

## The composition of fatty acids in female gametes of *Arbacia dufresnii* (Arbaciidae: Arbacioida) in the population of Bahía Norte, Nuevo gulf indicates good nutritional conditions

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**Abstract:** Here we study the population of *Arbacia dufresnii* in two locations within Nuevo Gulf (Bahía Norte and Punta Cuevas), Argentina, focusing on their size, density, and nutritional status through fatty acid (FA) profiles. Sea urchins were collected by scuba diving, analysing 4000 individuals over an entire year. The results showed that sea urchins in Bahía Norte are larger but less dense than those in Punta Cuevas. Polyunsaturated fatty acids (PUFA) and highly unsaturated fatty acids (HUFA) were predominant in Bahía Norte, indicating a diet rich in high-quality nutrients. *A. dufresnii* showed an omnivorous diet with a tendency towards carnivory in Bahía Norte. Additionally, significant differences in trophic markers were found between both locations, suggesting variations in food availability and quality. *A. dufresnii* proved to be an excellent bioindicator, reflecting environmental and nutritional changes in its FA profiles.

**Key words:** sea urchins, trophic dynamics, food quality, fatty acids, environmental bioindicators, gametes

**Resumen:** La composición de ácidos grasos en los gametos femeninos de *Arbacia dufresnii* (Arbaciidae: Arbacioida) en la población de Bahía Norte, golfo Nuevo, indica buenas condiciones nutricionales. Aquí estudiamos la población de *Arbacia dufresnii* en dos localidades dentro del Golfo Nuevo (Bahía Norte y Punta Cuevas), Argentina, centrándonos en su tamaño, densidad y estado nutricional a través de los perfiles de ácidos grasos (AG). Los erizos de mar fueron recolectados mediante buceo, analizando 4000 individuos a lo largo de un año. Los resultados mostraron que los erizos en Bahía Norte son más grandes, pero menos densos que los de Punta Cuevas. Los ácidos grasos poliinsaturados (PUFA) y los ácidos grasos altamente insaturados (HUFA) fueron predominantes en Bahía Norte, lo que indica una dieta rica en nutrientes de alta calidad. *A. dufresnii* mostró una dieta omnívora con tendencia a la una dieta carnívora en Bahía Norte. Además, se encontraron diferencias significativas en los marcadores tróficos entre ambas localidades, lo que sugiere variaciones en la disponibilidad y calidad del alimento. *A. dufresnii* demostró ser un excelente bioindicador, reflejando cambios ambientales y nutricionales en sus perfiles de ácidos grasos.

**Palabras clave:** erizos de mar, dinámica trófica, calidad del alimento, ácidos grasos, bioindicadores ambientales, gametas

### INTRODUCTION

*Arbacia dufresnii* is a sea urchin species distributed in the Argentine Sea, which has been extensively studied over the past 20 years (Brogger *et al.*, 2010, 2013; Epherra *et al.*, 2014, 2015; Parra *et al.*, 2015; Epherra, 2016; Rubilar *et al.*, 2016, 2017; Zárate *et al.*, 2016; Díaz de Vivar *et al.*, 2019; Fernández *et al.*, 2021; Sepúlveda *et al.*, 2021; Ancín *et al.*, 2021; Chaar *et al.*, 2021; Castro *et al.*, 2022; Garcés *et al.*, 2024; Martelli *et al.*, 2024; Vera-Piombo *et al.*, 2024). It is described

as omnivorous in all environments and seasons, with notable plasticity that allows it to adapt to diverse habitats. *A. dufresnii* competes with herbivores, thereby increasing the complexity of the benthic food web in the North Patagonian gulfs where it resides. Its tendency toward carnivory is related to the abundance of animal prey in its environment. In environments with high availability of macroalgae and animal prey, its diet tends to be more carnivorous, without a preference for native over invasive algae (Penchaszadeh & Lawrence, 1999; Epherra, 2016; Castro *et al.*,

2022). Studies on the reproductive and biochemical characteristics of *Arbacia dufresnii* also demonstrate its high plasticity (Epherra *et al.*, 2014; Parra *et al.*, 2015).

Populations of *A. dufresnii* have shown sizes according to the size-temperature rule and abundances were higher in areas with smaller animals, showing a density-dependent effect (Epherra, 2016). Smaller adults have been recorded by Morsan (2009), in the north of San Matias Gulf (~12 mm) although, the high presence of juveniles in San Matias Gulf, could result in a smaller average size (Epherra, 2016). Larger ones have been recorded on the coasts of Comodoro Rivadavia (~63 mm) by Epherra *et al.* (2014). Food availability is a crucial variable affecting size in sea urchins (Ebert, 1968; McShane & Anderson, 1997; Hill & Lawrence, 2003), although it has not yet been studied in *A. dufresnii*. This polymodal distribution has been observed in several populations of benthic invertebrates, including *A. dufresnii*, and aligns with the presence of multiple annual cohorts (Botsford *et al.*, 1994; Smith & Botsford, 1998; Beddingfield & McClintock, 2000; Epherra, 2016). The diversity of coexisting sizes is also associated with the seasonality of the reproductive cycle, with spawning occurring in spring and summer (Brogger *et al.*, 2010, 2013; Epherra *et al.*, 2014), as well as with the variety of available food sources (Epherra, 2016).

