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The dual functions of sea urchin gonads are reflected in the temporal variations of their biochemistry

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Abstract Fatty acid analyses are emerging as a powerful technique to probe trophic interactions between organisms. In this paper, the application of both this procedure and gonad index (GI) determination on two populations (intertidal and subtidal) of the echinoid *Psammechinus miliaris* is reported. The investigation spanned the 3-month spawning period of Scottish west coast populations. In both populations a progressive decrease in the GI was found, coupled with an increasing maturity stage (from mature to spent). Sexual maturation and decrease in GI was synchronous between the two populations. In conjunction, there were distinct changes in gonad biochemistry. Differences in the fatty acid composition of the gonad reflected the changes in sexual maturation. Mature males and females had significant differences in the fatty acid composition of their gonads, whereas post-spawned individuals showed no gender differences. Male urchins had higher levels of polyunsaturated fatty acids (PUFAs) compared to females, and there was a dramatic reduction in the fatty acids 22:6(*n*-3) and 20:5(*n*-3) with increasing maturity stage. Using multivariate statistical techniques, these changes in the fatty acid composition of the sea urchin gonad were linked to habitat related diet differences combined with gender differences. These changes in the

fatty acid signatures clearly reflect the dual function of the gonad as both a nutrient store and a reproductive organ.

Introduction

Understanding the trophic relationships that exist within and between species, and the way that these interactions vary along temporal and spatial scales has long been a principle component of marine ecological research (Elton 1927; Linderman 1942; Fretwell 1987; Menge 2000). Techniques have increased in variety and resolution to explore and quantify such interactions. At their simplest these include passive observations such as direct study of feeding behaviour (Zamon 2001) or examination of fecal pellets (Bonesi et al. 2004). Potentially powerful insights can be gained by invasive methods such as stomach content analysis (de la Moriniere et al. 2003) and biochemical sampling such as stable isotope analysis (Fry and Sherr 1984), carotenoid pigment analysis (Hudson et al. 2003) and fatty acid analysis (Pond et al. 1997).

Fatty acid analysis, the technique employed in the current study, has been used to study trophic interactions in a particularly wide range of habitats from terrestrial (McWilliams et al. 2004) to freshwater and marine (Go et al. 2002). It has been successfully applied to discriminate the trophic niches of co-occurring species (Howell et al. 2003) and diet changes within a species across a range of temporal and spatial scales (Cripps et al. 1999; Iverson et al. 1997; Phillips et al. 2003). It is also a useful method for examining the biochemical changes in marine invertebrates linked to their reproductive cycle. The link between the fatty acid signature of the gonad and the reproductive cycle has been demonstrated for the bivalves *Pecten maximus* (Pazos et al. 1997), *Crassostrea gigas* (Soudant et al. 1999), *Ruditapes decussates* (Ojea et al. 2004), the cephalopods *Octopus*

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vulgaris and *O. deflippi* (Rosa et al. 2004a), *Eledone cirrhosa* and *E. moschata* (Rosa et al. 2004b), and the crustaceans *Nephrops norvegicus* (Rosa and Nunes 2002) and *Aristeus antennatus* (Rosa and Nunes 2003). These studies have shown that there are major changes in the fatty acid signatures of the gonads during sexual maturation, and that two fatty acids, 20:5(*n*-3) and 22:6(*n*-3), are particularly important in this maturation cycle.

Our study focuses on a less frequent target taxon, the echinoderms, and specifically echinoids. Gonadal index (GI) studies have revealed that gonad mass relative to whole organism mass varies considerably along both spatial and temporal scales (McClanahan and Kurtis 1991; Kelly 2000). Echinoid gonads have a multifunctional role and are known to reflect both nutritive and reproductive status (Russell 1998). *Psammechinus miliaris*, a small and common coastal species, is known to be an opportunistic omnivore, which can occur at very high densities—upto 350 individuals per square meter (Kelly 2000) in Scottish sea lochs. It occurs from the low littoral to ~100 m depth (Kelly and Cook 2001) and in

our study region, West Scotland, *P. miliaris* typically occurs in the littoral and sub-littoral of sheltered sea lochs. Here we investigate changes in the gonadal indices and biochemistry across the spawning period. The study was designed to address two specific hypotheses: that changes in the GI over the spawning period were consistent between the intertidal and subtidal populations; and that the fatty acid signature of the gonad would reflect changes in gonad maturation.

Materials and methods

Study site

The study was conducted at two locations on the south shore of Loch Creran, west Scotland (Fig. 1). Loch Creran is a silled sea loch 12.8 km long, with four basins that reach a maximum depth of 49 m. It has been characterized as a well-mixed system with a flushing time of 3 days (Black et al. 2000). Two study locations separated by approximately 5 km, Rubha Garbh (RG) and

