J. Mar. Biol. Ass. U.K. (2000), **80**, 909–919
Printed in the United Kingdom

The reproductive cycle of the sea urchin *Psammechinus miliaris* (Echinodermata: Echinoidea) in a Scottish sea loch

Maeve S. Kelly

Scottish Association for Marine Science, PO Box 3, Oban, Argyll, PA34 4AD. E-mail mke@dml.ac.uk

The sea urchin *Psammechinus miliaris* was sampled at monthly intervals from two replicate sites at contrasting locations (littoral and subtidal habitats) on the west coast of Scotland. Samples were collected from November 1995 to October 1997. A study of gonad development showed a clearly defined annual cycle of gametogenesis with a single spawning period. Gonad indices (GIs) varied between locations, between sites at the same location and between study years. Gonad indices were maximal prior to the onset of the spawning period in June and July. Ripe gametes were shed by dissected urchins from June to September in 1996 and June to August in 1997 coinciding with the time of year when the gonad colour was best in terms of what is desired in the market place. Over winter the GIs were very low and gonads became very dark in colour. Higher summer GIs in the urchins from one littoral site suggested that the urchins there had a seasonal influx of a more nutritious or more abundant food resource. Histological determination of the reproductive state showed that for these food-limited populations high GIs do equate with the spawning period. The gametogenic cycle fits the six stage pattern described for other echinoid species. In females oocytes of various developmental stages were present throughout the annual cycle. Recruitment to the littoral location was monitored over two seasons by quantifying the number of urchins with a test diameter <5 mm appearing in the population. Recruitment appeared variable between sites and between seasons suggesting that the collection of juveniles resulting from natural settlement may not be a reliable alternative to hatchery produced spat. Although *P. miliaris* has commercial potential when in cultivation, this study provided no evidence of an opportunity for a commercial fishery.

INTRODUCTION

The echinoid *Psammechinus miliaris* (Gmelin) is found all around the British Isles, as far north as Scandinavia and south to Morocco, but is absent from the Mediterranean (Mortensen, 1927). In Scotland it occurs in dense but discrete populations, typically found in sheltered areas of sea lochs on the west coast (Davies, 1989; Holt, 1991). Its distribution frequently coincides with that of the brown macroalgae *Laminaria saccharina*. Its depth range extends to the littoral zone where populations can be found exposed on boulder shores at low water (Picton, 1993). *Psammechinus miliaris* will graze on living kelp plants as well as detached kelp (Bedford & Moore, 1985) but it also feeds on a wide selection of algae and encrusting organisms (Lawrence, 1975). As a consequence its distribution is thought to be limited more by physical parameters than food availability (Faller-Fritsch & Emson, 1972).

Psammechinus miliaris is one of three species of edible urchin native to the west coast of Scotland. *Paracentrotus lividus*, a species commonly sold for human consumption in Europe, is scarce and at the northernmost limits of its range (Picton, 1993). The larger and more abundant *Echinus esculentus* is generally considered to have poor quality roe unsuitable for European markets. While *Psammechinus miliaris* has never been fished for export from Scottish waters, it has been exported from Ireland and marketed in Paris (Read, 1994). It is reported to have a higher gonad index (GI) than *Paracentrotus lividus* and some authors (Le Gall, 1989) believe its gonad tastes superior to that of *P. lividus* and it can therefore command a higher price. The depletion of French and Irish urchin stocks due to unsustainable fishing practices has instigated considerable research effort into echinoculture, both countries having largely concentrated their research efforts on *P. lividus*. However, it is *Psammechinus miliaris* which is considered to have the greatest potential as an aquaculture species in Scotland (Kelly et al., 1998a,b).

The reproductive cycle of other commercial or potentially commercial species of echinoids have been described in detail (Gonor, 1973; Meidel & Scheibling, 1998). Some studies have included a recommendation on the feasibility or management of a fishery (Byrne, 1990; Laedsgaard et al., 1991; Guillou & Michel, 1993; King et al., 1994). Previously authors have commented on the local spawning period for *P. miliaris* (Jensen, 1969; Comely, 1979; Bedford & Moore, 1985), but there is no complete description of the reproductive cycle for this species. This investigation of the reproductive cycle in *P. miliaris* from two typical but contrasting habitats was conducted as part of a study on the commercial potential of this species. In addition an assessment of annual recruitment was made to assess the feasibility of using natural spat collection in place of hatchery production.

