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# Sex and reproductive cycle affect lipid and fatty acid profiles of gonads of *Arbacia dufresnii* (Echinodermata: Echinoidea)

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ABSTRACT: Analysis of the lipid composition of gonads allows differentiation between energy and structural lipids, which is important for understanding lipid utilization during gametogenesis. Fatty acids, which are frequently used as biomarkers in trophic ecology studies, are also a useful companion to lipid studies and provide a detailed understanding of a species' reproductive cycle. We examined the influence of sex and the reproductive cycle on the lipid and fatty acid profiles of gonads in the sea urchin *Arbacia dufresnii*. In a population from Nuevo Gulf, Argentina, we found significant changes in total lipid concentration, lipid profile and fatty acid profile throughout the reproductive cycle. Ovaries and testes containing fully mature gametes differed in total lipid concentration (higher in the ovaries), lipid profile (more energy lipids in ovaries and more structural lipids in testes) and fatty acid profile (differed by sex, not only in the variety of fatty acids present but in the degree of unsaturation). Our results show that differences in lipid and fatty acid profiles caused by sex and gonadal reproductive stage need to be considered when these tools are used as biomarkers in ecological studies.

KEY WORDS: Sea urchin · Gametogenesis · Lipid cycle · Fatty acid cycle

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## INTRODUCTION

Lipids are major sources of metabolic energy and essential constituents of cells, and they are of great importance in the physiology and reproductive processes of marine animals (Christie & Han 2010). The role of lipids in reproduction can be investigated at 2 levels: (1) the lipid profile = the analysis of the lipid composition of tissues with regard to the major lipid classes, differentiating between the 'neutral lipids' (energy-storage lipids) and the 'polar lipids' (main constituents of cellular membranes); and (2) the fatty acid (FA) profile or FA signature = the identification and quantification of the relative proportion and composition of the component FAs.

Sea urchin gonads have a multifunctional role as a nutritive store and as the reproductive organs, typically generating mature oocytes and sperm during an annual reproductive cycle (Walker et al. 2013). The lipid and FA profiles of gonads therefore reflect both their nutritive and reproductive status (Russell 1998, Walker et al. 2001, 2007, 2013, Hughes et al. 2006, 2011), as well as long-term trophic interactions (Hughes et al. 2006, Lawrence 2007). Recent studies have used FA profiles as tracers of the energy source of consumers in macroalgal-dominated ecosystems

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(Graeve et al. 2002, Hanson et al. 2010), as the profile is relatively specific at different trophic levels and most FAs are directly assimilated from diet to consumer (Kelly et al. 2009, Kelly & Scheibling 2012). FAs derived from specific sources can act as 'biomarkers', making it possible to identify a dietary source, and therefore the feeding habits, of the target species (Parrish et al. 2000, Kelly et al. 2008, Iverson 2009).

However, because of their dual role as nutritive/reproductive organs, sea urchin gonads have been suggested to undergo a greater biochemical and FA modification than the lipid storage organs of other herbivorous invertebrates (Cook et al. 2000, Hughes et al. 2006, Kelly et al. 2008, Carboni et al. 2013). For example, feeding experiments have shown that sea urchins are able to synthesize FAs that are not present in their diets (Liyana-Pathirana et al. 2002, Kelly et al. 2008), and lipid and FA composition can change throughout the reproductive cycle (Martínez-Pita et al. 2010a, Verachia et al. 2012, Carboni et al. 2013). Therefore, interpretation of habitat- or population-related diet differences using lipids and FAs first requires an understanding of changes in gonads during their reproductive cycle.

*Arbacia dufresnii* is the most abundant sea urchin in the northern Patagonian gulfs (Zaixso & Lizarralde 2000, Brogger et al. 2013). It can be considered as herbivorous, carnivorous or omnivorous depending on the food availability in the environment where it is found (Vásquez et al. 1984, Penchaszadeh & Lawrence 1999, Galván et al. 2009, Newcombe et al. 2012). As part of a broader study comparing the lipid and FA profiles of *A. dufresnii* to echinoids with more herbivorous diets, we examined changes during the annual reproductive cycle in Nuevo Gulf, Argentina. Gonad development proceeds through pregametic (austral autumn, May), growth (austral winter, July and August), premature and mature stages (austral spring, September to November), with an extended spawning season during the late spring and summer (2 partial spawning stages, December to February) and a reabsorption stage at the end of the summer (March) (Epherra et al. 2015). Peak lipid concentrations occur in this population during the mature and spawning stages in both ovaries and testes (Parra et al. 2015), and we investigated in detail how the lipid and FA profiles of *A. dufresnii* gonads vary with sex and throughout the gametogenic cycle. Our results suggest that both of these factors need to be considered when comparing lipid and FA profiles of echinoid gonads in trophic studies.

## MATERIALS AND METHODS

### Sampling area

Every 2 months from January 2011 to January 2012, individuals of *Arbacia dufresnii* were collected from Punta Cuevas (42° 46′ 44″ S, 64° 59′ 52″ W), Nuevo Gulf, Patagonia, Argentina, at 5–10 m depth by scuba diving. As described by Epherra et al. (2015), Punta Cuevas is a wave-protected, shallow rocky reef. The benthic community is dominated by the algae *Codium* spp., *Dictyota* sp. and *Ulva* spp. (Piriz et al. 2003). However, every late winter and spring, a dense forest of the invasive alga *Undaria pinnatifida* dominates the rocky reef (Casas & Piriz 1994, Casas et al. 2008), where *A. dusfresnii* is among the most common species.

To study lipid and FA profiles as well the total lipid concentration of gonads that contain mature gametes (premature stage), we used individuals taken in September 2009 and September 2010, when most of the population had mature gametes in their gonads (Epherra et al. 2015) and maximum lipid contents (Parra et al. 2015).

### Dissection

Each individual was blotted with filter paper to remove adhering water and then weighed to the nearest 0.01 g. Individuals were narcotized by immersion for 15 min in a 5% solution of $MgCl_2$ in filtered seawater. Gonads were dissected out and weighed to the nearest 0.01 g and then sorted by sex. In *A. dufresnii*, gonads are easily distinguished by their colouration, which does not change with the gametogenic cycle, even in the intergametic and pregametic stages: ovaries have a strong purple colour, whereas testes are a pale yellowish colour (Epherra et al. 2015, Parra et al. 2015).

Gonads were individually dried at 60°C to constant weight and ground to powder in a mortar for total lipid (TL) determination.

For lipid profile analysis, gonads were separately stored in 50 ml polypropylene tubes at −80°C until lyophilization. Gonads were individually lyophilized for 48 h to constant mass using a Labcono Freeze Dry System (FreeZone 6 l) and kept at −20°C until shipping to the University of Auckland, New Zealand.

For the FA profile analysis, mature gonads (n = 5 females and 5 males from September 2009; n = 7 females and 3 males from September 2010) were treated in the same way. Gonads collected during

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2011–2012 were pooled by sex each month (n ≈15 per sex) and pooled due to the small amount of gonad tissue per individual (i.e. a pooled male and a pooled female sample per month) to ensure enough material for the transmethylation procedure (see below).

### Lipid and FA analysis

TLs were measured in triplicate according to Zöllner & Kirsch (1962), with cholesterol as the standard. Results are expressed as the mean (±SD) of percent dry weight of tissues.

Lipid class determination was carried out for each sample of gonads using an Iatroscan Mark V$^{new}$ thin-layer chromatography/flame ionization detector system and silica gel S-III Chromarods following the protocols defined by Parrish (1987, 1999) as described by Sewell (2005) with the following modification: 250 µl of ultrapure water, 25 µl of ketone in chloroform (used as an internal standard, as natural concentrations are low in marine tissues), 100 µl chloroform and 250 µl methanol (final ratio of water:chloroform:methanol 2:1:2) were added to the V-vial before 20 min of sonication on ice followed by centrifugation at room temperature. Both the aqueous and chloroform fractions were taken. An additional 250 µl of water and 250 µl of chloroform were added, followed by shaking and subsequent centrifugation. Finally, the upper water:methanol fraction was removed. The lower chloroform layer was stored in a −20°C freezer until used for the Iatroscan analysis. All V-vials used in the extraction process were cleaned with 3 methanol and 3 chloroform washes as recommended by Parrish (1999), and all solvents used in lipid extraction were HPLC-grade.

Immediately before spotting onto the Chromarods, the lipid extract was dried down in $N_2$ and 10 µl of chloroform for the gonads were added. Chromarods were developed in hexane-based solvents as described by Sewell (2005), applying the same settings.