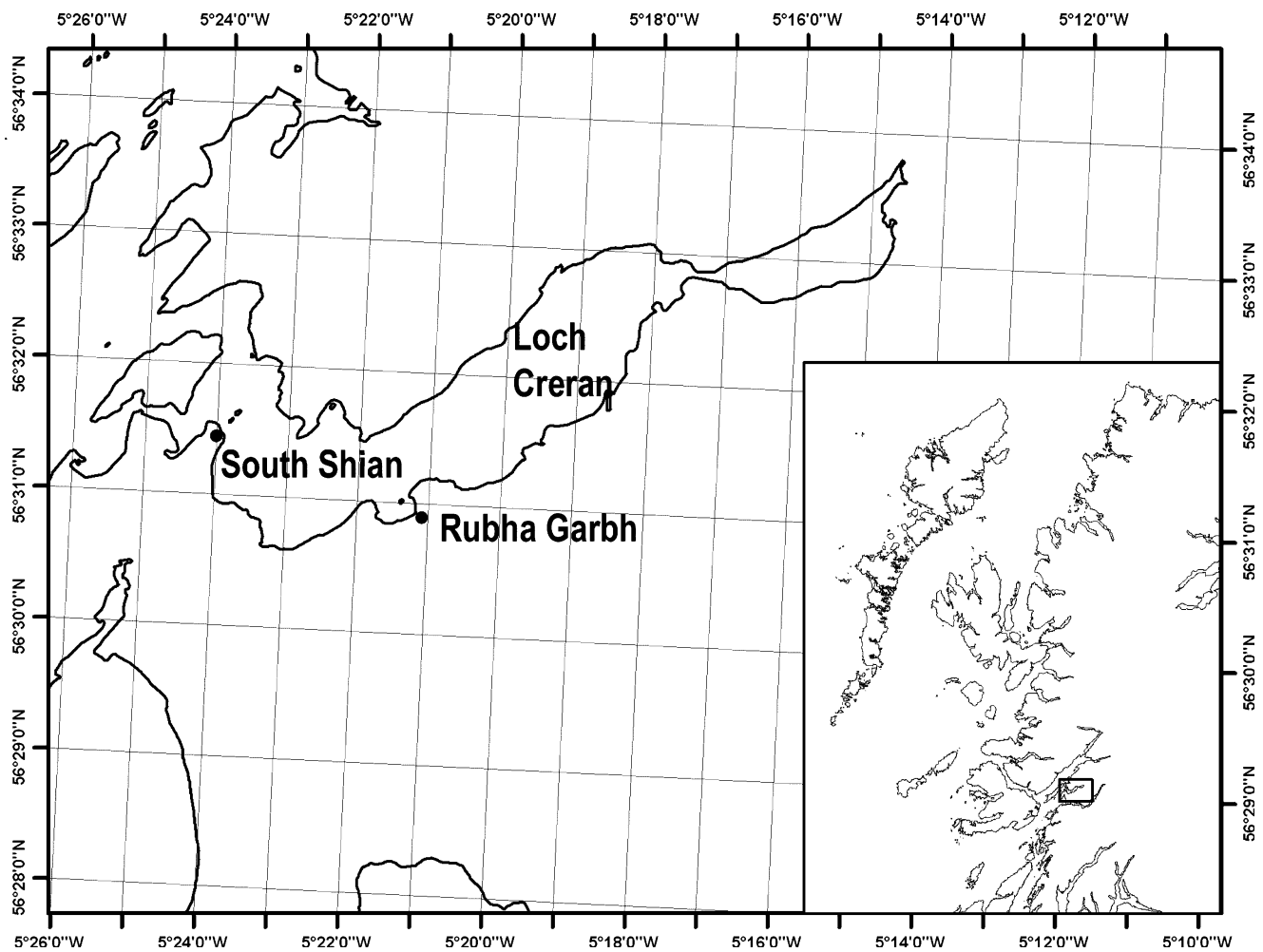


Fig. 1 The experimental location on the west coast of Scotland, showing the position of Loch Creran and the location of the two sampling sites

South Shian (SS), were selected. The substratum nature, rugosity and profile were similar at both locations, being boulders, cobbles and pebbles on mixed sand and mud. The most obvious macroscopic biota in the intertidal zone was furoid algae (*Fucus vesiculosus* and *F. serratus*) and in the shallow subtidal, *Laminaria saccharina*.

Field protocol

Five *P. miliaris* individuals of approximately equal test diameter were collected from each of two replicate sites, at each of two depths (intertidal and subtidal), and at each of two locations (Fig. 2). Those from the intertidal zone were collected from an area just above extreme low water springs (ELWS). By observation this level was determined to be exposed for approximately 2.5 h on three consecutive days on spring tides. *P. miliaris* individuals from the subtidal zone were collected (by snorkelling) from permanently submerged areas at depths of 2–3 m. The specimens from each replicate were collected from within a 1 m radius. The replicate sites were selected at each location to be approximately 10 m apart. The *P. miliaris* individuals were transferred to the laboratory and maintained in running sea water and without food until analysis (within 48 h). This was repeated each month, from July to September.

Laboratory analysis

Test diameter and wet mass for each individual *P. miliaris* was determined prior to the removal of the gonad. Test diameter was measured three times using adapted Vernier calipers, and the mean of the three measurements was used. The excised gonad was weighed, and a small sample was taken and used for microscopic determination of gender and maturity stage. The maturity stage was determined using a wet squash where a

small section of fresh tissue was placed between a slide and cover slip and examined under a microscope. The gonad was scored on a six point scale: stage 1 recovery, stage 2 early growth, stage 3 premature, stage 4 mature, stage 5 partially spawned and stage 6 spent (Kelly 2001). The GIs were calculated for each animal using the formula:

$$GI = \frac{\text{wet mass gonad}}{\text{wet mass of whole test}} \times 100$$

After dissection of the gonad, it was placed in chloroform:methanol (vol. 2:1), and stored under nitrogen until ready for lipid extraction (< 1 h).

Lipid extraction and fatty acid analysis

The gonad was homogenized in chloroform:methanol (2:1 10 cm³) and stored under nitrogen overnight at 4°C. Prior to storage, the fatty acid standard 23:0 (400 µl, 0.5 mg/g tissue) was added to each sample as an internal standard. The lipid was then extracted and transesterified to fatty acid methyl esters (FAMES). The FAMES were purified using thin layer chromatography (250 µm silica gel, Whitman®) and then stored in hexane under nitrogen at -16°C for no longer than a week prior to gas chromatography.

