexico. In: Proceedings of the 11th International Coral Reef Symposium, Fort Lauderdale

Herrero-Pérezrul MD, Reyes-Bonilla H, González-Azcárraga A, Cintra-Buenrostro CE, Rojas-Sierra (2008) Equinodermos. In: Danemann GD, Ezcurra E (eds) Bahía de Los Ángeles: Recursos naturales y comunidad, línea base 2007. México: Secretaría de Medio Ambiente y Recursos Naturales, Instituto Nacional de Ecología, Pronatura Noroeste and San Diego Natural History Museum, pp 339–357

Hinojosa-Arango G, Rioja-Nieto R, Suárez-Castillo ÁN, Riosmena-Rodríguez R (2014) Using GIS methods to evaluate rhodolith and *Sargassum* beds as critical habitats for commercially important marine species in Bahía Concepción, BCS, México. Cryptogamie Algol 35:49–65

Holguín-Quiñones O, Wrigth LH, Solís-Marín FA (2000) Asteroidea, Echinoidea y Holothuroidea en fondos someros de la Bahía de Loreto, B.C.S., México. Rev Biol Trop 48:749–757

Hyslop EJ (1980) Stomach contents analysis—a review of methods and their application. J Fish Biol 17:411–429

James DW (2000) Diet, movement, and covering behavior of the sea urchin *Toxopneustes roseus* in rhodolith beds in the Gulf of California, Mexico. Mar Biol 137:913–992

Jara-Marini ME, Soto-Jiménez MF, Páez-Osuna F (2009) Trophic relationships and transfer of cadmium, copper, lead and zinc in a subtropical coastal lagoon food web from SE Gulf of California. Chemosphere 77:1366–1373

Joly AB (1967) Generos de algas marinhas da costa Atlantica Latino-americana. Universidad de De Sao Paulo, Sao Paula

Klumpp DW, Salita-Espinosa JT, Fortes MD (1993) Feeding ecology and trophic role of sea urchins in a tropical seagrass community. Aquat Bot 45:205–229

Lawrence JM, Agatsuma Y (2013) *Tripneustes*. In: Lawrence JM (ed) Sea urchins: biology and ecology, developments in aquaculture and fisheries science, vol 38., Elsevier ScienceOxford, UK, pp 491–507

Lawrence JM, Jangoux M (2013) Cidaroids. In: Lawrence JM (ed) Sea urchins: biology and ecology, developments in aquaculture and fisheries science, vol 38. Elsevier Science, Oxford, pp 225–242

Lin HJ, Kao WY, Wang YT (2007) Analyses of stomach contents and stable isotopes reveal food sources of estuarine detritivorous fish in tropical/subtropical Taiwan. Estuar Coast Shelf S 73:527–537

Majer AP, Petti MAV, Corbisier TN, Ribeiro AP, Theophilo CYS, Ferreira PADL, Figueira RCL (2014) Bioaccumulation of potentially toxic trace elements in benthic organisms of Admiralty Bay (King George Island, Antarctica). Mar Pollut Bull 79:321–325

Manahan SE (2007) Introducción a la química ambiental. Editorial Reverté, Barcelona

Marconi M, Giordano M, Raven JA (2011) Impact of taxonomy, geography, and depth on δ^{13}C and δ^{15}N variation in a large collection of macroalgae. J Phycol 47:1023–1035

Mazariegos-Villarreal A, Piñon-Gimate A, Aguilar-Mora F, Medina M, Serviere-Zaragoza E (2013) Diet of the keyhole limpet *Megathura crenulata* (Mollusca: Gastropoda) in subtropical rocky reefs. J Shellfish Res 32:297–303

McPherson BF (1968a) Feeding and oxygen uptake of the tropical sea urchin *Eucidaris tribuloides* (Lamarck). Biol Bull 135:308–321

McPherson BF (1968b) Contributions to the biology of the sea urchin *Eucidaris tribuloides* (Lamarck). B Mar Sci 18:400–443