The nutrients assimilated by sea urchins directly reflect the food they access, accumulating in tissues such as the integument, gonads, intestines, and gametes (Scharm *et al.*, 2018; Zuo *et al.*, 2018). Fatty acid (FA) profiles are very useful for inferring nutritional conditions in aquatic environments (Powell *et al.*, 2020). Although the nutritional conditions of *A. dufresnii* populations has not been evaluated, its plastic feeding habits could modify its nutritional status according to its habitat. The density and size of individuals also help understand the species' strategy in different environments. For example, in San José Gulf, it exhibits an omnivorous habit with a tendency towards carnivory, with low-density populations (~3.8 ind/m<sup>2</sup>) and intermediate sizes in Punta Tehuelche (1-3 cm) and Zone 39 (1-5 cm). In contrast, in Nuevo Gulf, at Punta Cuevas, they are omnivorous with a tendency towards herbivory in dense populations (~9 ind/m<sup>2</sup>) of small size (15-25 cm) (Epherra, 2016).

Bahía Norte, a northern area of Nuevo Gulf, is characterized by its proximity to the Fishing Industrial Park and has been influenced by ef-

fluents from fish and shrimp meal processing plants for decades (Ambrosio *et al.*, 2004; Quille & Donaires, 2013; Mera Zambrano & Zambrano Rendón, 2023; Rueda, 2023). Although regulations now prevent liquid fish waste from being discharged into the sea, this was not the case for a long time. The aim of this study is to investigate a new population of *A. dufresnii* in Bahía Norte by evaluating its size, density, and nutritional status through fatty acid profiles.

## MATERIALS AND METHODS

**Sampling area.** A total of 4000 animals were collected through scuba diving in the subtidal zone of Bahía Norte (42°43'41.10"S-65°1'31.60"W) in Puerto Madryn, Argentina (Fig. 1) during February, March, July, August, September, and October 2022. The collection of gametes in autumn was excluded, as during this season the animals in the studied population are in the intergametic stage, and their gonads contain nutritive phagocytes and intermediate or senescent cells (Epherra, 2016). In this study, the FA analysis was performed on mature oocytes. In January 2023, a sampling was conducted in Bahía Norte (42°43'41.10"S-65°1'31.60"W) and Punta Cuevas (42°46'33.1"S-64°59'40.9"W) following the same method, along three transects perpendicular to the coast at depths between 5-10 m, where all animals were collected to estimate density (individuals/m<sup>2</sup>) and sizes. The transects were identified in three depth sections: 5 m, 10 m, and 15 m. The animals were transported to the pilot plant ERISEA S.A. in buckets with seawater following the protocols established by Crespi-Abril & Rubilar (2023) and placed in a recirculating system (isolated) until processed.

**Processing of Individuals.** The animals were placed in tanks in a closed recirculation system with constant temperature and salinity conditions; photoperiod (12:12 h.). Size was measured with a caliper (mm) and submerged weight (g) from 900 animals. Spawning was induced in the organisms by injecting 0.3 mL of KCl [0.5 M] into the peristomal membrane (Sun & Chiang, 2015). A pool of female gametes, free from seawater, was collected and stored (N<sub>2</sub> atmosphere; -20 °C). This process was repeated until 150 females were obtained each month (sourced from a mixed batch of 500 animals).

**Return of Individuals.** This study was conducted following new ethical standards for invertebrate research based on the 5Rs principle (Replacement, Reduction, Refinement,



Fig. 1. Map of Nuevo Gulf, Bahía Norte ( $42^{\circ}43'41.10''\text{S}$ - $65^{\circ}1'31.60''\text{W}$ ) and is situated in the northern area of the city, directly connected to the Fishing Industrial Park while Punta Punta Cuevas ( $42^{\circ}46'33.1''\text{S}$ - $64^{\circ}59'40.9''\text{W}$ ) is located in the southern area of the city, free from industrial influence.

Responsibility, and Respect) as outlined by Crespi-Abril & Rubilar (2023). Consequently, after obtaining and recording the necessary information, the sea urchins were not sacrificed but returned to the field in a nearby location to avoid recapture. The release of individuals after careful handling under a strict welfare protocol ensures negligible mortality resulting from this research.

**Obtaining Fatty Acid Methyl Esters (FAME).** A total of 100 mg (dried at  $40^{\circ}\text{C}$ ) of female gametes of *A. dufresnii* from each sampling pool ( $N = 900$  animals) were taken in each month ( $n = 150$ ): February, March, July, August, September, and October. They were subjected to a transmethylation reaction using the Lepage & Roy (1986) method. Identification and quantification of FA were performed by gas chromatography-mass spectrometry (GC-MS) Thermo Focus ISQ. Peaks corresponding to each FA molecule detected were identified by comparing relative retention times with authentic standards (a mixture of 37 FA species from Supelco 47885-U). Additionally, mass spectra were analyzed and compared with the NIST (National Institute of Standards and Technology, USA) library and The Lipid Web database for confirmation (Christie, 1998; Liebisch et al., 2020).