The purified FAMES were separated using a Perkin Elmer 8320 gas chromatograph equipped with split injector (100:1), flame ionization detector (FID) and a Zebtron ZB-WAX fused silica capillary column (30 m × 0.25 mm i.d., 0.25 µm film thickness). Helium was the carrier gas and the oven temperature was programmed to increase from 160–240°C at 4°C min⁻¹, and then held for 10 min. In order to store and integrate the chromatograms, the detector output was coupled to a data system (Varian Star™). The FAMES were identified by comparing their retention times with those of authentic standards with the exception of 16:0 dimethylacetal and 20:2 non-methylene interrupted dienes. These were identified using retention times from a previous study (Cook et al. 2000). All samples were run on the same column and under the same conditions. Individual fatty acids were identified and the relative content of each was determined using peak areas and expressed as the percentage by mass of the total fatty acids characterized. These values were then converted to relative mass per gram of wet mass of gonad tissue (mg g⁻¹) using the response of the internal standard.

Statistical analysis

Prior to investigation of the GI variability, the relationships between urchin test diameter and whole wet-mass, gonad wet-mass and GI were examined using Pearson's product-moment correlation. Prior to all parametric univariate analysis, data normality was tes-

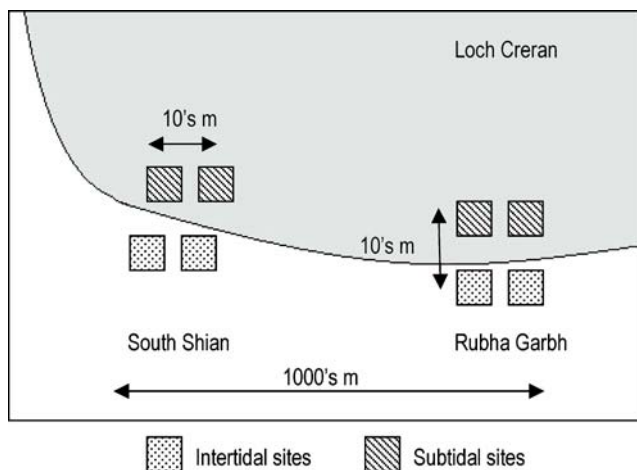


Fig. 2 Diagrammatic representation of the experimental layout used in this study showing the approximate distance between different sampling units

ted using the Kolmogorov–Smirnov test; any data found to significantly differ was log transformed to meet assumptions of normality. Data was also checked for homogeneity of variance using Cochran’s test. Pearson product moment correlation values (rho statistic) and associated probabilities demonstrated a significant relationship between *P. miliaris* test diameter and (a) whole wet mass ($\rho=0.834$, $p<0.001$) and (b) gonad wet mass ($\rho=0.416$, $p<0.001$) as expected. There was no significant correlation between test diameter and GI ($\rho=0.132$, $p=0.15$) thus establishing GI as a size-independent metric.

Differences in test diameter, and GI were tested using a four factor ANOVA with depth (two levels and fixed) being orthogonal to month (three levels and random) and location (two levels and random), and sites nested within location (two levels).

All multivariate analysis was carried out using the PRIMER v5 (Plymouth Routines In Multivariate Ecological Research) with the exception of the two-way SIMPER which used v6 β . Though this routine has no known issues connected with it, as it is a beta version of the software the results should be interrupted with a degree of caution (R. Gorley, personal communication). The data was left untransformed (Howell et al. 2003) and converted into similarity matrices using Euclidean distances as the metric. A single outlier from the third month which had very low quantities of all fatty acids, was removed from the analysis. If the procedure required a balanced data set, a dummy variable was created as the average of the other four fatty acid signatures from that replicate.

Permutation based analysis of similarity (ANOSIM) routines were used as the hypothesis testing framework. Differences in the fatty acid signatures with depth, gender, and maturity stage were explored using the similarity percentages routine (SIMPER), both one-way and crossed.

By matching the fatty acid signatures to the environmental/biological variables for each sample, it is possible to find which combination of the variables are best correlated (Spearman rank) with the fatty acid signatures (BIO-ENV). The five variables used were depth, site, month, gender, and maturity stage.

Results

From July to September, the gonadal indices of *P. miliaris* typically decreased across locations, and depths (Fig. 3). The largest magnitude of decline occurred in the intertidal at the SS site (which had the highest initial value), from a mean GI of 8.1% in July to 2.0% by September. The mean level of decrease between July and September across locations was 71% for both intertidal and subtidal. The maturity stage clearly progressed in both genders over the study months (Fig. 4). In July, the majority of male *P. miliaris* were at maturity stage 4 as opposed to 3 for females. This trend for males to be developmentally ahead continued for the remainder of the study, with a modal stage of 5 for males, and 4 for females during August, and 6 and 5, respectively, during September.

There was a significant difference between the GI for each maturity stage ($df=3,116$; $f=22.81$, $p<0.001$). Pair-wise comparison showed that stages 3 and 4 were similar and significantly different from stages 5 and 6. The highest mean GI was recorded for stage 4 with a mean of 5.95% and the lowest was stage 6 with a mean GI of 2.15% (Fig. 5).