Quantification of lipids in the samples was performed as described by Sewell (2005). Lipid classes were: wax esters (WE: miristyl dodecanoate), methyl esters (ME: methyl palmitate), ketones (KET: 3-hexadecanone), triacylglycerols (TAG: tripalmitin), free FAs (FFA: palmitic acid), sterols (ST: cholesterol), diacylglycerols (DAG: 1,2 dipalmitoyl-rac-glycerol), acetone-mobile polar lipids (AMPL: 1-monopalmitoyl-rac-glycerol) and phospholipids (PL: L-α-phosphoditylcholine). AMPL includes glycolipids, pigments and any remaining neutral lipids from the PL and is normally separated in the acetone development (Sewell 2005). We did not use the acetone third development (Parrish 1987); however, the second development was enough to separate a different peak from PL and it appeared in the same place as 1-monopalmitoyl-rac-glycerol, the standard used for AMPL, confirming the presence of possible glycolipids and monoacylglycerols. Thus, this extra peak that appears before PL is called AMPL, even though the acetone development was not used. Peak areas from the calibration curves were calculated based on the mean of 3 separate Chromarods ($r^2 > 0.9968$ for all lipid classes). The sum of all lipid classes was calculated for gonads from each sea urchin. The amount of energy lipids (ELs) was calculated by summing the amount of TAG, DAG, FFA, ME and WE, and the amount of structural lipids (SLs) was calculated by summing the amount of PL, ST and AMPL. Values are presented as the mean (±SE) of the different tissue samples (n = 6 for each gender), in units of µg lipid mg$^{-1}$ of lyophilized sample.

For FA analysis, every monthly pooled sample per sex was submitted to 2 lipid extractions each for 24 h following Bligh & Dyer (1959). Solvents were evaporated under $N_2$ and lipids were immediately transmethylated according to Lepage & Roy (1986) (with the minor modification of no standard acid addition) to obtain the FA methyl esthers (FAMEs). In the case of gonads collected in September 2009 and 2010 (mature gonads), lyophilized gonads (see 'Dissection' above) were analysed on an individual basis.

FAMEs were separated and quantified using a gas chromatograph (GC 7890 Agilent system) equipped with a mass spectrometry detector (MSD 5975c). Separation was performed with a 35 m fused silica column with an internal diameter of 0.32 mm. The column was wall-coated with 0.20 mm SP-2330. Helium was used as a carrier gas. After injection at 60°C, the oven temperature was raised to 150°C at a rate of 40°C min$^{-1}$, then to 230°C at 3°C min$^{-1}$, and finally held constant for 30 min. FAME peaks were identified by comparing their retention times with those of authentic FA standards (Supelco 37 component FAME mix). The mass spectra of FAMEs not present in the standard mix were compared with those from the National Institute of Standards and Technology mass spectra library (NIST MS Search 2.0), together with the Lipid Library (Christie 2012). Each value is presented as the percent of the total identified FAs. FAs were grouped into saturated FAs (SFAs: those FAs with simple covalent C-C bonds in the hydrocarbon chain), monounsaturated FAs (MUFAs: those FAs with only 1 double covalent C-C

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bond in the hydrocarbon chain), polyunsaturated FAs (PUFAs: those FAs with 2 or 3 double covalent C-C bonds in the hydrocarbon chain) and highly unsaturated FAs (HUFAs: those FAs with 4 or more double C-C bonds in the hydrocarbon chain).

### Statistical analysis

To evaluate changes in the mature gonad wet weight between September 2009 and September 2010, the mean adjusted gonad weights (AGWs) were obtained through a series of generalized linear model ANCOVAs, with urchin test diameter as the covariate and gender and sampling years as factors, due to an allometric relationship between organ weight and test diameter. Changes in the gonad wet weight among the different months were also analysed using AGW as recommended by Grant & Tyler (1983) and Packard & Boardman (1999).

Lipid and FA profiles of *A. dufresnii* gonads were compared between sexes using Primer v6.1.12 (Clarke & Gorley 2006) with the permutational multivariate ANOVA PERMANOVA+ v1.0.1 add-on (Anderson 2003). The data were left untransformed and converted into similarity matrices using Euclidean distances. Similarity patterns in the data were visualised using multidimensional scaling (MDS). Multivariate 2-way PERMANOVA was performed to examine differences in the lipid and FA profiles between sexes, years and months. Univariate PERMANOVA was used to test differences in the SL and EL levels as well as the total percentage of SFAs, MUFAs, PUFAs and HUFAs. This test avoids the assumptions of the traditional 1-way ANOVA (Underwood 1997) and assumes only that the samples are exchangeable under a true hypothesis (Anderson 2003). Multivariate PERMANOVA was used to test differences in the profiles of SFAs, MUFAs, PUFAs and HUFAs between sex and years, and between sex and months. Pairwise comparisons were conducted when there was a significant effect between months using an unrestricted permutation of raw data. The similarity percentages (SIMPER) procedure was used to explore the differences between sexes and years as well as between sexes and months by determining which individual SLs and ELs as well as individual SFAs, MUFAs, PUFAs and HUFAs contributed most to the differences in the multivariate profile. Univariate 1-way PERMANOVA was used to test the differences of each of these important FAs.

## RESULTS

### Mature gonads

Means of the AGW (test diameter was held constant at 29.16 mm) did not vary between years (2009, 2010; $F_{1.17} = 2.13$; p = 0.16), but varied between sexes ($F_{1,17} = 8.56$, p = 0.0094); testes weights were higher than ovaries. The Year × Sex interaction was not significant ($F_{1,17} = 0.0007$, p = 0.9797).

The TL concentration (as % of dry weight) in gonads was statistically different between sexes ($F_{1,17} = 0.77$, p = 0.009425), with the ovaries having higher values (19.29 ± 1.52) than the testes (10.49 ± 0.63). The ratio % lipids of females:males was 1.84.

The lipid profile was statistically different between sexes (pseudo-$F_{1,14} = 45.97$, p(perm) = 0.0002). Eight lipid classes were found in the ovaries of *Arbacia dufresnii*, with a preponderance of PL, TAG and ST, while AMPL, WE, ME and DAG were found in very low amounts. DAG and FFA were not detected in the testes (Table 1). The sum of all the lipid classes was also different between sexes (pseudo-$F_{1,14} = 104.59$,

Table 1. Lipid classes (in µg mg$^{-1}$ of dry weight [DW] and in % of sum of all lipid classes) present in gonads of *Arbacia dufresnii* (September 2009 and 2010). Structural lipids (SL): sterols (ST), acetone-mobile polar lipids (AMPL) and phospholipids (PL). Energy lipids (EL): free fatty acids (FFA), triacylglycerols (TAG), wax esters (WE), methyl esters (ME) and diacylglycerols (DAG); nd: not detected. Data represent the mean (±SE) of 5 samples (2009) or 6 samples (2010) for each sex. As there were no significant differences in the lipid profiles between 2009 and 2010 (pseudo-$F_{1,14} = 1.54$, p(perm) = 0.2213), these samples were pooled

| Lipid class | Ovaries | | Testes | |
|---|---|---|---|---|
| | µg mg$^{-1}$ DW | % | µg mg$^{-1}$ DW | % |
| ST | 16.76 ± 5.38 | 9.86 ± 2.31 | 23.56 ± 4.39 | 28.10 ± 5.49 |
| AMPL | 5.24 ± 0.98 | 3.08 ± 0.68 | 3.50 ± 1.93 | 4.17 ± 2.57 |
| PL | 74.12 ± 6.54 | 43.60 ± 2.45 | 47.03 ± 8.75 | 56.09 ± 6.44 |
| SL | 96.12 ± 10.78 | 56.54 ± 1.71 | 74.09 ± 8.73 | 88.36 ± 6.27 |
| FFA | 10.92 ± 6.36 | 6.42 ± 4.38 | nd | nd |
| TAG | 51.96 ± 16.56 | 30.57 ± 7.03 | 7.90 ± 4.61 | 9.42 ± 4.92 |
| WE | 2.74 ± 0.99 | 1.61 ± 0.79 | 1.20 ± 0.64 | 1.43 ± 0.66 |
| ME | 5.92 ± 2.45 | 3.48 ± 1.85 | 0.66 ± 0.70 | 0.79 ± 0.84 |
| DAG | 2.33 ± 0.96 | 1.37 ± 0,51 | nd | nd |
| EL | 73.87 ± 9.92 | 43.45 ± 1.56 | 9.76 ± 5.70 | 11.62 ± 6.10 |
| EL/SL | | 0.77 ± 0.51 | | 0.132 ± 0.08 |
| Sum of lipid classes | 169.99 ± 20.01 | | 83.85 ± 11.15 | |

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p(perm) = 0.001), being higher in the ovaries. Testes were characterized by a high concentration of SLs that were also dominant in the ovaries; however, their concentration was significantly lower than in the testes (pseudo-$F_{1,14}$ = 318.78, p(perm) = 0.0001).

SIMPER analysis revealed that TAG and PL were the lipid classes that contributed to the differences between sexes (66.05 and 25.11%, respectively). Significant differences were observed in the concentrations of TAG between sexes (pseudo-$F_{1,17}$ = 52.76; p(perm)=0.0003), being present at higher concentrations in the ovaries. The concentrations of PL were also significantly different between sexes (pseudo-$F_{1,17}$ = 56.72, p(perm) = 0.0001), with higher concentrations in the ovaries (Table 1). However, when considering the proportion of each lipid class over the sum of lipid classes determined, PL were more abundant in the lipids of testes. The EL:SL ratio was greater in ovaries (Table 1).