**Fatty Acid Profile analysis.** The concentration of fatty acids (FA) in the female gametes

of *A. dufresnii* was calculated and expressed as [ $\mu\text{g}\cdot\text{mg}^{-1} \pm \text{SD}$ ], averaging the results (FA) for the months of the seasons: Summer (February and March); Winter (July and August); and Spring (September and October). Each analysis was performed in triplicate. FAs were expressed with their common name and abbreviation.

The abundance (%) of the identified FA was calculated for the gametes of *A. dufresnii* studied in Bahía Norte. The sum of FA was classified by the number of carbon chain unsaturations, expressed by their acronyms as:  $\Sigma\text{SFA}$ ;  $\Sigma\text{MUFA}$ ;  $\Sigma\text{HUFA}$ ;  $\Sigma\text{PUFA}$ ; and UFA (Table 1).

**Trophic Markers.** FA relevant as trophic markers indicating (a) diversity of food sources were selected: brown algae ( $\text{C}_{20:4}(\text{n}-5)$  ARA) (Epherra et al., 2017; Dellatorre et al., 2020); green algae ( $\text{C}_{18:2}(\text{n}-6)$  LA +  $\text{C}_{18:3}(\text{n}-3)$  ALA) (Schram et al., 2018); red algae ( $\text{C}_{20:5}(\text{n}-3)$  EPA) (Jenzri et al., 2025) and carnivorous diets ( $\text{C}_{22:6}(\text{n}-3)$  DHA) (Zhukova, 2022). Additionally, the (b) abundance (%) of FA from the nutritional metabolic pathways from ( $\omega$  omega):  $\Sigma(\omega-3)$  (ALA  $\rightarrow$  EPA  $\rightarrow$  DHA) and  $\Sigma(\omega-6)$  (LA  $\rightarrow$  ARA) was calculated, along with the ratio of  $(\omega-3)/(\omega-6)$ .

**Statistical analysis.** A one-way analysis of variance (ANOVA) was used to compare densities, diameter, height/diameter ratio, and body weight between sites, only when normality (Shapiro-Wilk Test) and homoscedasticity (Levene's Test) assumptions were met. When significant differences ( $p < 0.05$ ) were found, comparisons were made using Tukey's test. The Chi-square analysis was used to study differences in sex ratios within the studied populations. Least squares fit of the data to a power function ( $P(g) = aT(\text{mm})^b$ ) was used to describe the relationship between individual wet weight (g) and size (diameter of the shell in mm) of *A. dufresnii* between Bahía Norte and Punta Cuevas in Nuevo Gulf. The comparison between both regressions was made by testing the equality of slopes. The log-transformed size was used to ensure linearity of the relationship, and the slopes were compared using a parametric t-test (Di Rienzo et al., 2011).

Fatty acids were compared as families of molecules grouped by the number of unsaturations, as previously described, and the trophic chain markers through analysis of variance between seasons. The fatty acid profile expressed as % abundance was compared using Primer v7.0.13 (Clarke et al., 2014), working with the Euclidean distances of the raw values and visualized using non-metric multidimensional scaling (NMDS) and respective ANOSIM analysis. A one-way

Table 1. FA Classification. Methodological considerations of FA selection according to the number of saturated and unsaturated links in the carbonated chain. (Christie, 1998; Liebisch *et al.* 2020).

INITIALS OF THE GROUP	FA NAME OF GROUP	CHARACTERISTICS OF THE CARBONATED CHAIN	NUMBER OF DOUBLES LINKS (C=C)
SFA	Saturated Fatty Acids	Only simple links	0
MUFA	Monounsaturated Fatty Acids	One unsaturated link	1
PUFA	Poliunsaturated Fatty acids	Two or three unsaturated links	2 o 3
HUFA	Highly unsaturated Fatty Acids	High number of unsaturated links	≥4
UFA	Unsaturated Fatty Acids	More than one unsaturated link	≥1

permutational multivariate analysis of variance (PERMANOVA) was performed in Primer v7.0.13 with the PERMANOVA +1 add-on (M.J. Anderson, 2017). Differences between FA profiles in the sampling seasons were examined by comparing distances between centroids, resulting in a more detailed analysis of lipid and FA profiles, supported by SIMPER. All multivariate PERMANOVA (main test) were performed using Type III sums of squares (partial) fixed effects (seasons) summing to zero with 9999 permutations using an unrestricted permutation of raw data.

## RESULTS

**Size and Density.** A total of 405 sea urchins were collected for size and weight estimation. Size and weight varied significantly between sites (two-way ANOVA  $F_{1,403} = 101.49$ ,  $p < 0.0001$ ). In Punta Cuevas, smaller individuals were found with an average size of 24.68 mm (Standard Deviation 7.75 mm) and an average weight of 7.5 g (Standard Deviation 5.6 g). In Bahía Norte, no individuals were found at 5 m depth, and the average size was larger at 30.99 mm (Standard Deviation 4.81) with an average weight of 14.4 g (Standard Deviation 8.25 g). Densities and biomass were significantly higher in Punta Cuevas (two-way ANOVA  $F_{1,16} = 5.43$ ,  $p = 0.0333$ ). On the other hand, no significant differences were found in the sex ratio in both populations (Punta Cuevas  $X^2 = 3.500$ ,  $p = 0.213$ ; Bahía Norte  $X^2 = 1.200$ ,  $p = 0.242$ ) (Table 2).