Fatty acid analysis

The predominant fatty acid for all months and depths (Table 1) was 20:5($n-3$) being highest in the subtidal in

Fig. 3 The mean GI for each location and depth from July to September. Error bars show 95% confidence intervals *filled circle* RG Intertidal, *open circle* RG Subtidal, *filled triangle* SS Intertidal, *open triangle* SS Subtidal

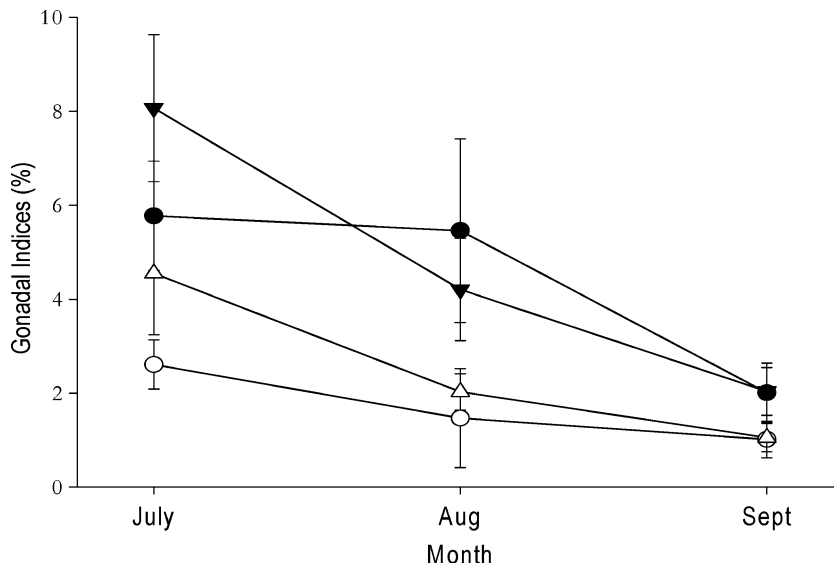
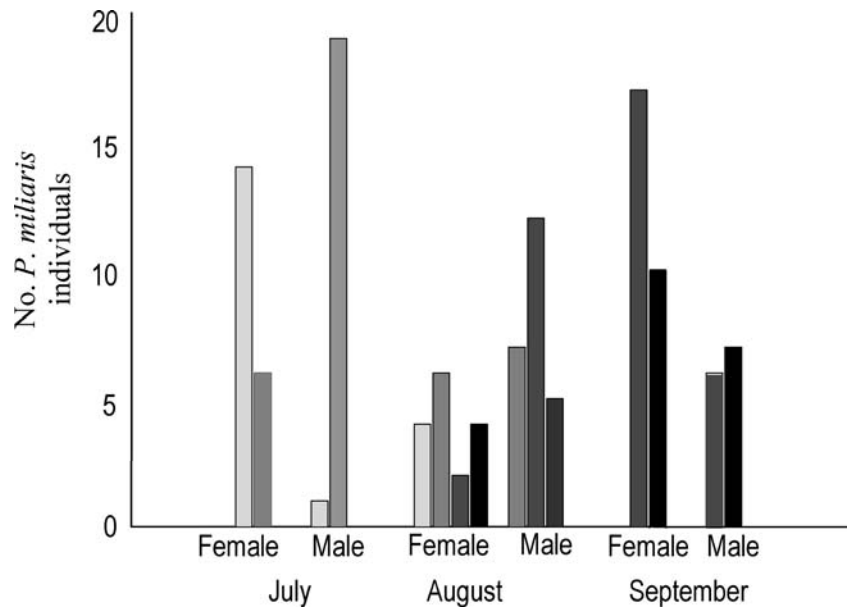


Fig. 4 The number of urchins recorded at each maturity stage and gender for each month. *Light shade*, Stage 3, *Medium shade*, Stage 4, *Dark shade*, Stage 5, *Darker shade*, Stage 6



July (17.88%), followed by 20:4($n=6$) and 16:0. Poly-unsaturated fatty acids [PUFAs: 18:2($n=6$), 18:2($n=3$), 18:3($n=6$), 18:3($n=3$), 18:4($n=3$), 20:2($n=6$), 20:4($n=6$), 20:3($n=3$), 20:4($n=3$), 20:5($n=3$), 22:2($n=6$), 22:5($n=3$), 22:6($n=3$)] formed the largest class ranging between 35.9 to 45.5%. The saturated fatty acids (SFAs: 14:0, 15:0, 16:0, 17:0, 18:0, 20:0, 21:0) were the second largest class ranging between 17.1 and 19.9%. Mono-unsaturated fatty acids [MUFAs: 16:1($n=7$), 18:1($n=9$), 18:1($n=7$), 20:1($n=9$), 20:1($n=7$), 22:1($n=9$)] were the smallest class. The total FAMES (mg/g tissue) varied little between habitats but decreased between July and August, and remained static after that. The relationship between the fatty acid signatures of each month shows a clear seg-

regation in the signature, confirmed by the ANOSIM results (Table 2). Post hoc pair-wise testing showed differences between all pairs of months.

In order to examine the role that the recorded change in maturity stage had on the fatty acid signature, a crossed ANOSIM was used to test for significance differences in the fatty acid signature of each gender at each maturity stage, averaged over the months (Table 3, 4). The test confirmed that there was a significant difference between the months when averaged across gender and maturity stages ($r=0.157$, $p=0.014$), and a significant difference between gender/maturity stage when averaged across months ($r=0.269$, $p<0.001$). Post hoc pair-wise testing was used to examine the relation between the

Fig. 5 The mean GI for each maturity stage (error bars = 95% confidence interval CI)