The FA profile showed a total of 43 FAs in *A. dufresnii* gonads; 40 FAs were identified in the ovaries and 30 in the testes (Table 2). The FA profile was statistically different between sexes (pseudo-$F_{1,16}$ = 29.379, p(perm) = 0.0001; Fig. 1). The FA profile differed by sex not only in the variety of FAs present but in the degree of unsaturation. Significant differences were observed in SFA profiles between sexes (pseudo-$F_{1,19}$ = 8, p(perm) = 0.011), with higher values in the testes (Table 2). In contrast, the PUFA profiles significantly differed between sexes (pseudo-$F_{1,19}$ = 20.03, p(perm) = 0.0003), with higher values in the ovaries (Table 2). No significant differences were seen in the MUFA (pseudo-$F_{1,19}$ = 0.001, p(perm) = 0.97) or HUFA profiles (pseudo-$F_{1,19}$ = 1.5, p(perm) = 0.23) between sexes.

Table 2. Fatty acid (FA) composition (% of total FA) of *Arbacia dufresnii* gonads (September 2009 and 2010). Data represent the mean (±SE) of 5 individuals per sex for 2009 and 7 females and 3 males for 2010. As there were no significant differences in the FA profiles between 2009 and 2010 (pseudo-$F_{1,16}$ = 2.20, p(perm) = 0.1145), these samples were pooled. SFA: saturated FA, MUFA: monounsaturated FA, PUFA: polyunsaturated FA, HUFA: highly unsaturated FA; nd: not detected

| FA | Ovaries | Testes |
|---|---|---|
| C14:0 | 3.51 ± 0.87 | 1.44 ± 0.22 |
| 12-MeC14:0 | 0.17 ± 0.06 | nd |
| C15:0 | 0.94 ± 0.23 | 0.45 ± 0.06 |
| C16:0 | 16.44 ± 2.08 | 18.01 ± 3.15 |
| C16:1 | 0.15 ± 0.06 | nd |
| C16:1 | 0.16 ± 0.03 | nd |
| C16:1(n-7) | 2.76 ± 0.65 | 0.71 ± 0.21 |
| C16:1 | 0.27 ± 0.04 | nd |
| C17:0 | 0.50 ± 0.07 | 0.56 ± 0.04 |
| C16:2(n-6) | 0.30 ± 0.12 | nd |
| C18:0 | 5.51 ± 1.63 | 11.39 ± 1.55 |
| C16:3(n-3) | 0.53 ± 0.16 | nd |
| C18:1(n-9t) | 1.58 ± 1.00 | 0.35 ± 0.30 |
| C18:1(n-9c) | 3.60 ± 0.50 | 2.86 ± 0.44 |
| C18:2(n-6c) | 1.49 ± 0.43 | 1.49 ± 0.19 |
| C19:1 | 0.47 ± 0.11 | 0.29 ± 0.25 |
| C18:3(n-6c) | 0.37 ± 0.17 | 0.35 ± 0.24 |
| C18:3(n-3c) | 1.37 ± 0.63 | 0.34 ± 0.22 |
| C20:0 | 1.25 ± 0.27 | 1.96 ± 0.23 |
| C20:1(n-15) | 6.25 ± 1.02 | 7.08 ± 0.33 |
| C18:4(n-3) | 5.76 ± 1.20 | nd |
| C20:1(n-11) | nd | 1.74 ± 0.78 |
| C20:1(n-9) | 1.20 ± 0.30 | 1.26 ± 0.27 |
| C20:2(n-9) | 4.97 ± 0.63 | 3.34 ± 0.55 |
| C20:2(delta 5,9) | 0.55 ± 0.11 | 0.46 ± 0.11 |
| C20:2(n-6c) | 2.06 ± 0.62 | 2.38 ± 0.64 |
| C21:0 | 0.35 ± 0.13 | nd |
| Unknown PUFA | nd | 0.24 ± 0.10 |
| C20:3(n-9) | 1.13 ± 0.37 | 1.06 ± 0.94 |
| C21:1 | 0.54 ± 0.14 | nd |
| C20:3(n-6) | 0.48 ± 0.20 | 0.48 ± 0.07 |
| C20:4(n-6) | 9.01 ± 1.43 | 13.21 ± 1.75 |
| C20:3 (n-3c) | 1.66 ± 0.41 | 1.12 ± 0.25 |
| C22:0 | 0.22 ± 0.13 | 0.24 ± 0.11 |
| C20:4(n-3) | 0.36 ± 0.14 | nd |
| C22:1 | 0.15 ± 0.19 | nd |
| C22:1(n-9) | 3.24 ± 0.80 | 6.54 ± 0.95 |
| C20:5(n-3) | 16.68 ± 1.62 | 18.25 ± 1.37 |
| C22:4 | 0.50 ± 0.29 | nd |
| C22:4(n-7) | 0.73 ± 0.70 | nd |
| C24:0 | nd | 0.25 ± 0.25 |
| C24:1(n-9) | 0.97 ± 0.73 | 0.47 ± 0.52 |
| C22:6(n-3c) | 1.39 ± 0.77 | 1.65 ± 0.46 |
| SFA | 29.03 ± 3.61 | 34.31 ± 4.76 |
| MUFA | 21.33 ± 1.92 | 21.30 ± 2.27 |
| PUFA | 14.91 ± 2.3 | 11.03 ± 1 |
| HUFA | 34.44 ± 2.5 | 33.12 ± 2.16 |

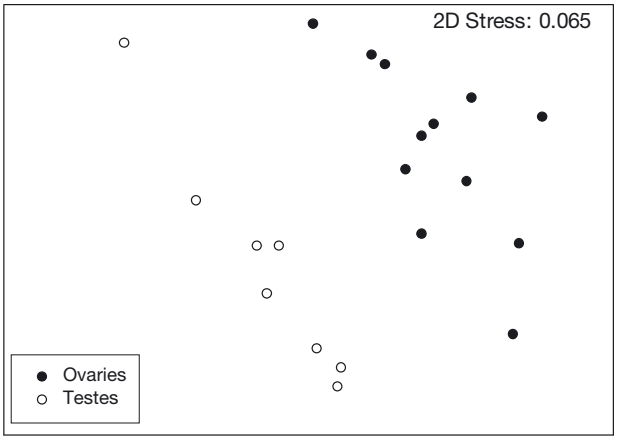


Fig. 1. Multidimensional scaling plot of Euclidean similarities of the gonadal fatty acid profile of *Arbacia dufresnii* ovaries and testes. Data from September 2009 and 2010 were combined (see legend of Table 1)

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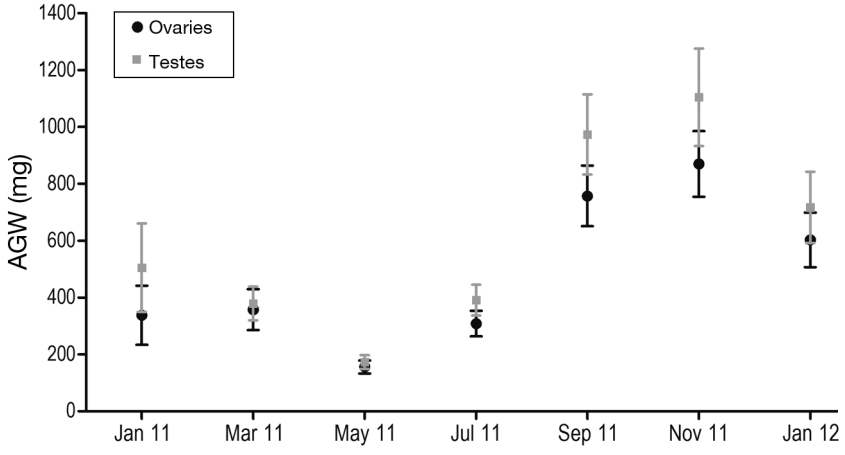


Fig. 2. Size-adjusted gonad weight (AGW) of ovaries and testes of *Arbacia dufresnii* by month. Data are the adjusted means (±0.95 confidence interval, n = 50), if test diameter was held constant at the overall mean of 27.57 mm

Ovaries contained 29% of SFAs and testes 34%, with the dominant SFA being C16:0 (palmitic acid), C18:0 (stearic acid) and a minor concentration of C14:0 (myristic acid), regardless of sex. MUFAs were about 21% of total FAs regardless of sex, with C20:1(n-15), C22:1(n-9), C18:1(n-9c) (oleic acid) and C16:1(n-7) (palmitoleic acid) as the dominant MUFAs. PUFAs comprised 15% in the ovaries and 11% in the testes of the total FAs. The concentration of the dominant HUFAs differed by sex: C20:5(n-3) (eicosapentaenoic acid, EPA) was the dominant HUFA, with C18:4(n-3) (stearidonic acid) found only in the ovaries and a significantly higher concentration of C20:4(n-6) (arachidonic acid, AA) in the testes (Table 2).

SIMPER analysis revealed that the FAs that contributed >10% to the differences between sexes were stearic acid (~24%) and AA (~14%), which were present in higher percentages in the testes, and stearidonic acid (~21%), which was present only in the ovaries. The concentration of these 3 FAs also varied significantly between sexes (Table 3).