The length-weight relationships adjusted to a power function with  $R^2$  values close to 1. In Punta Cuevas, the  $R^2$  coefficient was greater than 0.8, while in Bahía Norte, it was greater than 0.7 (Fig. 2). The comparison test of slopes between the regressions of both sites did not show significant differences (t-Student  $p > 0.05$ ), indicating that the length-weight relationship is similar between the two studied sites.

In general, it can be observed that the sizes of the specimens were larger at 5 m depth. In Bahía Norte, a single mode of larger individuals ( $31 \pm 4.8$  mm). In Punta Cuevas, presence of two modes: ( $24 \pm 7.7$  mm); and a small group around 10 mm, recruitment of new cohorts into the population (Fig. 3).

**Fatty Acid Concentration** in Bahía Norte. The concentration of 24 different fatty acids (FA) in female gametes of *A. dufresnii* from the Bahía Norte population was obtained, expressed in [ $\mu\text{g}\cdot\text{mg}^{-1} \pm \text{DE}$ ] for the seasons (Table 3). The FA profile of female gametes in Bahía Norte did not vary between seasons within each FA species, allowing the use of the average concentration value of each FA species for analyses (PERMANOVA pseudo- $F_{(2,3)} = 0.8651$ ;  $p = 0.6632$ ).

Based on the Abundance (%) of each FA, the degree of unsaturation in the female gametes of *A. dufresnii* in the Bahía Norte samples for 2022 was explored (Fig. 4). In descending order, they were  $\Sigma\text{SFA}$ ,  $\Sigma\text{MUFA}$ ,  $\Sigma\text{HUFA}$ ,  $\Sigma\text{PUFA}$ , and UFA (Table 4). Within each described group, the highest abundance (%) could be assigned to a specific FA for the female gametes of *A. dufresnii* in Bahía Norte (Table 5).

**Seasonal Variation of FAs** in Bahía Norte. The non-metric multidimensional scaling (NMDS) presented a stress of zero, indicating excellent data ordering without forcing the fit (Fig. 5). The sampling months were grouped according to the seasons and showed similarity in their values, not indicating significant differences (ANOSIM  $R = 0.444$ ;  $\rho = 0.2$ ). According to the SIMPER analysis, there were relevant and homogeneous FA for each season. At the same time, the FA that differentiated the seasons were more distanced between summer and winter (Table 6).

**Trophic Markers** in Bahía Norte. The abundance (%) of specific FA considered for trophic markers in function of the seasons indicated that *A. dufresnii* accesses diverse (a) food sources throughout the year. The % abundance of



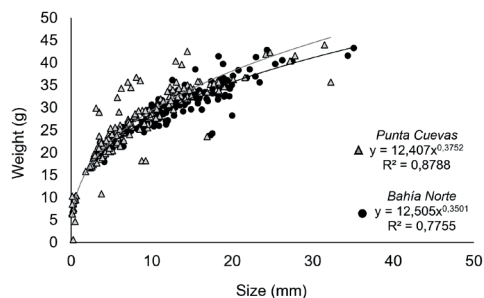


Fig. 2. Size and Weight Relationship of *Arbacia dufresnii* in Bahía Norte (circles) Punta Cuevas (triangles).

the trophic marker average was graphed based on the sampled seasons. Brown algae 2.62 %, (C20:4(n-5) ARA) ( $F_{2;0.05} = 0.08$ ;  $p = 0.925$ ); Red algae 10.25 %, (C20:5(n-3) EPA) ( $F_{2;0.05} = 1.26$ ;  $p = 0.401$ ); Green algae 4.73 %, (C18:2(n-6) LA + C18:3(n-3) ALA) ( $F_{2;0.05} = 7.16$ ;  $p = 0.072$ ); Carnivorous diet 3.40 %, (C22:6(n-3) DHA) ( $F_{2;0.05} = 0.36$ ;  $p = 0.726$ ), indicating that the food accessed by *A. dufresnii* in Bahía Norte is consistent between seasons. The results regarding the metabolic pathways (b), studied for the accumulation of omegas  $\Sigma$  ( $\omega$ -3),  $\Sigma$  ( $\omega$ -6), and the proportionality of  $\Sigma$  ( $\omega$ -3) /  $\Sigma$  ( $\omega$ -6) in female gametes of *A. dufresnii* for Bahía Norte are shown in Table 7. There were no nutritional differences regarding the metabolic pathways of omegas ( $\omega$ ) 3 or 6, nor concerning the ratio between them.

## DISCUSSION

**Sizes and density.** This study provides a unique opportunity to compare two populations of the same species, *Arbacia dufresnii*, in nearby locations with different contamination characteristics. Sea urchins in the Punta Cuevas population exhibited a size and density distribution similar to previously reported (Epherra, 2016). Conversely, in Bahía Norte, the animals are larger ( $31 \pm 4.8$  mm) but less dense ( $< 2$  ind/m<sup>2</sup>). This distribution is similar to that observed in Zone 39 of San José Gulf, where animals averaged  $29 \pm 4.5$  mm and had a density of 3.7 ind/m<sup>2</sup> (Epherra, 2016).