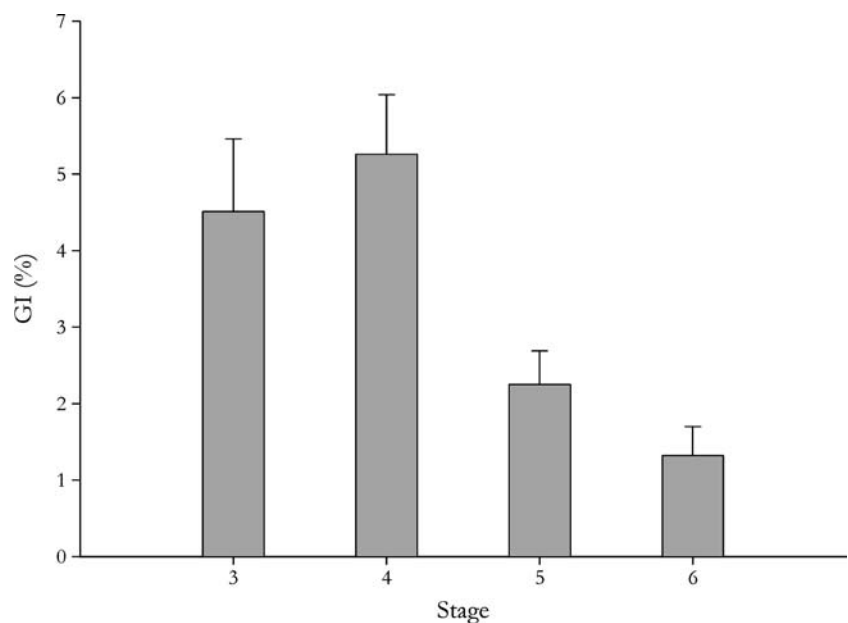


Table 1 The relative abundances (%) of the identified fatty acids from the sea urchin gonads, separated by month and depth

Fatty acid	July		August				September					
	Intertidal	Subtidal	Intertidal	Subtidal	Intertidal	Subtidal	Intertidal	Subtidal	Intertidal	Subtidal		
	Mean	95% CI	Mean	95% CI	Mean	95% CI	Mean	95% CI	Mean	95% CI	Mean	95% CI
14:0	3.32	0.55	3.60	0.57	2.10	0.41	3.04	0.46	3.02	0.28	3.52	0.83
a15:0	0.13	0.03	0.08	0.03	0.11	0.05	0.04	0.04	0.13	0.05	0.10	0.04
15:0	0.64	0.07	0.58	0.09	0.56	0.11	0.54	0.13	0.91	0.10	0.66	0.13
16:0	10.21	0.26	10.92	0.37	8.77	0.98	9.81	1.25	10.53	0.51	10.54	1.53
16:1(n-7)	2.37	0.52	2.81	0.74	1.19	0.29	1.64	0.45	1.73	0.25	2.17	0.45
16:2	0.58	0.16	0.32	0.14	0.49	0.17	0.11	0.09	0.82	0.62	0.19	0.09
17:0	0.37	0.08	0.27	0.23	0.51	0.10	0.16	0.07	0.47	0.26	0.15	0.06
16:0 DMA ^a	3.36	0.31	4.39	0.48	4.04	0.53	3.98	1.07	4.14	0.64	4.13	0.90
16:4	0.19	0.08	0.19	0.10	0.12	0.05	0.04	0.04	0.10	0.04	0.04	0.03
18:0	3.49	0.52	3.76	0.53	4.51	0.62	4.49	0.48	3.43	0.35	2.86	0.44
18:1(n-9)	1.50	0.20	1.57	0.33	0.91	0.14	1.95	0.36	3.41	1.01	5.29	2.26
18:1(n-7)	2.76	0.20	2.59	0.20	2.53	0.16	3.09	1.68	2.55	0.21	2.57	0.66
18:2(n-6)	1.19	0.15	1.39	0.17	0.82	0.13	1.23	0.19	1.61	0.26	1.75	0.66
18:2(n-3)	0.08	0.05	0.10	0.11	0.07	0.04	0.01	0.01	0.41	0.11	0.30	0.11
18:3(n-6)	0.52	0.06	0.42	0.13	0.60	0.09	0.39	0.12	0.56	0.07	0.63	0.30
18:3(n-3)	1.52	0.27	1.44	0.32	1.07	0.33	0.89	0.21	1.96	0.38	0.86	0.33
18:4(n-3)	2.92	0.87	2.81	0.87	1.23	0.49	1.40	0.47	2.57	0.56	1.65	0.58
20:0	0.49	0.04	0.55	0.09	0.51	0.04	0.60	0.16	0.45	0.11	0.56	0.17
20:2 NMID ^b	6.06	0.47	6.52	0.84	6.84	0.44	8.55	0.62	6.32	0.76	7.53	1.25
20:1(n-9)	1.89	0.16	2.60	0.66	1.53	0.13	2.68	0.43	1.91	0.24	2.19	0.41
20:1(n-7)	1.18	0.08	0.87	0.15	1.30	0.10	0.85	0.13	1.12	0.18	0.96	0.18
20:2(n-6)	2.42	0.24	2.71	0.30	2.67	0.33	2.56	0.27	2.43	0.21	1.65	0.24
21:0	0.25	0.03	0.16	0.07	0.21	0.06	0.14	0.06	0.27	0.07	0.20	0.08
20:4(n-6)	11.32	0.89	13.35	1.62	11.61	0.89	16.57	1.16	11.60	1.25	12.64	1.92
20:3(n-3)	1.99	0.20	1.78	0.19	2.04	0.37	1.33	0.15	2.01	0.49	0.82	0.20
20:4(n-3)	0.71	0.14	0.49	0.16	0.56	0.14	0.34	0.10	0.71	0.19	0.40	0.16
20:5(n-3)	15.70	1.18	17.88	1.16	16.57	1.25	16.16	1.18	13.26	0.94	12.28	1.95
22:1(n-11)	0.15	0.04	0.07	0.04	0.17	0.05	0.15	0.08	0.27	0.11	0.22	0.08
22:1(n-9)	3.68	0.30	3.80	0.25	3.07	0.38	4.03	0.41	3.25	0.24	3.66	0.65
22:2(n-6)	0.09	0.02	0.12	0.12	0.14	0.03	0.01	0.02	0.51	0.32	0.46	0.36
22:5(n-3)	1.27	0.22	0.85	0.18	1.61	0.21	0.70	0.22	1.11	0.18	0.50	0.17
24:1	0.00	0.00	0.00	0.00	0.02	0.02	0.01	0.01	0.01	0.01	0.07	0.12
22:6(n-3)	4.84	1.44	2.15	0.33	6.55	1.30	2.53	0.52	4.10	1.15	1.94	0.53
Σ MUFA	13.52	0.98	14.31	1.14	16.97	0.55	13.76	2.40	14.25	1.26	17.05	1.97
Σ PUFA	44.58	1.87	45.49	1.97	45.20	2.45	41.77	4.59	42.86	1.54	35.88	3.23
Σ SFA	18.18	0.43	19.85	0.74	17.17	1.02	17.87	2.29	19.07	0.66	18.48	1.85
Total FAMES (mg/g)	16.59	3.48	15.17	3.46	8.37	2.05	7.73	2.88	7.48	1.47	8.90	1.89