Méndez-Rodríguez L, Palacios E, Acosta B, Monsalvo-Spencer P, Álvarez-Castañeda T (2006) Heavy metals in the Clam *Megapitaria squalida* collected from wild and phosphorite mine-impacted sites in Baja California, Mexico. Biol Trace Elem Res 110:275–287

Muñetón-Gómez MS, Hernández-Carmona G (1993) Crecimiento estacional de *Sargassum horridum* (Setchell y Gardner) Phaeophyta, en la Bahía de La Paz, BCS, México. Investigaciones Marinas CICIMAR-IPN, vol 8, pp 23–31

Neff JM (2002) Bioaccumulation in marine organisms. Effect of contaminants from oil well produced water. Elsevier Science, Oxford

Ng JS, Wai TC, Williams GA (2007) The effects of acidification on the stable isotope signatures of marine algae and molluscs. Mar Chem 103:97–102

Núñez-López RA, Casas-Valdez MM (1996) Fenología de las especies de *Sargassum* (Fucales: Sargassaceae) en tres zonas de Bahía Concepción, BCS, México. Rev Biol Trop 44:455–464

Pacheco-Ruíz I, Zertuche-González JA, Espinoza-Ávalos J, Riosmena-Rodríguez R, Galindo-Bect L, Gálvez-Télles A, Meling-López AE, Orduña-Rojas J (2008) Macroalgas. In: Danemann GD, Ezcurra E (eds) Bahía de Los Ángeles: Recursos naturales y comunidad, línea base 2007. México: Secretaría de Medio Ambiente y Recursos Naturales, Instituto Nacional de Ecología, Pronatura Noroeste y San Diego Natural History Museum, pp 181–214

Peterson BJ, Fry B (1987) Stable isotopes in ecosystem studies. Annu Rev Ecol Evol Syst 18:293–320

Pinkas L, Oliphant MS, Iverson ILK (1971) Food habits of albacore, bluefin tuna, and bonito in California waters. Calif Dep Fish Game Fish Bull 152:1–105

Pinnegar JK, Polunin NV (2000) Contributions of stable-isotope data to elucidating food webs of Mediterranean rocky littoral fishes. Oecologia 122:399–409

Ray S (1984) Bioaccumulation of cadmium in marine organisms. Exp Suppl 40:14–23

Reaka-Kudla ML, Feingold JS, Glynn W (1996) Experimental studies of rapid bioerosion of coral reefs in the Galapagos Islands. Coral Reefs 15:101–107

Reyes-Bonilla H, Calderon-Aguilera LE (1999) Population density, distribution and consumption rates of three corallivores at Cabo Pulmo Reef, Gulf of California, Mexico. Mar Ecol 20:347–357

Riley JP, Chester R (1971) Introduction to marine chemistry. Academic Press, London

Ríos-Jara E, Galván-Villa CM, Solís-Marín FA (2008) Echinoderms of the National Park Isla Isabel, Nayarit, Mexico. Rev Mex Biodivers 79:131–141

Ruelas-Inzunza JR, Páez-Osuna F (2008) Trophic distribution of Cd, Pb and Zn in a food web from Altata-Ensenada del Pabellón subtropical lagoon, SE Gulf of California. Arch Environ Contam Toxicol 54:584–596

Ruitton S, Francour P, Boudouresque CF (2000) Relationships between algae, benthic herbivorous invertebrates and fishes in rocky sublittoral communities of a temperate sea (Mediterranean). Estuar Coast Shelf S 50:217–230

Sabiha-Javied Mehmood T, Chaudhry MM, Tufail M, Irfan N (2009) Heavy metal pollution from phosphate rock used for the production of fertilizer in Pakistan. Microchem J 91:94–99

Sadiq M, Mian AA, Saji AP (1996) Metal bioaccumulation by sea urchin (*Echinometra mathaei*) from the Saudi coastal areas of the Arabian Gulf: 2. Cadmium, copper, chromium, barium, calcium, and strontium. Bull Environ Contam Toxicol 57:964–971