The density-dependent size relationship has been described for the genus *Arbacia* (*A. punctulata* in the Gulf of Florida Hill & Lawrence, 2003; *A. lixula* in the Mediterranean Sala et al., 1998; Hereu et al., 2012). The causes of this relationship have been based on variation in food availability and quality, differential recruitment, or the presence/absence of predators (Levitan

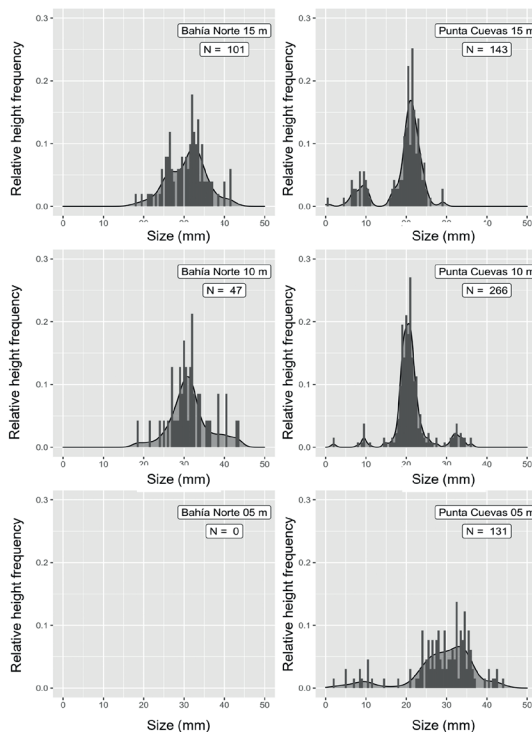


Fig. 3. Size structure (mm) of *Arbacia dufresnii* individuals in Bahía Norte and Punta Cuevas for each of the depths (15 m, 10 m and 5 m).

1988, 1989; Brey et al., 1995; Hill & Lawrence 2003; Tuya et al., 2004; Muthiga & Jaccarini 2005; Cabanillas-Terán 2009; Paredes, 2010). This study reinforces the idea that less dense populations of *A. dufresnii* possess larger individuals. However, no correlation was found in the size-temperature relationship, as suggested by Epherra (2016) in their comparative study of populations at different latitudes. This may be because, although both locations are practically at the same latitude and within the same gulf, Punta Cuevas and Bahía Norte have different food offers due to the contamination observed in Bahía Norte, generating a different population structure.

**Nutritional Status.** The results of this study show a higher concentration of fatty acids (FA) in *A. dufresnii* gametes in Bahía Norte compared to Punta Cuevas (Díaz de Vivar et al., 2019). The percentage of polyunsaturated fatty acids (PUFA) abundance in Bahía Norte is 3.20 times higher than in Punta Cuevas. This ratio reverses for highly unsaturated fatty acids (HUFA), being 2.73 times higher in Bahía Norte. For monounsaturated (MUFA) and saturated (SFA) fatty ac-

Table 2. Density, biomass and sex ratio data of *Arbacia dufresnii* in Bahía Norte and Punta Cuevas. In the columns: DEPTH (m); DENSITY (N° individuals /m<sup>2</sup> ± SD); BiOMASS (g /m<sup>2</sup> ± SD); H % = population proportion of females and M % population proportion of males.

PLACE	DEPTH	DENSITY		BIOMASS		H %	M %
Punta Cuevas	5	1.5	0.36	14.21	12.13	53 %	47 %
	10	5.5	3.22	31.16	21.82	64 %	36 %
	15	2.13	1.91	7.29	6.31	45 %	55 %
Bahía Norte	5	0	0	0	0	-	-
	10	0.43	0.32	7.22	3.04	50 %	50 %
	15	1.8	2.08	24.26	27.02	50 %	50 %

Table 3. FA concentration (mean ± SD; µg·mg<sup>-1</sup>) in the female gametes of *Arbacia dufresnii* in the Bahía Norte population during the 2022 seasons. The nd: indicates an undetected FA. The FA marked in the table are: those that stand out in the season (\*) The FA that characterizes the season and makes it more dissimilar (not different). (bold). All FA have a *cis*-spatial configuration.