^a dimethylacetal^b non-methylene interrupted dienes

genders at each maturity stage, when averaged over the months. Only at maturity stage 4, was there a significant difference in the fatty acid signatures between the genders. A two-way SIMPER was used to see which specific fatty acids were contributing to this difference. It was shown that 20:5(n-3), 20:4(n-6), and 22:6(n-3) were all found in higher proportions in the males, and contributed a total of 59% of the difference between them.

The different fatty acid classes varied as much as 5% in their mean levels for each maturity stage and gender (Fig. 6). The total of the PUFA decreased over the maturity stages for both sexes, whilst the SFA showed only a reduction for the males. The levels of 20:5(n-3) reduced with increasing maturity stage in both male and female (Fig. 7). A rapid reduction in this fatty acid occurred in females between stages 4 and 5, and in males between stages 5 and 6. In the fatty acid 20:4(n-6) there was a dramatic reduction between stage 3 and 4 in

females followed by a slight increase, but only slight changes in males. An inverse pattern to that of 20:4(n-6) is seen for 22:6(n-3), with an increase for females between stages 3 and 4 followed by a reduction between stages 4 and 6. For the males, there was an increase from stages 4 to 5, and a reduction from stages 5 to 6.

Table 2 One way ANOSIM with post hoc pair wise tests for differences between fatty acid signature for each month

Month	<i>r</i>	<i>P</i>
All months	0.172	<i>P</i> < 0.001
1 and 2	0.111	<i>P</i> < 0.001
1 and 3	0.184	<i>P</i> < 0.001
2 and 3	0.228	<i>P</i> < 0.001

Table 3 One way ANOSIM of differences between the genders for each maturity stage based on the fatty acid signatures

Stage	R	P
3	0.168	23.3
4	0.398	< 0.00
5	0.167	9.00
6	0.055	22.0

Table 4 Two way SIMPER analysis of the fatty acids contributing to the difference between the fatty acid signatures of the genders at maturity stage 4 when averaged over the months

Variable	Mean		Contrib%	Cum.%
	Female	Male		
20:5(n-3)	14.8	18.6	22.65	22.65
20:4(n-6)	10.3	14.3	22.24	44.89
22:6(n-3)	3.66	4.94	14.21	59.10
18:4(n-3)	3.48	1.13	8.19	67.30
16:1(n-7)	2.98	1.41	5.14	72.44
18:0	2.84	4.91	4.71	77.15
14:0	3.91	2.24	4.14	81.29
16:0 DMA	3.65	4.07	3.58	84.87
20:2 NMID	6.4	6.86	2.97	87.83
20:1(n-9)	1.54	2.49	2.35	90.19

Of the five variables measured in *P. miliaris* individuals (depth, gender, maturity stage, month and location), depth and gender had the highest correlation ($\rho = 0.305$) with the fatty acid signatures.

Discussion

The findings of the current study supported the hypothesis that changes in the GI of the study species, *P. miliaris*, over the spawning period were consistent between intertidal and subtidal populations. Although clear and significant differences were found between individuals at these two depths, both populations showed similar reductions over the study months. Comparable differences between the nutritional state of urchins from different habitats have been recorded for other echinoid species elsewhere. For example, *Anthocidaris crassispina* from sargassum beds had a higher GI when compared to those from areas dominated by coralline algae (Yatsuya and Nakahara 2004), and *Centrostephanus rodgersii* from barren areas had a lower GI compared to those from algal dominated areas (Byrne et al. 1998). Both studies found that gonad development was synchronous between the habitats, as described in the current study. The reduction in GI was coupled with a progressive increase in maturity stage of *P. miliaris* individuals from the study populations. A similar pattern was suggested for this species by Kelly (2000), who noted a dramatic reduction in GI during the month of August, when all of the females had reached maturity stage 5. Although GI has been questioned as a reliable

predictor of maturity stage (Lozano et al. 1995), the study population showed a significant difference in GI between pre-spawned (stages 3 and 4), and post-spawned (stages 5 and 6). This difference is very likely to be due to the loss of reproductive material as a result of spawning.

Fatty acid analysis

Studies to date have suggested that echinoids have similar fatty acid compositions, from which this study found *P. miliaris* differs little. Using all soft body parts of *Paracentrotus lividus*, Serrazanetti et al. (1995) found the dominant fatty acids to be 14:0, 16:0 and 20:5(n-3). Similarly Floreto et al. (1996), using all the soft tissue of *Tripneustes gratilla*, found PUFA to be the dominant class, and 16:0, 20:4(n-6) 20:5(n-3) to be the primary fatty acids. The testis of *Echinus esculentus* collected from southern England again showed a similar pattern but showed much higher levels of 22:6(n-3) than those of *P. miliaris* in our study (Allen 1968). The study by Cook et al. (2000) of the fatty acid composition of west coast Scottish *P. miliaris* found similar overall patterns with those observed in this study except that their wild populations had lower values of total PUFA. This is probably due to habitat differences between the sample populations of the two studies.