### Seasonality of gonads

The AGW means reflected a seasonal reproductive pattern in both sexes. Significant differences were found between sexes ($F_{1,264}$ = 5.54, p = 0.0193) and also between months ($F_{6,264}$ = 41.26, p = 0.00000), with testes weights higher than ovaries throughout the reproductive cycle. The Month × Sex interaction was not significant ($F_{6,264}$ = 0.18, p = 0.983). The mean AGW values increased from austral autumn (May) to austral spring (September–November), when maximum values occurred. Values of the average AGW decreased during austral summer (January; Fig. 2).

Significant differences were found in the TL between sexes ($t_{70}$ = 5.34, p < 0.0001). Even though significant differences in the ovaries were not found with time ($F_{5,30}$ = 1.54, p = 0.2061), their lipid concentration fluctuated, showing 2 declines from March to May and from July to September, and a slight rise in November. In contrast, the seasonal TL in the testes was significantly different between months, where the values dropped continuously from March to November ($H_5$ = 22.89, p = 0.0004; Fig. 3).

Table 3. Contribution of individual fatty acids (FAs) to multivariate differences between *Arbacia dufresnii* ovaries and testes by SIMPER. Pseudo-*F*, p(perm) and Unique perm were obtained in 2-way permutational ANOVA on a single variable. Significant results (p < 0.05) are shown in **bold**

| Average squared distance | 161.1 | | |
|---|---|---|---|
| FA | C18:0 | C18:4(n-3) | C20:4(n-6) |
| Avg. value ovaries | 5.51 | 5.76 | 9.01 |
| Avg. value testes | 11.4 | 0 | 13.2 |
| Avg. sq. dist. | 39.1 | 34.5 | 22.2 |
| Sq. dist/SD | 1.58 | 2.27 | 1.28 |
| Contribution % | 24.27 | 21.41 | 13.78 |
| Cumulative % | 24.27 | 45.68 | 59.46 |
| Pseudo-*F* | 34.76 | 181.66 | 34.682 |
| p(perm) | **0.0001** | **0.001** | **0.0001** |
| Unique perm | 9444 | 828 | 9443 |

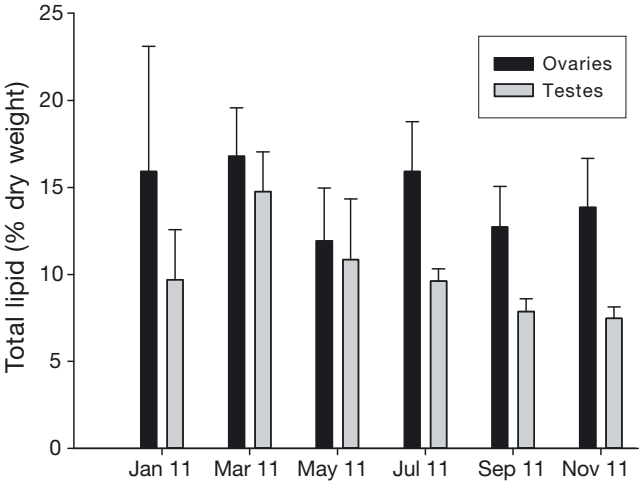


Fig. 3. Seasonal total lipid in ovaries and testes of *Arbacia dufresnii*. Data represent the mean (+SE) of 6 samples for each sex per month

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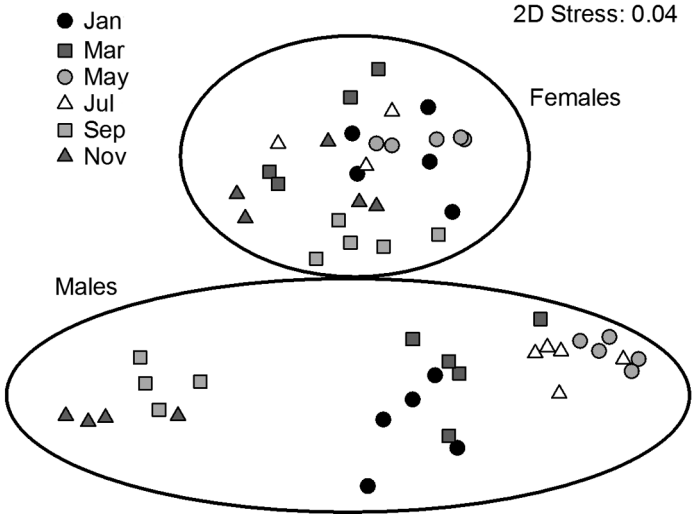


Fig. 4. Multidimensional scaling plot of Euclidean similarities of *Arbacia dufresnii* ovaries and testes lipid profiles among different months during 2011

The lipid profile was significantly different between months (pseudo-$F_{5,43}$ = 29.86, p(perm) = 0.0001) and also between sexes (pseudo-$F_{1,3}$ = 109.55, p(perm) = 0.0001). However, the interaction term was also significant (pseudo-$F_{5,43}$ = 19.53, p(perm) = 0.0001; Fig. 4). Seven lipid classes were detected in *A. dufresnii* gonads during the reproductive cycle, and their concentrations were different between months (Table 4, Fig. 5). Due to the significant interaction term, the analysis of lipid profiles by month was performed separately for ovaries and testes.

The seasonal cycle of the main lipid classes showed clear differences between sexes. The proportion of ELs in the ovaries varied from ~40 to ~55%, while in the testes, it ranged between ~9 and ~46%. The proportion of SLs ranged from ~45 to ~63% in the ovaries and from ~54 to ~90% in the testes (Table 3).

### Ovaries

Significant differences were found in the lipid profile of *A. dufresnii* ovaries between months (pseudo-$F_{5,21}$ = 3.82, p(perm) = 0.0003). The differences were observed between January and September ($t_8$ = 1.93, p = 0.025); January and November ($t_8$ = 2.18, p = 0.033); March and May ($t_7$ = 2.35, p = 0.029); March and September ($t_7$ = 2.04, p = 0.009); May and September ($t_8$ = 3.15, p = 0.008); May and November ($t_8$ = 3.44, p = 0.009); and July and September ($t_6$ = 2.09, p = 0.016; Fig. 6).

PL, TAG and FFA were the lipid classes with the highest proportions throughout the reproductive cycle (Table 3, Fig. 5). SIMPER analysis revealed that PL was the lipid class that contributed most (>25%) to the differences between all months, where its concentration was significantly different between months (pseudo-$F_{5,21}$ = 3.43, p(perm) = 0.0227). The rest of the SL (ST and AMPL) also contributed to the differences (>15 and >10% of contribution, respectively) and their concentrations were also different between months (pseudo-$F_{5,21}$ = 5.45, p(perm) = 0.0038 and pseudo-$F_{5,21}$ = 8.13, p(perm) = 0.0003, respectively). ST proportion also varied between months. TAG and FFA as the dominant ELs also contributed to the differences between months. TAG contributed with >15%, and its concentration was statistically different between months (pseudo-$F_{5,21}$ = 4.54, p(perm) = 0.0063). FFA

Table 4. Lipid classes (in % of the total of all lipid classes) present in gonads of *Arbacia dufresnii* (January to November 2011). See Table 1 for definitions of lipids; DAG not detected. nd: not detected

| Lipid | Jan | Mar | May | Jul | Sep | Nov |
|---|---|---|---|---|---|---|
| %ST | | | | | | |
| Female | 5.38 | 5.07 | 3.95 | 4.18 | 8.73 | 9.37 |
| Male | 24.98 | 16.38 | 13.55 | 19.04 | 18 | 20.5 |
| %AMPL | | | | | | |
| Female | 5.67 | 5.04 | 2.1 | 2.43 | 9.29 | 8.63 |
| Male | 12.52 | 8.63 | 6.73 | 5.81 | 5.82 | 8.28 |
| %PL | | | | | | |
| Female | 38.07 | 42.92 | 38.54 | 43.76 | 44.71 | 42.5 |
| Male | 49.14 | 48.73 | 33.53 | 38.58 | 63.9 | 62.33 |
| %SL | | | | | | |
| Female | 49.12 | 53.04 | 44.59 | 50.37 | 62.73 | 60.50 |
| Male | 86.65 | 73.74 | 53.81 | 63.42 | 87.72 | 91.11 |
| %FFA | | | | | | |
| Female | 13.76 | 11.76 | 18.43 | 14.67 | 11.12 | 12.63 |
| Male | nd | nd | nd | nd | nd | nd |
| %TAG | | | | | | |
| Female | 26.51 | 26.58 | 28.59 | 25.34 | 21.55 | 22.20 |
| Male | 10.2 | 19.07 | 33.54 | 28.38 | 9.7 | 6.65 |
| %WE | | | | | | |
| Female | 5.22 | 4.93 | 5.11 | 5.27 | 2.42 | 2.5 |
| Male | 1.84 | 4.66 | 7.27 | 4.46 | 1.24 | 1.06 |
| %ME | | | | | | |
| Female | 5.39 | 3.69 | 3.28 | 4.35 | 2.17 | 2.17 |
| Male | 1.46 | 2.54 | 5.38 | 3.73 | 1.34 | 1.18 |
| %EL | | | | | | |
| Female | 50.88 | 46.96 | 55.41 | 49.63 | 37.27 | 39.25 |
| Male | 13.5 | 26.26 | 46.19 | 36.58 | 12.28 | 8.89 |
| EL/SL | | | | | | |
| Female | 1.04 | 0.89 | 1.24 | 0.99 | 0.59 | 0.12 |
| Male | 0.16 | 0.36 | 0.86 | 0.58 | 0.14 | 0.1 |