COMMON NAME	FAs CODE	SUMMER	WINTER	SPRING
Lauric acid	C12:0	0.22 ± 0.08	0.40 ± 0.08	0.21 ± 0.10
Myristic acid	C14:0	5.59 ± 0.22	4.92 ± 1.27	4.78 ± 1.83
Pentadecylic acid	C15:0	1.91 ± 0.10	1.04 ± 0.39	1.13 ± 0.62
Palmitic acid	C16:0	11.96 ± 0.27	9.46 ± 1.95	*9.09 ± 2.78
Palmitoleic acid (PA)	C16:1 (n-7)	4.05 ± 0.01	3.45 ± 1.08	3.68 ± 1.46
Margaric acid	C17:0	0.97 ± 0.01	0.62 ± 0.19	0.72 ± 0.46
Ginkgolic acid	C17:1 (n-7)	0.39 ± 0.08	nd	nd
Stearic acid	C18:0	3.65 ± 0.04	2.98 ± 0.81	2.79 ± 1.05
Oleic acid ( <b>OA</b> )	C18:1 (n-9)	<b>2.49 ± 0.04</b>	<b>4.66 ± 1.27</b>	<b>4.40 ± 1.92</b>
Linoleic acid ( <b>LA</b> )	C18:2 (n-6)	1.66 ± 0.02	0.97 ± 0.35	1.38 ± 0.63
gamma-Linolenic acid (GLA)	C18:3 (n-6)	*1.52 ± 0.08	1.34 ± 0.43	1.65 ± 0.83
Alpha-Linolenic acid ( <b>ALA</b> )	C18:3 (n-3)	1.61 ± 0.18	0.96 ± 0.33	1.00 ± 0.47
Arachidic acid	C20:0	1.44 ± 0.09	1.54 ± 0.31	1.23 ± 0.44
Gondoic acid	C20:1(n-9)	2.00 ± 0.15	1.53 ± 0.27	2.20 ± 1.54
Eicosadienoic acid	C20:2 (n-6)	2.07 ± 0.03	1.68 ± 0.35	1.77 ± 0.52
Heneicosanoic acid	C21:0	0.17 ± 0.02	0.30 ± 0.05	0.29 ± 0.07
Sciadonic acid (ETA)	C20:3 (n-6)	0.82 ± 0.03	0.79 ± 0.08	0.85 ± 0.38
Arachidonic acid (ARA)	C20:4 (n-6)	1.45 ± 0.04	1.21 ± 0.30	1.09 ± 0.07
Dihomo-alpha-linolenic acid	C20:3 (n-3)	<b>4.57 ± 0.08</b>	<b>*2.17 ± 1.12</b>	<b>3.09 ± 1.39</b>
Eicosapentaenoic acid (EPA)	C20:5 (n-3)	<b>4.69 ± 0.26</b>	5.38 ± 0.94	<b>* 5.00 ± 0.98</b>
Behenic acid	C22:0	0.30 ± 0.08	nd	nd
Erucic acid	C22:1 (n-9)	*1.49 ± 0.08	1.36 ± 0.18	1.28 ± 0.36
Cervonic acid ( <b>DHA</b> )	C22:6 (n-3)	1.76 ± 0.04	1.65 ± 0.36	1.64 ± 0.36
Nervonic acid	C24:1 (n-9)	0.66 ± 0.01	0.69 ± 0.11	1.17 ± 1.07

ids, the ratio is approximately 1.5 times higher in Bahía Norte. This ratio is important because the increase of HUFA and PUFA favors reproductive success, as these values increase with the maturation of gametes, providing cell membrane fluidity and acting as signals for endogenous reactions (Martinez-Pita *et al.*, 2010; Ranguelov *et al.*, 2022). This could positively influence gametogenesis, which continues during spring (Epherra *et al.*, 2015).

In Bahía Norte, the analysis of Unsaturated FA (UFA) showed that these are predominantly high despite the higher proportion of saturated FA (SFA). The UFA group is more abundant with homogeneous PUFA, HUFA, and MUFA, suggesting a diet rich in high-quality nutrients (Zuo *et al.*, 2018). Additionally, when comparing the ratio between unsaturated (UFA) and saturated (SFA) FA, it was found that in Bahía Norte, UFA predominate over SFA, also indicating a good nu-

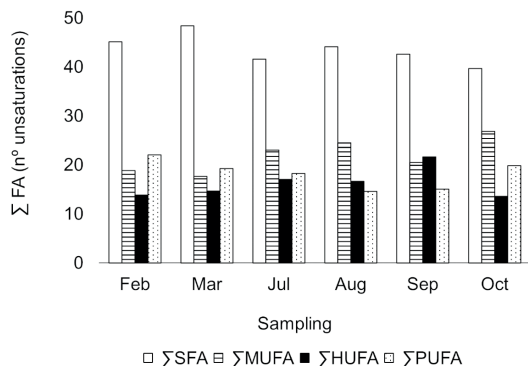


Fig. 4. Sum of Abundance (%), FA in the female gametes of *Arbacia dufresnii* in the Bahía Norte sampling in 2022. Acronyms for groups:  $\Sigma$ SFA, saturated FA (smooth white);  $\Sigma$ MUFA, monounsaturated FA;  $\Sigma$ PUFA, polyunsaturated FA (dotted plot);  $\Sigma$ HUFA, highly unsaturated FA (line plot).

tritional status of the population (Hughes *et al.*, 2011; Murzina *et al.*, 2021).

These results contrast with those reported for Punta Cuevas, where HUFA are more prevalent (Díaz de Vivar *et al.*, 2019). All this evidence suggests differences in food quality and availability between populations. The lack of quality food availability in Punta Cuevas has been reported for both *A. dufresnii* and other echinoderm species (Pastor-de-Ward *et al.*, 2007).