Temporal variation

Crucially, all temporal variation observed in the fatty acid signature of wild populations of urchins can be confounded by the dual role of the urchin gonad. Because the organ is both the reproductive and nutritive store, any observed differences in the fatty acid signature over time may be a result of both changes in diet and changes in the reproductive maturity of the animal. The current study showed clear differences over the experiment duration in the fatty acid signature and this was consistent over depth and location. An unknown proportion of the difference can be attributed to diet variation, as when averaged over the maturity stage there were still significant differences between the months.

Differences in fatty acid profile between testes and ovaries have been previously discussed for this population of urchins (Hughes et al. 2005). However, it appears that these gender specific differences are transient and may be the result of the annual gametogenic cycle. Significant differences existed between genders prior to spawning but not for the post spawning stages. Thus the difference in the fatty acid signature between male and female gonads at stage 4 would seem likely to be the result of the presence of high levels of gametes, and that these gametes have different fatty acid compositions. There were distinct changes in the fatty acid composition of gonads with increased maturity stage. PUFAs have been associated with reproductive capac-

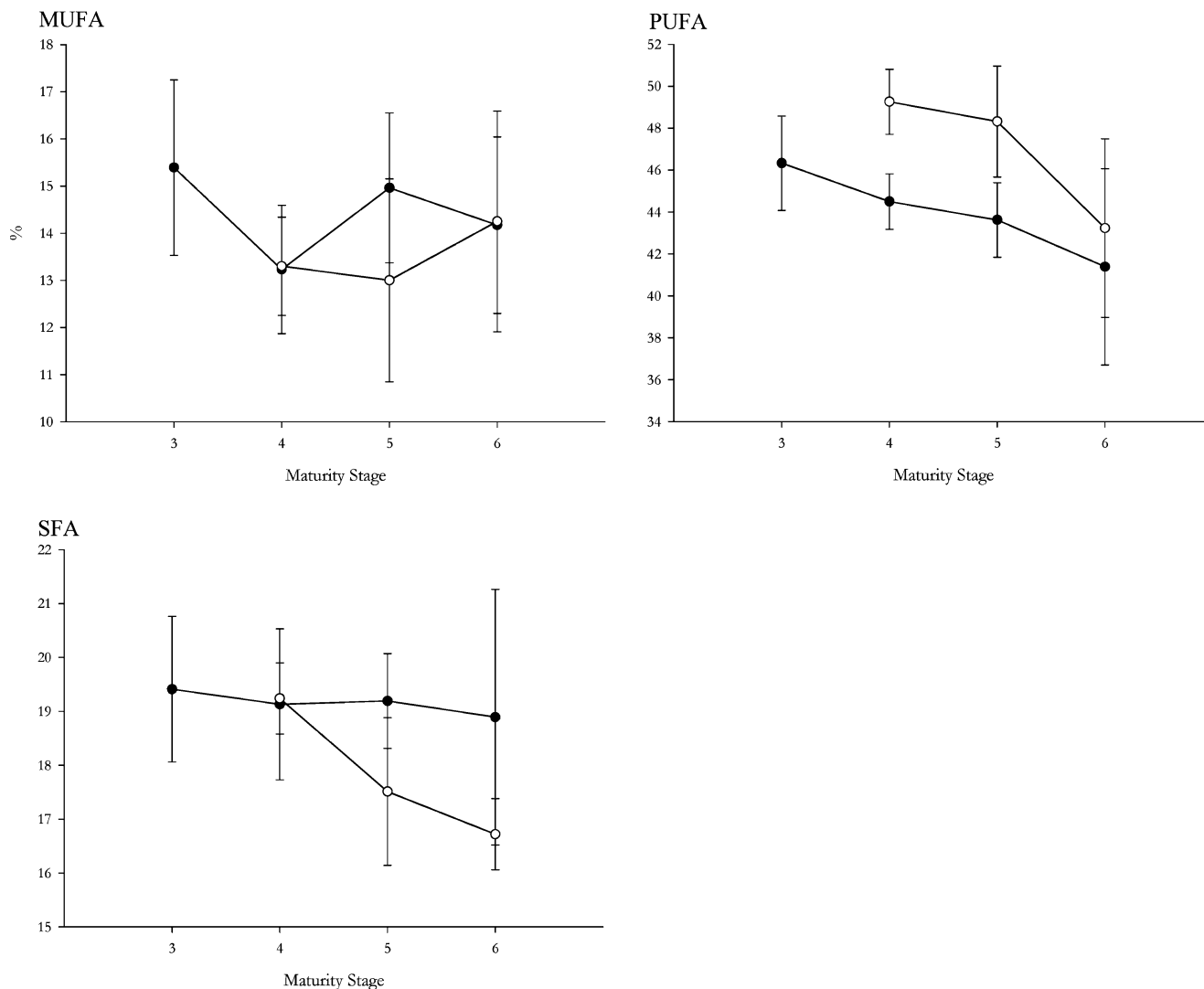


Fig. 6 The variation in classes of fatty acid between genders at each different maturity stage

ity in shrimps (Rosa and Nunes 2003) and more specifically with structural elements in the phospholipid membranes that have been shown to be prominent components in spermatozoa of marine invertebrates (Cook and Gabbott 1972). PUFAs were found at higher levels in the gonad of male limpets compared to the females (Morais et al. 2003) as also reported in the current study. We suggest that this was as a result of a reduction in male gametes in the gonad during spawning. In contrast MUFAs have been linked to energy reserves necessary for vitalogenesis, and significantly higher concentrations have been reported for female gonads (Brazao et al. 2003). The current study found no clear pattern in *P. miliaris*—similar levels occurred in both males and females and there was no noticeable reduction with maturity stage. It is possible that the multifunctional role of the sea urchin gonad may lead to a more complicated interruption of the variation in the fatty acid signature of the gonad. The fatty acids 20:5($n-3$) and 22:6($n-3$) have been linked

to energy storage in teleost eggs (Fraser et al. 1988), and as an energy source during embryogenesis (Xu et al. 1994). It has also been hypothesised that 22:6($n-3$) plays an important role in the membrane of eggs (Pazos et al. 1997). There was a rapid reduction in these fatty acids from stage 4 to 5 in the female gonads of *P. miliaris*. This reduction may be as a result of the release of oocytes rich in 20:5($n-3$) and 22:6($n-3$).