Sánchez-Rodríguez I, Cervantes-Duarte R (1999) Longitud y biomasa de *Sargassum sinicola* Setchell et Gardner (Phaeophyta) en Bahía Magdalena, Baja California Sur, México. Hidrobiologica 9:117–124

Santos CP, Coutinho AB, Hajdu E (2002) Spongivory by *Eucidaris tribuloides* from Salvador, Bahia (Echinodermata: Echinoidea). J Mar Biol Assoc UK 82:295–297

Schintu M, Marras B, Durante L, Meloni P, Contu A (2010) Macroalgae and DGT as indicators of available trace metals in marine coastal waters near a lead–zinc smelter. Environ Monit Assess 167:653–661

Schlacher TA, Connolly RM (2014) Effects of acid treatment on carbon and nitrogen stable isotope ratios in ecological samples: a review and synthesis. Methods Ecol Evol 5:541–550

Schlosser SC, Lupatsch I, Lawrence JM, Lawrence AL, Shpigel M (2005) Protein and energy digestibility and gonad development of the European sea urchin *Paracentrotus lividus* (Lamarck) fed algal and prepared diets during spring and fall. Aquac Res 36:972–982

Serrano O, Serrano L, Mateo MA, Colombini I, Chelazzi L, Gagnarli E, Fallaci M (2008) Acid washing effect on elemental and isotopic composition of whole beach arthropods: implications for food web studies using stable isotopes. Acta Oecol 34:89–96

Servicio Geológico Mexicano (2008) Panorama minero del Estado de Baja California Sur. Secretaria de Economía, Servicio Geológico Mexicano, Coordinación General de Minería, Secretaría de Gobernación, Mexico

Setchell WA, Gardner NL (1924) The marine algae: expedition of the California Academy of Sciences to the Gulf of California in 1921. Proc Calif Acad Sci 12:695–949

Seymour S, Paul NA, Dworjanyn SA, de Nys R (2013) Feeding preference and performance in the tropical sea urchin *Tripneustes gratilla*. Aquaculture 400–401:6–13

Solís-Marín FA, Honey-Escandón MB, Herrero-Pérezrul MD, Benitez-Villalobos F, Díaz-Martínez JP, Buitrón-Sánchez BE, Durán-González A (2013) The echinoderms of Mexico: biodiversity, distribution and current state of knowledge. In: Alvarado JJ, Solis-Marin FA (eds) Echinoderm research and diversity in Latin America. Springer, Berlin, pp 11–65

Solís-Marín FA, Laguarda-Figueras A, Honey-Escandón MB (2014) Biodiversity of echinoderms (Echinodermata) in Mexico. Rev Mex Biodivers 85:S441–S449

Sonnenholzner JI, Montaño-Moctezuma G, Searcy-Bernal R, Salas-Garza A (2011) Effect of macrophyte diet and initial size on the survival and somatic growth of sub-adult *Strongylocentrotus purpuratus*: a laboratory experimental approach. J Appl Phycol 23:505–513

StatSoft (2007) STATISTICA (data analysis software system), version 8.0. StatSoft, Tulsa

Steneck RS (2013) Sea urchins as drivers of shallow benthic marine community structure. In: Lawrence JM (ed) Sea urchins: biology and ecology, developments in aquaculture and fisheries science, vol 38. Elsevier Science, Oxford, pp 195–212

Stephens TA, Hepburn CD (2014) Mass-transfer gradients across kelp beds influence *Macrocystis pyrifera* growth over small spatial scales. MEP 515:97–109

Stephenson RL, Tan FC, Mann KH (1984) Stable carbon isotope variability in marine macrophytes and its implications for food web studies. Mar Biol 81:223–230

Stimson J, Cunha T, Philippoff J (2007) Food preferences and related behavior of the browsing sea urchin *Tripneustes gratilla* (Linnaeus) and its potential for use as a biological control agent. Mar Biol 151:1761–1772