MATERIALS AND METHODS

Psammechinus miliaris were collected at approximately monthly intervals from November 1995 to October 1997 from Loch Creran, on the west coast of Scotland. Loch Creran is 13 km long, with a maximum depth of 49 m and a mean depth at low water of 13.4 m. The intertidal area represents some 12% of the total surface area of the loch basin which has a flushing time of 3 d (Edwards & Sharples, 1986). Urchins were collected from two typical but contrasting habitats. Urchins from a littoral location in the lower loch basin (56°32′N 05°21′W) were collected when exposed at low water spring tides. This location is characterized by pebbles overlying finer sediments with occasional small boulders (<50 cm diameter exposed) and mostly covered by the macroalgae *Fucus vesiculosis, Fucus serratus,* and *Ascophyllum nodosum* with a narrow zone of *Laminaria saccharina*. In the summer months there was a dense covering of *Enteromorphora* and *Pilayella* spp. on the boulders on the lower intertidal zone. Ten urchins were collected from each of two sites (LA and LB), 200 m apart, at this location. A gradual change in cover was observed horizontally along the shore as boulder size reduced from site LA to LB. A diverse community of encrusting invertebrates including barnacles and sponges, the mussels *Mytilus edulis* and *Modiolus modiolus* were also found in greater abundance at site LA than at LB. Urchins were collected from between rocks, among the fucoids and laminarians, and beneath pebbles on the beach.

At the second location in the upper loch basin (56°33′N 05°17′W) urchins from a subtidal population were collected from a depth of 3 m below low water spring tides by snorkelling or by SCUBA. At this sheltered location there were boulders with a covering of *L. saccharina* from 1 to 4 m below low water spring tide. *Psammechinus miliaris* were collected from silted rock surfaces beneath the *L. saccharina* and from the *Laminaria* fronds. There was no evidence of encrusting invertebrate communities as found at the littoral site. A brackish water layer was visible at the surface on some collection days. Ten urchins were collected from each of two sites (SA and SB), 150 m apart, at this location.

At both locations urchins in the size range 20–30 mm were collected to minimize the influence of size on the GI (Gonor, 1972). There was no significant correlation between test diameter and GI within this size range for urchins collected when GI were high (June 1996 Pearson correlation, $P=0.352$) or when GI were low (October 1996; Pearson correlation, $P=0.652$) suggesting GI in *P. miliaris* is asymptotic in this size range. The urchins were collected from two sites (replicates) at each location to permit legitimate interpretation of whether any variability between the populations could be ascribed to the differences in habitat at these locations. The water temperature was measured on each sampling day and the number of hours of daylight calculated from Meteorological Office data.

After collection urchins were maintained in seawater aquaria until they were dissected (within 24 h). The test diameter of each individual was measured using calipers adapted by the addition of stainless steel pins that fitted between the urchins' spines. The diameter of each individual was measured three times and the mean calculated. The weights of gonad, alimentary canal (washed and empty) and the eviscerated test were recorded. The GI was calculated as the wet weight of the gonad divided by the weight wet of the eviscerated test expressed as a percentage. An alimentary index (AI) was calculated as an additional indicator of sequestered nutrient stores (Klinger et al., 1988; Kelly et al., 1998b). The AI was calculated as for GI but based on dry weights to improve accuracy of weighing after the gut had been washed to remove the contents.

The statistical significance of differences in GI and AI values on any given sample date was determined using nested analysis of variance (ANOVA) (Sokal & Rohlf, 1995) and Tukey's test. To ensure compliance with the assumptions of ANOVA, percentage data were arcsine transformed and tested for normality (Anderson–Darling test) and homogeneity of variance (*F*-test). All analysis was performed using the software package Minitab 12.

Gonad colour was recorded upon dissection using a Sea Urchin Colour Card (University of Maine and Maine Department of Marine Resources Fisheries Technology service). Colours were then scored as acceptable (orange) or unacceptable (dull shades, grey or brown) to the consumer market. Extrusion of gametes upon dissection, either directly from the gonad or the gonopores, was recorded. The mean diameter of 100 shed eggs from each of four females from each of the four sites was determined using a microscope and eye piece graticule.

From January 1996 to January 1997 one of the gonads from each of six urchins from each location was preserved in Bouin's fluid for histological examination of the reproductive state. A central portion of the preserved gonads were dehydrated in alcohol, embedded in paraffin wax and sectioned at 7 μm. The sections were stained with haematoxylin and eosin (H/E). The reproductive state of the urchins was then assigned a stage from I to VI (stage I, recovery; stage II, early growth; stage III, premature; stage IV, mature; stage V, partially spawned; and stage VI, spent) (Fuji, 1960; Byrne, 1990).