On the other hand, the balance of metabolic pathways between omega-3 ( $\omega$ -3) and omega-6 ( $\omega$ -6) fatty acids, ideally 4:1, indicates an optimal nutritional status in nature (Simopoulos 2002; Sanna *et al.*, 2017). In Bahía Norte, the balance is approximately 2:1 throughout the year, possibly promoting growth (Guillou *et al.*, 2000) or the reproductive maturity of *A. dufresnii* (Epherra *et al.*, 2015). As gametogenesis progresses in autumn and winter, this organism accumulates EPA and DHA, which are intermediate metabolites of the  $\omega$ -3 pathway resulting from the healthy assimilation of essential fatty acids like ALA. Thus, the  $\omega$ -3/ $\omega$ -6 ratio found in Bahía Norte is favorable, indicating a balanced and healthy diet for *A. dufresnii*. This ratio is crucial for the health and development of marine organisms, and its positive value in Bahía Norte supports the hypothesis that this population has good nutritional quality (Hughes *et al.*, 2011).

Additionally, the FA profiles varied significantly between Bahía Norte and other sites. Specific FA by their common name, cervonic acid (DHA),  $\alpha$ -linoleic acid (ALA), and linoleic acid (LA), were higher in Bahía Norte than those

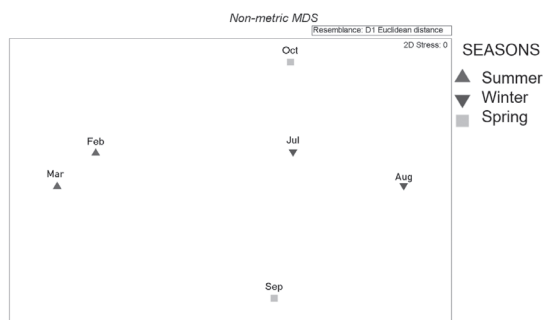


Fig. 5. NMDS analysis. NMDS analysis (Primer v 7 ®) of fatty acids in the female gametes of *Arbacia dufresnii* between the seasons Summer (triangle); Winter (inverted triangle); Spring (square).

reported in Punta Cuevas (Díaz de Vivar *et al.*, 2019), suggesting a difference in food sources and nutritional quality between sites. The concentrations of FA in Bahía Norte showed seasonal variations, reflecting changes in food availability and quality throughout the year.

**Trophic Markers.** Differences in FA trophic markers between Punta Cuevas (from Díaz de Vivar *et al.*, 2019) and Bahía Norte within the same gulf were evidenced. In Punta Cuevas, the dominant habit of *A. dufresnii* is herbivory (Epherra *et al.*, 2015), with a consumption of red algae (29%) and brown algae (13%), while green algae and carnivorous diet are around 2% (Díaz de Vivar *et al.*, 2019). In Bahía Norte, the percentage levels of trophic markers remain constant throughout the year, indicating that *A. dufresnii* accesses a diversity of foods in all seasons, unlike what is observed in Punta Cuevas.

During the summer, the fatty acids OA and GLA become abundant due to the assimilation of FA from brown algae, coinciding with the presence of *Undaria pinnatifida* in the area (Dellatorre *et al.*, 2020). The increase in EPA in spring could be a delayed effect of the transfer from gonads to gametes at the end of summer and autumn (Zárate *et al.*, 2016), coinciding with the seasonality of *Gracilaria gracilis* (Piriz *et al.*, 2003). The constant assimilation of LA and ALA comes from the contribution of green algae such as *Codium* sp. and *Ulva* sp. (Graham *et al.*, 2018; Castro *et al.*, 2022). DHA, a robust marker due to its low convertibility rate (Rangelov *et al.*, 2022), remains at similar levels throughout the year, indicating a constant consumption of

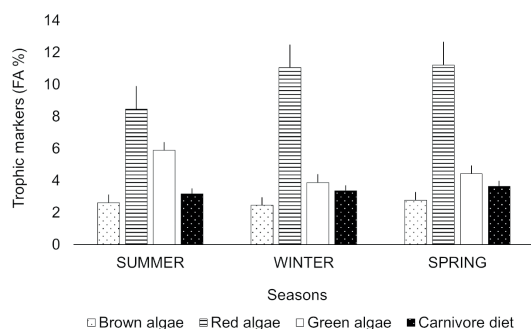


Fig. 6. Trophic markers. FA present in female gametes of *Arbacia dufresnii*, as trophic markers that indicate diversity of food sources. Brown algae (dotted pattern; Red algae (lined pattern); Green algae (plain white); Meat diet (black dotted pattern).

a carnivorous diet in Bahía Norte. The herbivorous habit of *A. dufresnii* is more pronounced in summer in Bahía Norte due to the increase in the biomass of macroalgae available for its diet (Dellatorre *et al.*, 2020). In winter, the tendency towards carnivory intensifies, reflected in an increase in saturated FA, although it is present throughout the year.

*A. dufresnii* has been proposed as an effective biomarker due to its ability to reflect changes in food availability and environmental quality through FA profiles in its gonads (Zárate *et al.*, 2016). The results of this study reinforce the idea of using *A. dufresnii* as a valuable indicator for biomonitoring studies in marine ecosystems impacted by human activities.