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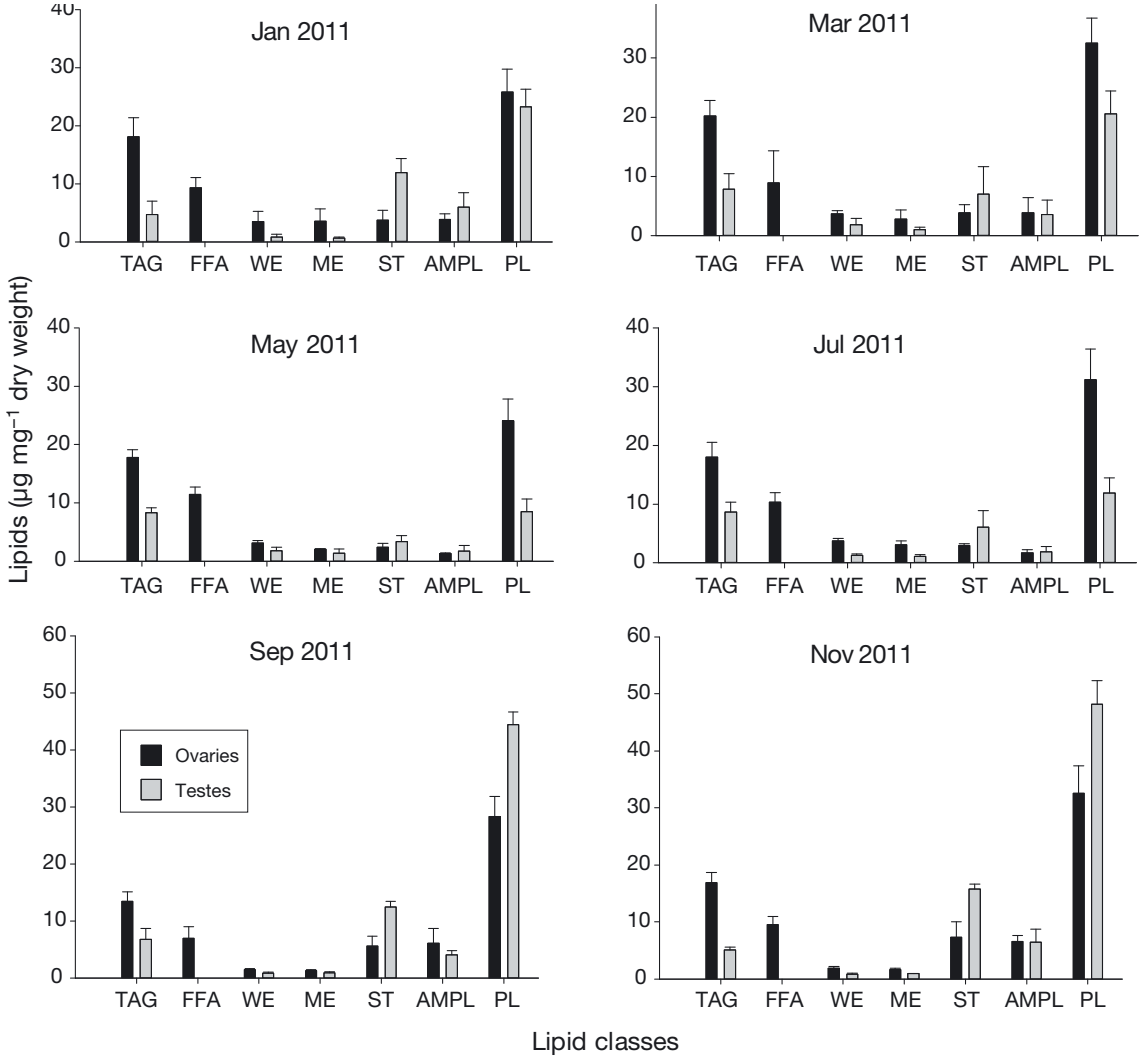


Fig. 5. Seasonal concentration of lipid classes present in ovaries and testes of *Arbacia dufresnii*. TAG: triacylglycerols, FFA: free fatty acids, WE: wax esters, ME: methyl esters, ST: sterols, AMPL: acetone-mobile polar lipids, PL: phospholipids. Data represent the mean (+SE) of 5 samples per month for each sex

contribution was >10%; however, no statistical differences were found in the concentration of this EL between months (pseudo-$F_{5,21}$ = 1.69, p(perm) = 0.1758). WE and ME were present in relatively low proportions (Table 4).

The proportion of TAG and FFA decreased from the maximum value at the beginning of gametogenesis (May, pregametic stage) to September (mature stage), because of the concomitant rise in the proportion of PL and ST. However, the proportion of TAG and FFA was never lower than 30% in the ovaries (Table 4). The other lipid classes were in very low proportions, although a drop in WE and ME was seen from July (growth stage) towards September (mature stage).

The ratio of EL:SL was 1.24 at the beginning of gametogenesis (pregametic stage, May) and dropped continuously to 0.59 in September and 0.12 in November (mature stage) (Table 4).

### Testes

The lipid profile was significantly different between months (pseudo-$F_{5,22}$ = 56.51, p(perm) = 0.0001). Pairwise tests revealed that the differences were between all months ($p < 0.05$) except for September and November, which were not significantly different ($t = 1.97$, $p = 0.11$). SIMPER analysis showed that the lipid classes that contributed to the differ-

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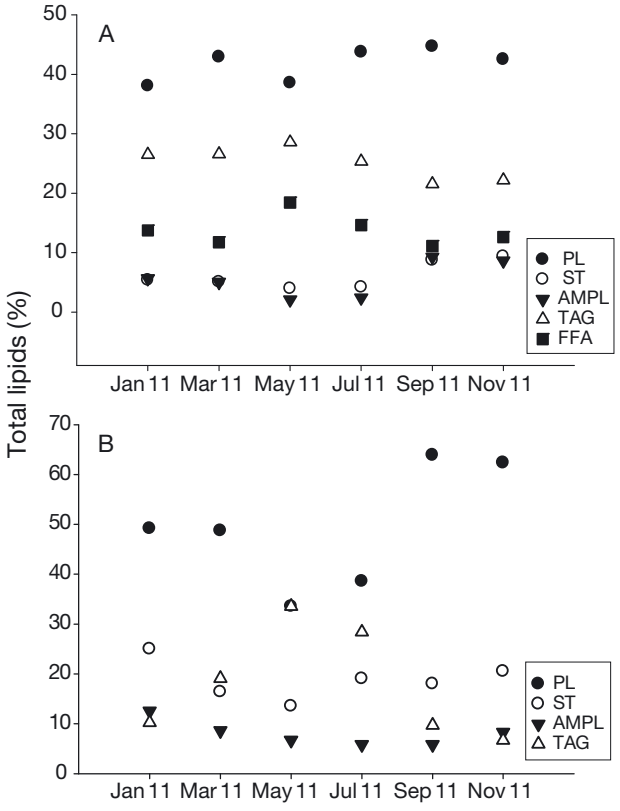


Fig. 6. Seasonal variation in lipid classes of *Arbacia dufresnii* (A) ovaries and (B) testes that contributed to the differences between months. Data are presented as % of the total lipids. PL: phospholipids, ST: sterols, AMPL: acetone-mobile polar lipids, TAG: triacylglycerol, FFA: free fatty acid

ences between months were the SLs. PL had a contribution of 24% between January and March, but between the rest of the months the contribution was >65%. ST contributed with more than 10% between most of the months, and AMPL only contributed with 14% to the difference between January and March. The concentration of PL was significantly different between months (pseudo-$F_{5,22}$ = 126.78, p(perm) = 0.0001). There were also significant differences of the concentration of ST ($F_{5,22}$ = 14.38, p(perm) = 0.0001) between months, as well as the concentration of AMPL ($F_{5,22}$ = 5.55, p(perm) = 0.0023). The only EL that contributed to the difference was TAG, with a contribution of 18% between January and March. Its concentration was also significantly different between months ($F_{5,22}$ = 3.97, p(perm) = 0.0085).

PL and TAG were the lipid classes with the higher proportions at the beginning of the reproductive cycle (May, pregametic state), but TAG proportion dropped markedly from 33.5 to 6.6%, concomitant with the rise of PL and ST proportions (Table 4, Fig. 6). The other lipid classes were in very low proportions (2.2 to 12.6%) throughout the cycle, although a drop in WE and ME was seen from July (growth stage) to September–November (premature stage). FFA were not detected.

The ratio of EL:SL was maximum (0.86) at the onset of the gametogenesis (May, pregametic stage) and dropped to 0.14 in September and 0.10 in November (premature stage) (Table 4).