Storelli MM, Storelli A, Marcotrigiano GO (2001) Heavy metals in the aquatic environment of the Southern Adriatic Sea, Italy: macroalgae, sediments and benthic species. Environ Int 26:505–509

Suárez-Castillo ÁN (2008) Fauna asociada a mantos de *Sargassum* (Ochrophyta: Fucales) en El Sauzoso, Baja California Sur, México. Master's Thesis, Instituto Politécnico Nacional, Centro Interdiciplinario de Ciencias Marinas, La Paz

Suárez-Castillo ÁN, Riosmena-Rodríguez R, Rojo-Amaya M, Torre-Cosio J, Rioja-Nieto R, Hudson-Weaver A, Pfister T, Hernández-Carmona G, Hinojosa-Arago G, Aburto-Oropeza O, Figueroa-Cárdenas AL (2013) Bosques de algas pardas en el golfo de California: *Sargassum*, un hábitat esencial. CONABIO. Biodiversitas 108:12–16

Tertschnig WP (1988) Diet activity patterns and foraging dynamics of the sea urchin *Tripneustes ventricosus* in a tropical seagrass community and a reef environment (Virgin Islands). Mar Ecol 10:3–21

Tomas F, Alvarez-Cascos D, Turon X, Romero J (2006) Differential element assimilation by sea urchins *Paracentrotus lividus* in seagrass beds: implications for trophic interactions. Mar Ecol Prog Ser 306:125–131

Vanderklift MA, Kendrick GA, Smit AJ (2006) Differences in trophic position among sympatric sea urchin species. Estuar Coast Shelf S 66:291–297

Voulgaropoulos A, Paneli M, Papaefstathiou E, Stavroulias S (1991) Comparative determinations of cadmium and lead in phosphorites dissolved in nitric acid and aqua regia using differential pulse anodic stripping voltammetry and atomic absorption spectrophotometry. Fresenius J Anal Chem 341:568–569

Walker CW, Lesser MP, Unuma T (2013) Sea urchin gametogenesis—structural, functional and molecular/genomic biology. In: Lawrence JM (ed) Sea urchins: biology and ecology, developments in aquaculture and fisheries science, vol 38. Elsevier Science, Oxford, pp 25–43

Wang WL (2002) Interactions of trace metals and different marine food chains. Mar Ecol Prog Ser 243:295–309

Wang WL, Yeh HW (2003) $\delta^{13}C$ values of marine macroalgae from Taiwan. Bot Bull Acad Sin 44:107–112

Warnau M, Ledent G, Temara A, Alva V, Jangoux M, Dubois P (1995a) Allometry of heavy metal bioconcentration in the echinoid *Paracentrotus lividus*. Arch Environ Contam Toxicol 29:393–399

Warnau M, Ledent G, Temara A, Jangoux M, Dubois P (1995b) Experimental cadmium contamination of the echinoid *Paracentrotus lividus*: influence of exposure mode and distribution of the metal in the organism. Mar Ecol Prog Ser 116:117–124

Warnau M, Teyssié JL, Fowler SW (1995c) Effect of feeding on cadmium bioaccumulation in the echinoid *Paracentrotus lividus* (Echinodermata). Mar Ecol Prog Ser 126:305–309

Watts S, Lawrence AL, Lawrence JM (2013) Nutrition. In: Lawrence JM (ed) Sea urchins: biology and ecology, developments in aquaculture and fisheries science, vol 38. Elsevier Science, Oxford, pp 155–169

Casas-Valdez M, Sanchez I, Hernández G (1993) Evaluación de *Sargassum* spp. en la costa oeste de Bahía Concepción, BCS, México. Investigaciones Marinas CICIMAR-IPN, vol 8, pp 61–68

Zar JH (2010) Biostatistical analysis, 5th edn. Prentice Hall, Upper Saddle River