**Environmental Conditions Between Populations.** In Bahía Norte, environmental problems have persisted for decades, receiving effluents from fishing plants and shrimp and fish meal producers (Torres & Caille, 2009; Díaz *et al.*, 2002). Despite changes in regulations and controls, clandestine effluents still exist. The most recent incident occurred in February 2023 and was investigated by the regional ecological authority due to its magnitude; the judicial process is ongoing. During the judicial investigation, records of various environmental variables such as total suspended solids, biological and chemical oxygen demand, ammonia and phosphate concentrations, and bacteriological analyses were collected but not disclosed to the public. These are in a confidential report for legal purposes. However, concerning contamination levels in the spilled waters have been reported, significantly exceeding the limits established by provincial environmental law (Law 24.051 and Provincial Decree Chubut 1540/16 of Law XI No. 35).

Table 4. Percentage abundance of FA in *Arbacia dufresnii* in Bahía Norte grouped by the number of unsaturations. The FA were grouped according to the degree of unsaturation of the carbon chain ( $\Sigma$ SFA;  $\Sigma$ MUFA;  $\Sigma$ HUFA;  $\Sigma$ PUFA; UFA) detected in the female gametes.

% FA IN BAHIA NORTE	%
$\Sigma$ SFA	44
$\Sigma$ MUFA	23
$\Sigma$ HUFA	19
$\Sigma$ PUFA	16
UFA (MUFA+HUFA+PUFA)	58

Although this study was not conducted after this spill, it can be assumed that the values of fishing liquid wastes were similar or higher during the decades they were received, causing eutrophication and environmental changes (Islam & Tanaka 2004; Ngatia *et al.*, 2019) compared to nearby populations that did not experience this environmental stress.

The *A. dufresnii* population in Bahía Norte has been subjected to this eutrophication for many decades, which has likely allowed it to gradually adapt to these changes, showing very different characteristics from other populations at similar latitudes. This population has managed to take advantage of a greater diversity of food, increasing its nutritional level and producing larger individuals compared to nearby populations that have not experienced this eutrophication condition. There is a constant trend towards carnivory throughout the year and greater utilization of the invasive macroalga *U. pinnatifida* during summer, more evident than in Punta Cuevas. This could generate greater reproductive success or longer gamete production; the reproductive cycle in this area has not yet been investigated.

## CONCLUSION

This study highlights the importance of *A. dufresnii* as a resilient organism capable of adapting to different feeding conditions, making it an excellent bioindicator for biomonitoring studies in eutrophicated areas. The significant differences in fatty acid profiles and nutritional status between the Bahía Norte and Punta Cuevas populations demonstrate the influence of food availability and quality. Additionally, the



Table 5. Abundances of specific FA for each group of unsaturations in *Arbacia dufresnii* in Bahía Norte. It is a detailed description of the % composition within the unsaturation groups and the predominance of FA (%) within each group.

FA GROUP (UNSATURATIONS)	FA	FA %	% FA (INSIDE THE LIPID PROFILE)
SFA	C16:0	20	80
	C14:0	9,80	40
MUFA	C18:1(n-9)	7.14	90
	C16:1(n-7)	7.10	89
PUFA	C20:3(n-3)	6.14	85
	C20:2(n-6)	3.60	50
HUFA	C20:4(n-6) ARA	10.25	85
	C20:5(n-3) EPA	3.40	35
	C22:6(n-3) DHA	2.62	20

Table 6. SIMPER. Seasonality of FA that contribute to the differences and similarities (shaded diagonal) in the female gametes of *Arbacia dufresnii* from the Bahía Norte population. The corresponding percentage is found in the lower right margin.

SIMPER	SUMMER	WINTER	SRPING
SUMMER	C18:3 (n-6) GLA C22:1 (n-9)		
			76
WINTER	C18:1 (n-9) OA C20:3 (n-3)	C20:3(n-3)	
			61 80
SPRING	C20:5 (n-3) C18:1 (n-9)	C20:3 (n-3) C18:1 (n-9) OA	C20:5(n-3) EPA C16:0
			38.8 40.39 56

Table 7. Metabolic pathways of FA in the female gametes of *Arbacia dufresnii* in Bahía Norte. There were no differences in the seasonality of the grouped omegas:  $\Sigma(\omega-3)$ ;  $\Sigma(\omega-6)$ ; and the ratio of  $\Sigma(\omega-3) / \Sigma(\omega-6)$ . Table 7 also indicates the value of the analysis of variance; there were no differences between the samples. (ANOVA  $F_{2,0.05}$ ; p).

METABOLIC PATHWAY	SUMMER	WINTER	SPRING	$F_{2,0.05}$	p
$\Sigma(\omega-3)$	22.77	23.31	22.13	0.22	0.816
$\Sigma(\omega-6)$	13.52	12.56	12.98	0.34	0.734
$\Sigma(\omega-3) / \Sigma(\omega-6)$	1.68	1.86	1.71	0.21	0.825

ability of *A. dufresnii* to maintain a diverse diet and adapt to changing environmental conditions underscores its value as an indicator of marine ecosystem health. These findings suggest that *A. dufresnii* can reflect changes in environmental quality and food resource availability, providing crucial information for the management and conservation of marine ecosystems.

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