### Seasonality of FA profiles

As there was a single pooled sample per sex each month, it was not possible to perform a multivariate analysis on changes in FA profiles over time. There were, however, changes in FA profiles with month (Table 5, Fig. 7).

HUFAs were the main FA class throughout the reproductive cycle in the gonads of *A. dufresnii*, followed by SFAs and MUFAs. The (n-3) FAs were higher in the ovaries, whereas (n-6) FAs were higher in the testes. Three FAs were the most abundant throughout the reproductive cycle: AA, EPA and palmitic acid, each exceeding 10% of total FAs, regardless of the month (Table 5).

The SFA cycle was similar for ovaries and testes, (Fig. 7A), inverse to the AGW cycle (Fig. 2). MUFAs varied very little throughout the reproductive cycle in both sexes (Fig. 7B). PUFAs varied like AGW in the ovaries but not in the testes (Fig. 7C), whereas HUFAs showed similar cycles in both sexes, following the AGW cycle (Fig. 7D).

EPA and stearidonic acid followed AGW, while AA had a somewhat inverse cycle in both sexes. The dominant SFAs—palmitic acid (both sexes) and stearic acid (in the testes)—had the same cycle, inverse to the AGW cycle. Stearidonic cycle (only found in the ovaries) also followed AGW (Table 5).

## DISCUSSION

Our examination of the lipid and FA profiles of *Arbacia dufresnii* gonads revealed 2 important findings: first, a clear differentiation in both profiles between sexes, and second, seasonal variation in some lipid and FA components related to the gametogenic stage of the gonads. These findings have important implications for understanding the trophic dynamics and feeding of *A. dufresnii*; comparisons of lipid and FA profiles with other populations or species will require the use of sex-specific profiles at similar points in the gametogenic cycle.

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Table 5. Seasonal fatty acid (FA) composition (% of the total FA) of *Arbacia dufresnii* ovaries and testes. Data represent the results of 12 individuals per sex pooled each month. IG: intergametic, PG: pregametic, G: growth, PM: premature, M: mature, pSP: partial spawing, nd: not detected, SFA: saturated FA, MUFA: monounsaturated FA, PUFA: polyunsaturated FA, HUFA: highly unsaturated FA

| FA | Mar 2011 IG | | May 2011 PG | | Jul 2011 G | | Sep 2011 PM | | Nov 2011 M | | Jan 2012 pSP | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Female | Male | Female | Male | Female | Male | Female | Male | Female | Male | Female | Male |
| C12:0 | 0.02 | 0.04 | 0.02 | 0.02 | 0.02 | 0.03 | 0.01 | 0.04 | 0.03 | 0.05 | 0.02 | 0.02 |
| C13:0 | 0.03 | 0.05 | 0.02 | 0.05 | 0.02 | 0.04 | 0.02 | 0.07 | 0.04 | 0.06 | 0.03 | 0.04 |
| C14:0 | 3.15 | 1.54 | 3.18 | 1.92 | 4.10 | 1.95 | 3.46 | 1.29 | 3.95 | 1.17 | 3.66 | 0.84 |
| 13-Me-C14:0 | 0.36 | 0.13 | 0.20 | 0.16 | 0.16 | 0.12 | 0.20 | 0.08 | 0.28 | 0.09 | 0.33 | 0.07 |
| 12-MeC14:0 | 0.06 | 0.03 | 0.04 | 0.03 | 0.02 | 0.05 | 0.05 | 0.02 | 0.07 | 0.03 | 0.07 | 0.03 |
| C15:0 | 1.20 | 0.60 | 1.04 | 0.73 | 0.88 | 0.63 | 0.85 | 0.49 | 1.01 | 0.47 | 1.15 | 0.33 |
| 14-MeC15:0 | 0.13 | nd | 0.09 | nd | 0.10 | nd | 0.07 | nd | 0.13 | nd | 0.14 | nd |
| C16:0 | 17.57 | 15.68 | 17.06 | 15.53 | 14.11 | 12.87 | 12.81 | 11.93 | 14.50 | 12.08 | 13.88 | 11.52 |
| C16:1 | 0.33 | 0.14 | 0.24 | 0.18 | 0.16 | 0.07 | 0.19 | 0.08 | 0.22 | 0.08 | 0.20 | 0.07 |
| C16:1 | 0.24 | 0.11 | 0.23 | 0.13 | 0.18 | 0.12 | 0.19 | 0.05 | 0.23 | 0.09 | 0.18 | 0.08 |
| C16:1(n-7) | 2.35 | 0.77 | 2.19 | 0.85 | 3.07 | 1.40 | 3.54 | 0.92 | 3.14 | 0.52 | 3.63 | 0.40 |
| 14-Me 16:0 | 0.20 | 0.19 | 0.22 | 0.24 | 0.22 | 0.16 | 0.27 | 0.09 | 0.22 | 0.05 | 0.28 | 0.25 |
| C17:0 | 0.67 | 0.68 | 0.53 | 0.60 | 0.42 | 0.40 | 0.41 | 0.61 | 0.35 | 0.66 | 0.46 | 0.57 |
| C16:2(n-6) | 0.49 | nd | 0.15 | nd | 0.13 | nd | 0.07 | nd | 0.32 | nd | 0.21 | nd |
| C17:1(n-7) | 0.18 | nd | 0.24 | nd | 0.41 | nd | 0.41 | nd | 0.37 | nd | 0.44 | nd |
| C18:0 | 4.44 | 9.87 | 4.57 | 8.49 | 2.97 | 6.64 | 2.77 | 6.75 | 3.51 | 7.07 | 3.10 | 10.69 |
| C18:1(n-13) | nd | 0.46 | nd | 0.41 | nd | 0.36 | nd | 1.06 | nd | 1.69 | nd | 0.33 |
| C18:1(n-9t) | 3.42 | 1.10 | 1.72 | 0.70 | 1.05 | 0.66 | 0.90 | 0.28 | 1.44 | 0.26 | 1.83 | 0.83 |
| C18:1(n-9c) | 2.63 | 2.22 | 2.74 | 2.33 | 3.88 | 3.96 | 4.85 | 3.51 | 3.62 | 2.19 | 3.45 | 2.34 |
| C18:2(n-6c) | 3.12 | 2.31 | 1.74 | 1.66 | 1.01 | 1.32 | 1.33 | 0.79 | 1.75 | 0.62 | 1.98 | 1.65 |
| C19:1 | 0.48 | 0.74 | 0.51 | 0.72 | 0.41 | 0.30 | 0.12 | 0.27 | 0.43 | 0.18 | 0.53 | 0.70 |
| C18:3(n-6c) | 0.53 | 0.21 | 0.38 | 0.20 | 0.34 | 0.24 | 0.01 | 0.12 | 0.44 | 0.06 | 0.53 | 0.10 |
| C18:3(n-3c) | 2.40 | 0.42 | 1.27 | 0.28 | 1.14 | 1.66 | 1.96 | 0.45 | 1.61 | 0.41 | 2.25 | 0.63 |
| C20:0 | 0.98 | 1.47 | 1.28 | 1.74 | 1.27 | 1.61 | 0.81 | 0.54 | 1.18 | 1.19 | 1.04 | 1.62 |
| C20:1(n-15) | 5.66 | 7.93 | 6.85 | 8.49 | 6.57 | 8.48 | 3.50 | 2.54 | 5.74 | 3.77 | 5.85 | 9.22 |
| C20:1 | nd | 0.49 | nd | 0.64 | nd | 1.23 | nd | 4.98 | nd | 3.21 | nd | 0.26 |
| C18:4(n-3) | 4.61 | nd | 4.10 | nd | 5.52 | nd | 6.98 | nd | 6.81 | nd | 4.98 | nd |
| C20:1(n-11) | 2.11 | 2.98 | 1.77 | 3.12 | 0.92 | 2.00 | 2.54 | 3.51 | 0.85 | 5.13 | 1.53 | 2.04 |
| C20:1(n-9) | 0.57 | 0.95 | 0.87 | 0.97 | 1.39 | 1.04 | 1.42 | 2.03 | 0.57 | 1.99 | 1.05 | 1.11 |
| C20:2(n-9) | 5.11 | 3.55 | 5.01 | 4.49 | 5.49 | 5.11 | 3.81 | 1.68 | 4.87 | 2.11 | 4.84 | 2.87 |
| C20:2(delta 5,9) | 0.21 | 0.00 | 0.38 | 0.00 | 0.63 | 0.00 | 2.54 | 0.00 | 0.50 | 0.00 | 0.44 | 0.00 |
| C20:2(n-6c) | 2.58 | 2.72 | 1.96 | 2.17 | 1.48 | 2.76 | 2.48 | 2.18 | 2.16 | 2.62 | 2.18 | 3.09 |
| Unknown PUFA | 0.29 | 0.23 | 0.36 | 0.33 | 0.16 | 0.34 | 0.80 | 0.90 | 0.37 | 0.57 | 0.23 | 0.25 |
| C21:1 | 1.30 | 1.34 | 1.15 | 1.19 | 0.93 | 0.57 | 0.97 | 0.97 | 0.88 | 0.37 | 1.22 | 1.13 |
| C20:3(n-6) | 0.48 | 0.40 | 0.51 | 0.51 | 0.51 | 0.68 | 0.55 | 0.96 | 0.62 | 0.27 | 0.87 | 0.80 |
| C20:2 | 0.17 | 0.26 | 0.38 | 0.22 | 0.56 | 0.29 | 0.40 | 0.04 | 0.50 | 0.03 | 0.37 | 0.21 |
| C20:4(n-6) | 13.16 | 16.41 | 12.41 | 15.90 | 9.97 | 14.81 | 8.34 | 18.09 | 10.35 | 19.17 | 11.63 | 19.33 |
| C20:3 (n-3c) | 0.85 | nd | 1.11 | nd | 1.10 | nd | 3.34 | nd | 1.47 | nd | 1.51 | nd |
| C22:0 | 0.13 | 0.31 | 0.21 | 0.31 | 0.12 | 0.10 | 0.08 | 0.09 | 0.20 | 0.15 | 0.09 | 0.17 |
| C22:1 | 0.29 | 0.19 | 0.33 | 0.24 | 0.29 | 0.12 | 0.52 | 0.33 | 0.29 | 0.22 | 0.36 | 0.10 |
| C22:1(n-9) | 2.35 | 6.76 | 3.15 | 5.19 | 3.28 | 3.17 | 2.56 | 4.71 | 3.24 | 5.99 | 3.06 | 4.68 |
| C20:5(n-3) | 11.57 | 11.89 | 16.30 | 14.99 | 20.44 | 21.39 | 20.14 | 23.32 | 17.81 | 21.48 | 16.65 | 17.45 |
| C22:4 | 0.27 | 0.37 | 0.24 | 0.29 | 0.29 | 0.37 | 0.58 | 0.36 | 0.20 | 0.35 | 0.31 | 0.45 |
| C24:0 | 0.22 | 0.00 | 0.43 | 0.00 | 0.67 | 0.00 | 0.44 | 0.00 | 0.57 | 0.00 | 0.39 | 0.00 |
| C23:1 | 0.31 | 0.73 | 0.65 | 0.77 | 0.63 | 0.57 | 0.78 | 0.51 | 0.90 | 0.51 | 0.59 | 0.26 |
| Unknown PUFA | nd | 0.22 | nd | 0.35 | nd | 0.44 | nd | 0.37 | nd | 0.33 | nd | 0.44 |
| Unknown PUFA | nd | 0.04 | nd | 0.03 | nd | 0.23 | nd | 0.06 | nd | 0.07 | nd | 0.31 |
| C24:1(n-9) | 0.45 | 0.43 | 0.88 | 0.21 | 1.47 | 0.14 | 0.13 | 0.31 | 0.50 | 0.29 | 1.00 | 0.25 |
| C22:6(n-3c) | 2.37 | 3.04 | 1.28 | 2.64 | 1.50 | 1.59 | 1.78 | 2.61 | 1.79 | 2.38 | 1.44 | 2.46 |
| SFA | 29.14 | 30.59 | 28.90 | 29.83 | 25.09 | 24.59 | 22.27 | 22.01 | 26.03 | 23.07 | 24.64 | 26.14 |
| MUFA | 22.49 | 27.35 | 23.29 | 26.11 | 24.25 | 24.18 | 22.22 | 26.04 | 22.06 | 26.47 | 24.50 | 23.82 |
| PUFA | 15.93 | 9.91 | 12.90 | 9.55 | 12.39 | 12.29 | 16.49 | 6.30 | 14.23 | 6.17 | 15.20 | 9.66 |
| HUFA | 32.27 | 32.15 | 34.70 | 34.50 | 37.87 | 38.94 | 38.62 | 45.65 | 37.33 | 44.29 | 35.24 | 40.38 |
| PUFA+HUFA | 48.20 | 42.06 | 47.60 | 44.05 | 50.25 | 51.23 | 55.11 | 51.95 | 51.57 | 50.46 | 50.44 | 50.04 |
| PUFA+ HUFA(n-3) | 21.81 | 15.56 | 24.07 | 18.26 | 29.69 | 25.08 | 34.20 | 26.75 | 29.49 | 24.61 | 26.84 | 20.98 |
| PUFA+HUFA (n-6) | 20.36 | 22.05 | 17.16 | 20.43 | 13.43 | 19.82 | 12.78 | 22.15 | 15.64 | 22.74 | 17.40 | 24.97 |