Recruitment to the littoral site was quantified in 1996 and 1997 by conducting population surveys at intervals before the spawning period in June of each year and then in the winter months, December and January, when it was possible to collect new recruits. A 0.25×0.25 m quadrat was positioned randomly at approximately 4 m intervals following a transect parallel to the waters edge at low water spring tides. A second set of quadrats was placed along a transect 4 m higher up the shore than the first. All urchins in each of the eight quadrats at each site were then counted, the test diameter measured and returned to the shore. The urchins were often well hidden; larger rocks were overturned and the underside examined, boulders were not moved. Cook et al. (1998) found hatchery reared juvenile *P. miliaris* in a tank-based trial and fed an algal diet reached a test diameter of 9.22 mm (±2.06 SE) in the six months post settlement. A degree of caution was used in ascribing tank-based growth rates to field populations and in the current study urchins of <5 mm were judged to be recently settled juveniles and to represent that season's recruits. No attempt to assess recruitment was made at the subtidal site where fine silt covering the surfaces of the boulders presented an obstacle to the accurate quantification of small urchins.

RESULTS

External appearance

The general appearance of the urchins collected from the two contrasting locations differed. The urchins from the littoral population were typically dark in colour, a deep purplish-brown and showed no variation between the colour of the test and the spines. The spines were often short, blunted or broken. In some individuals areas of the test were denuded of appendages; this was attributed to mechanical abrasion they received living in the intertidal zone. The urchins from the subtidal population were a much paler colour, with a light green test, and spines that were long and sharp with vivid purple tips. The mean test diameters of the urchins collected from each of the four sites were not significantly different, the mean size (±SD) collected at the four sites was LA 26.9 ±1.88 mm, LB 24.91 ±2.12 mm, SA 24.12 ±3.22 mm, SB 24.42 ±3.11 mm (N=190 for each population).

Gonad indices

In 1996 and 1997 urchins from both the littoral and subtidal locations showed an annual spawning cycle (Figure 1). The GI began to increase from its average over-winter value (<5%) in May and peaked in late June of each season studied (mean peak GI=LA, 28.3 ±4.09 SD, N=10). The GI did not return to its over-winter low value until October of each year but had dramatically declined by August. There was a variation in GI both between the littoral and subtidal locations, between sites at the same location and between years, however there was a synchronicity in attaining peak GI (June and July) over all the sites examined. Figure 1 illustrates replicate sites at each location rather than pooled replicates because of the intrasite variation. In both early and late June and August of 1996 and in June 1997 urchins from site LA had significantly higher GI than those from the other three sites (5 June 1996, $P<0.003$, $F=6.89$, df 2,36, Tukey's test LA $P<0.01$; 29 June 1996, $P<0.001$, $F=8.56$, df 2,36, Tukey's test LA $P<0.01$; 28 August 1996, $P<0.01$, $F=15.13$, df 2,36, Tukey's test LA $P<0.001$; 24 June 1997, $P<0.002$, $F=7.16$, df 2,36, Tukey's test LA $P<0.05$). In July 1996 there were again significant differences in GI between urchins from different sites at the same location; urchins from sites LA and SA had significantly higher GI than those from sites LB and SB ($P<0.003$, $F=6.76$, df 2,36, Tukey's test LA, SA $P<0.05$). There was however no significant effect of location (e.g. 29 June, July 1996 littoral vs subtidal $P>0.05$). In June 1997 the differences in GI of the urchins from the subtidal sites were not significant. The GI were minimal throughout the winter months, the minimum mean recorded was for site SB in February, 1996 (GI=1.88 ±0.78 SD, $P=10$).

In both the littoral and subtidal locations the GI were maximal 1–2 months before the highest water temperatures were recorded. Gonad indices began to increase dramatically once day length had increased to approximately 16 h d^{-1}. The spawning period coincided with the longest days.

Gonad colour

There was considerable variation in gonad colour. From October to May, when the GI was low, the most common gonad colours were dull grey, tan or a very dark brown. From May to October the colour range included more dull orange, pale grey (usually males) and bright orange colours. The highest percentage of acceptable gonad colours occurred from June to September in 1996 and from May to July in 1997 (Figure 2).