Further studies are required to differentiate between the role of diet and sexual maturation in influencing the biochemical composition of the echinoid gonad. Ojea et al (2004) concluded that changes in the fatty acid composition of the clam *Ruditapes decussatus* appeared to be more closely related to diet than to endogenous factors. The current study showed that the variables of depth and gender were best correlated with that of the fatty acid signature. Variation in the fatty acid signature as related to depth has been linked to differences in diet between the intertidal and subtidal populations with intertidal populations having a diet richer in inverte-

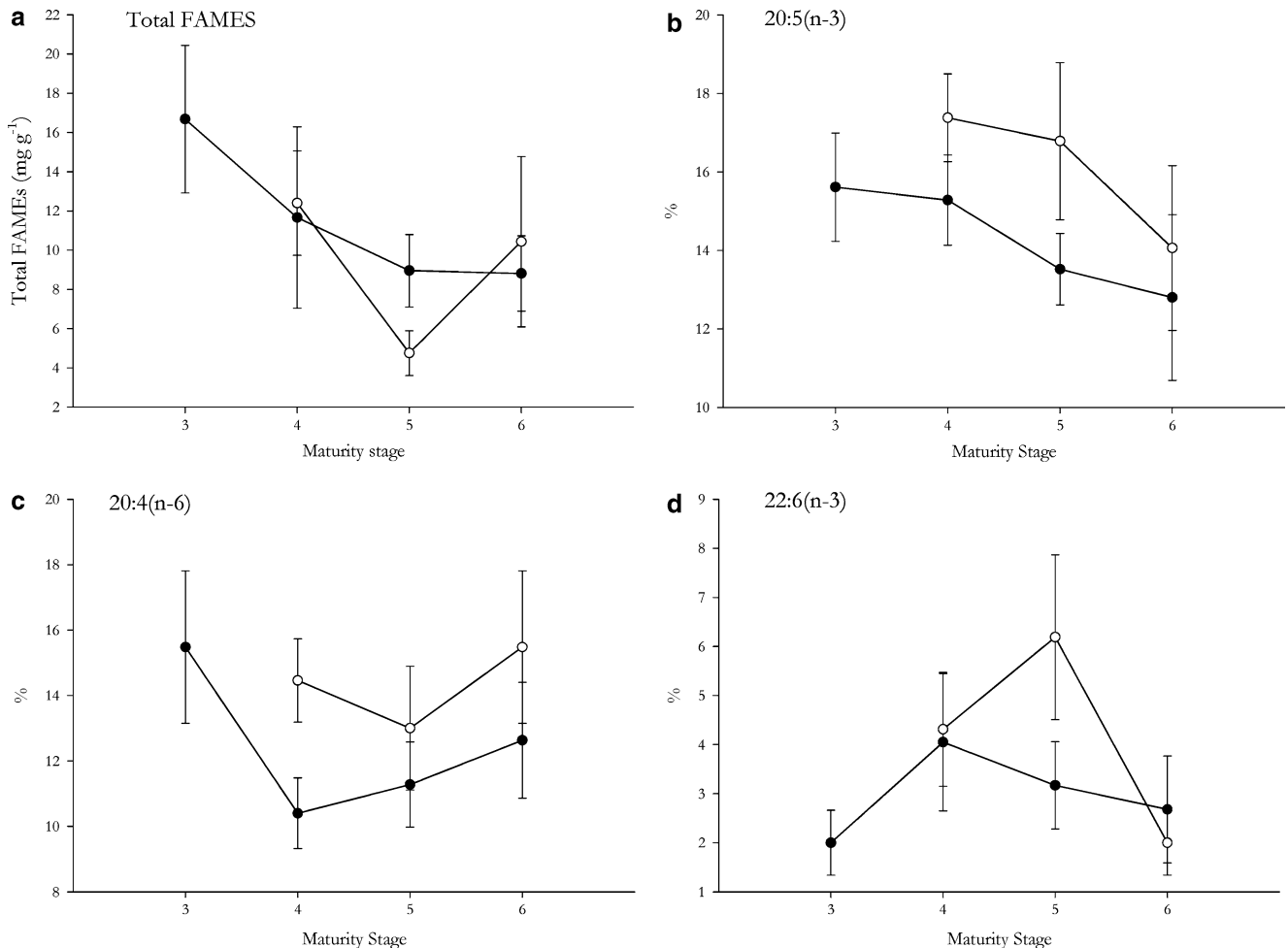


Fig. 7 The mean percentage (error bars = 95% CI) of the **a** total FAMES and the fatty acids **b** 20:5(*n*-3), **c** 20:4(*n*-6) and **d** 22:6(*n*-3)

brates and ephemeral green algae (Hughes et al. 2005). As such, depth differences in fatty acid signatures can be attributed to nutritional sources, while gender differences are likely to be due to the presence of gametes. This suggests that the duality of function of the gonad is strongly reflected in its fatty acid signature; habitat related diet differences combined with gender differences give the best correlation with the fatty acid signature.

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