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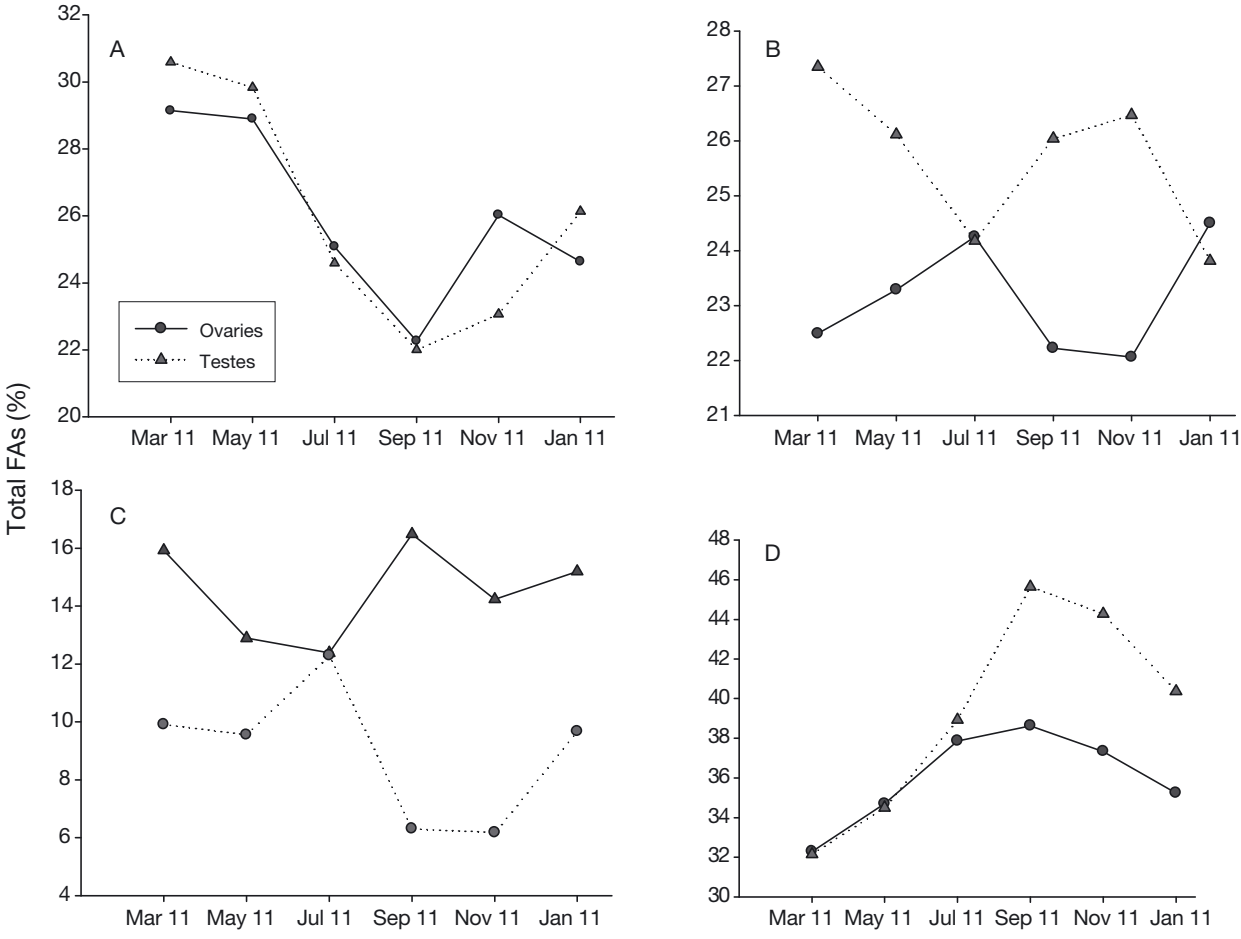


Fig. 7. Seasonal percentage of the total fatty acids (FAs) present in ovaries and testes of *Arbacia dufresnii*: (A) saturated FAs (SFAs), (B) monounsaturated FAs (MUFAs), (C) polyunsaturated FAs (PUFAs) and (D) highly unsaturated FAs (HUFAs)

The lipid profile of mature *A. dufresnii* gonads differed by sex in the number of lipid classes present (ovaries with 8 lipid classes, testes with 6; FFA and DAG were not detected in testes), and in the patterns occurring during the seasonal cycle. Lipids accumulated at the intergametic stage (March, when gonads were in reabsorption), and nutrients were stored either by autophagia or in the nutritive phagocytes (Parra et al. 2015), and became depleted at the onset of the gametogenesis (May, pregametic stage). In the latter stage, in the ovaries, the proportion of ELs reached the maximum value, mainly due to TAG and FFA, with low proportions of WE and ME. In contrast, in the same period, TAG represented only a third of TLs in the testes, and WE and ME, although low, were at their maximum values. These data indicate that differences between the sexes in the allocation of energy are evident from the very onset of gametogenesis. In the pregametic stage, ELs might have been allocated into the nutritive phagocytes, which started to fill the lumen in ovaries and were abundant in testes (Epherra et al. 2015), and may then have been used as energy to fuel the initial gonad development from July to September.

During the growth stage (winter), when the average wet weight of ovaries increased, the proportion of SLs exceeded that of ELs. This was due to the decrease in the proportion of TAG and FFA, partially used as fuels for gonadal growth, and the marked increase of PL and ST, while other classes remained nearly unchanged or in relatively low proportions. A similar pattern was observed in the testes, with a decrease in the proportion of TAG, WE and ME, and an increase in PL and ST. As new primary oocytes and new spermatogenic columns appear in the ovaries and testes during the growth phase (Epherra et al. 2015), we hypothesize that ELs, such as TAG and FFA, may be used for the synthesis of SLs, which are the main components of cellular membranes (Iverson 2009, Christie & Han 2010) in these new cells.

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In the mature stage (September–November), testes had a higher concentration of SLs, most likely reflecting the increased number of spermatozoa in the testes. In contrast, there was an accumulation of ELs in the ovaries during the spring and summer when fully developed gonads, with abundant mature gametes, are seen in the Nuevo Gulf population (Epherra et al. 2015). Maximal TLs coincident with mature gonads and high gonadal index is a pattern previously described in *Strongylocentrotus droebachiensis* (Liyana-Pathirana et al. (2002) and *Evechinus chloroticus* (Zárate 2014).

SLs dominated the lipid profile of *A. dufresnii* gonads, reaching ca. 62% in mature testes and ca. 42% in mature ovaries. In contrast, ovaries showed a higher proportion of ELs, mainly driven by TAG and FFA concentrations. This is expected, as it is generally recognized that male gametes are composed mostly of SLs, especially PL, which are essential parts of the cell membrane (Kozhina et al. 1978, Mita & Ueta 1989), whereas female gametes present more ELs playing a reserve function (Morais et al. 2003). Similar lipid profiles have been observed in gonads of *E. chloroticus* (Verachia et al. 2012, Zárate, 2014) and *S. droebachiensis* (Liyana-Pathirana et al. 2002), although none of these studies differentiated by sex.

The FA profile of *A. dufresnii* was dominated by HUFAs, followed by SFAs and MUFAs, as previously found in other temperate sea urchins (De la Cruz-García et al. 2000, Liyana-Pathirana et al. 2002, Hughes et al. 2005, 2006, 2011, González-Durán et al. 2008, Martínez-Pita et al. 2010a,b, Arafa et al. 2012, Carboni et al. 2013, Angioni & Addis 2014). Clear differences were also seen in the FA profile between sexes, with differences in the dominant FAs and with stearidonic acid [C18:4 (n-3)] absent in the testes. This may reflect metabolic differences in gonad tissue related to the specific requirements of spermatogenesis and oogenesis (Martínez-Pita et al. 2010a,b). In the ovaries, the high proportion of HUFAs and PUFAs was mainly due to C18:4(n-3), the higher percentage (although low) of 18:3(n-3) and somewhat higher percentages of the C20 PUFAs other than AA. C18:3(n-3) has been considered as a precursor for the synthesis of the long-chain essential EPA (Bell et al. 2001); thus the higher level of C18:3(n-3) in ovaries is consistent with the slightly lower value of EPA. The dominant MUFA C20:1(n-15) in both sexes suggests the presence of an active Δ-5 desaturase, as found in *Psammechinus miliaris* (Bell et al. 2001), and this feature was considered characteristic of echinoids by Takagi et al. (1986). Nevertheless, the corresponding non-methylene interrupted C20 and C22, usually found in sea urchins (Takagi et al. 1986, Cook et al. 2000, Liyana-Pathirana et al. 2002, Castell et al. 2004, Hughes et al. 2005) were not detected in the present study, although some of their precursors (C20:1(n-9); 22:1(n-9)) were found.

Seasonal variation in FA profiles has been studied for several temperate sea urchin species (Liyana-Pathirana et al. 2002, Hughes et al. 2006, Martínez-Pita et al. 2010a, Arafa et al. 2012, Carboni et al. 2013), although not all studies differentiated by sex. In *A. dufresnii*, HUFAs were the main FA class throughout the reproductive cycle, particularly in the testes, and the sum of PUFAs plus HUFAs accounted for nearly 50% of total FAs. These FA classes, however, showed slightly different patterns throughout the reproductive cycle. The HUFAs closely followed the AGW cycle from the onset of gametogenesis in May until the mature stage, when AGWs were higher in both sexes. In testes, there was a closer resemblance between the cycles of SLs and HUFAs. Considering the different lipid classes that comprise the SLs, PL and AMPL showed similar patterns as PUFAs+HUFAs in ovaries. In testes, the cycle of PL was close to the HUFA cycle, while AMPL had a similar cycle to that of MUFAs. Although these similarities were not statistically significant, they may be indicative of the distribution of FAs among the different lipid classes. Liyana-Pathirana et al. (2002) attributed the peak of unsaturated FAs in the winter to the need to increase the fluidity of cellular membranes when the seawater temperature was low. This hypothesis was not supported in the present study, where the peak of unsaturated FAs, and particularly the HUFAs, did not occur in the coldest season but at the beginning of the spring, when the gonads were filled with a large number of fully developed gametes.

When the PUFAs and HUFAs are split into (n-3) and (n-6) families, again distinct patterns arise. In the ovaries, the (n-3) FAs followed the AGW cycle, increasing as gametogenesis progressed, to show the maximum at the mature stage. This cycle was close to that of PL, thus suggesting that these FAs are important constituents of that lipid class. This pattern was similar but less marked in the testes. Similar variations were found for *Paracentrotus lividus* (Martínez-Pita et al. 2010a, Carboni et al. 2013) and *A. lixula* (Martínez Pita et al. 2010a).

The (n-3) pattern was clearly driven by the cycle of EPA. Martínez-Pita et al. (2010a) related the decrease in EPA percentage in the summer to the need to counteract the disorder in membrane lipids caused

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by higher temperatures. However, Hughes et al. (2006) attributed the decrease in EPA percentage during the final stages of the reproductive cycle of *P. miliaris* to the release of gametes during spawning. In the case of the population under study, we tend to prefer the second hypothesis since the EPA and the (n-3) patterns followed the AGW cycle, thus showing a decrease during spawning.

The (n-6) family did not show marked variation throughout the reproductive cycle in ovaries, but varied inversely to the AGW cycle in testes. A distinct cycle was found for AA, the dominant (n-6) HUFA, with the lowest percentage of AA in the winter, a continuous increase over time, and no depletion at the beginning of the spawning period, suggesting that it might not be exclusively accumulated in gametes but may also be present in other gonadal cells. A similar pattern was seen for ovaries, with a steady reduction in the AA percentage from the reabsorption stage to maturity, as previously observed for *P. miliaris* (Hughes et al. 2006), *P. lividus* and *A. lixula* (Martínez-Pita et al. 2010a). In these sea urchins, the final accumulation of AA was attributed to further needs for eicosanoid synthesis or to a stress response. In this light, it is interesting to consider the cycle of stearidonic acid which, despite being a precursor of EPA biosynthesis, showed the same pattern as EPA and was similar to that of AGW in the ovaries of *A. dufresnii*. By contrast, stearidonic acid appeared to be used for the synthesis of longer-chain FAs in the gonads of *P. lividus*, but not in *A. lixula* (Martínez-Pita et al. 2010a). The fact that the proportion of stearidonic acid did not drop with the proportional increase in EPA in *A. dufresnii* ovaries might suggest a dietary input and/or preferential retention in this species.

The SFA cycle was the opposite to AGW and to the cycle of PUFA+HUFA, accumulating in the reabsorption and pregametic stages when nutrients increased in the nutritive phagocytes (Parra et al. 2015), and at a minimum in the spring, when maturity was achieved in both sexes (Epherra et al. 2015). The fact that these FAs had a similar pattern to ELs, especially to TAG in the ovaries and in the testes, suggests that these lipid classes might be constituted mainly by saturated chains. Similar variations were apparent when the cycles of the dominant SFA palmitic acid and stearic acid were considered, as found for *A. lixula* and for *P. lividus* (Martínez-Pita et al. 2010a).

MUFAs remained nearly constant throughout the cycle in both sexes, as a result of inverse patterns followed by individual MUFAs. C20:1(n-15) was the dominant MUFA throughout the cycle, as found for *S. droebachiensis* (Liyana-Pathirana et al. 2002). The drop in the C20:1(n-15) percentage observed at the mature stage (more markedly in testes) was compensated with the opposite cycle shown by oleic acid, palmitoleic acid, C20:1(n-11) and C20:1(n-9) with a slight increment when gonads reached the mature stage.

In conclusion, this study has highlighted that sex and reproductive stage can significantly influence the lipid and FA profiles of *A. dufresnii*. In future research on echinoids, it will be very important to consider both factors when comparing lipid and FA profiles of echinoid gonads between populations and/or species when they are used as ecological tools.

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