Gamete availability

The extrusion of gametes from the gonopores or from the gonad directly upon dissection occurred from June to September in 1996 and from June to August in 1997

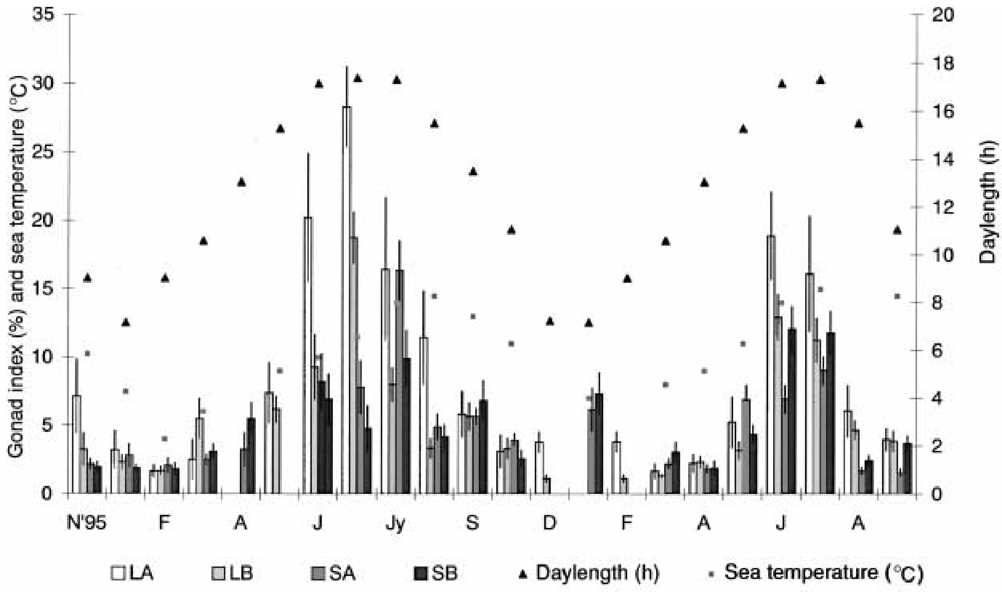

Figure 1. Gonad indices (GI) (N=10) for four populations of *Psammechinus miliaris*. Seawater temperature (°C) and daylength (h) are illustrated. Error bars represent 95% confidence limits.

(there was no sampling in September 1997) (Figure 2). Higher percentages of urchins extruded gametes on dissection in 1997 (100% of urchins examined in June and 97.5% of those examined in July) than in 1996 (72.5% and 62.5% for June and July respectively). Males extruded gametes earlier in the season than females and continued extrude to gametes after the females had ceased. There was no significant difference in the size of the eggs shed by urchins from the littoral or subtidal locations. Mean ±SD egg diameter was 96.3 ±10.69 μm.

Alimentary indices

There was not a clearly defined annual cycle of AIs. As there were differences in the AI of urchins from different sites at the same location data for each site rather than pooled replicates are illustrated (Figure 3). The AI for urchins from site LA were significantly higher than those of LB and SA (but not SB) on 5 June 1996 ($P<0.027$, $F=4.01$, df 2,36, Tukey's test LA $P<0.05$). In July 1996 the pattern of AI matched that of the GI, AIs for sites LA and SA were higher than those of urchins from sites LB and SB ($P<0.001$, $F=19.85$, df 2,36, Tukey's test LA, SA $P<0.001$). There appeared to be a general reduction in AI from June to August which coincided with the spawning period.

Histology

The pattern of gametogenesis in *Psammechinus milaris* can be described in terms of the six stages described for other species (Byrne, 1990). Differential staining with H/E showed the distribution of gametic tissue, which was basophilic and stained dark purple, compared to the nutritive phagocytes which were strongly eosinophilic and stained pink. The gametogenic cycle was synchronous across the two habitat types studied, although individuals from more than one stage of development could be found at any one site on any given sample day. The stage which described 60% or more of the urchins examined on any given sample date is presented (Figure 2). There were no obvious differences in the pattern of gamete development between the locations studied, although the detection of subtle differences between locations may have required a larger sample size. Sex determinations were always possible as in females oocytes of various developmental states were present all year.

Ovaries

From January to March the females were in the recovery phase (stage I). Primary oocytes were present along the ascinal walls (10–20 μm diameter) and the nutritive phagocytes formed an open meshwork across the ascinus (Figure 4A). The early growth phase (stage II) commenced in late April (Figure 4B). The primary oocytes remained around the outer wall of the ascini but increased in size (20–80 μm diameter) as vitellogenesis commenced. At this stage the nutritive phagocytes became more dense and stained more intensely pink. Females (two of the six examined) representative of stage I and VI respectively were also recorded in April 1996. The majority of females examined had premature ovaries

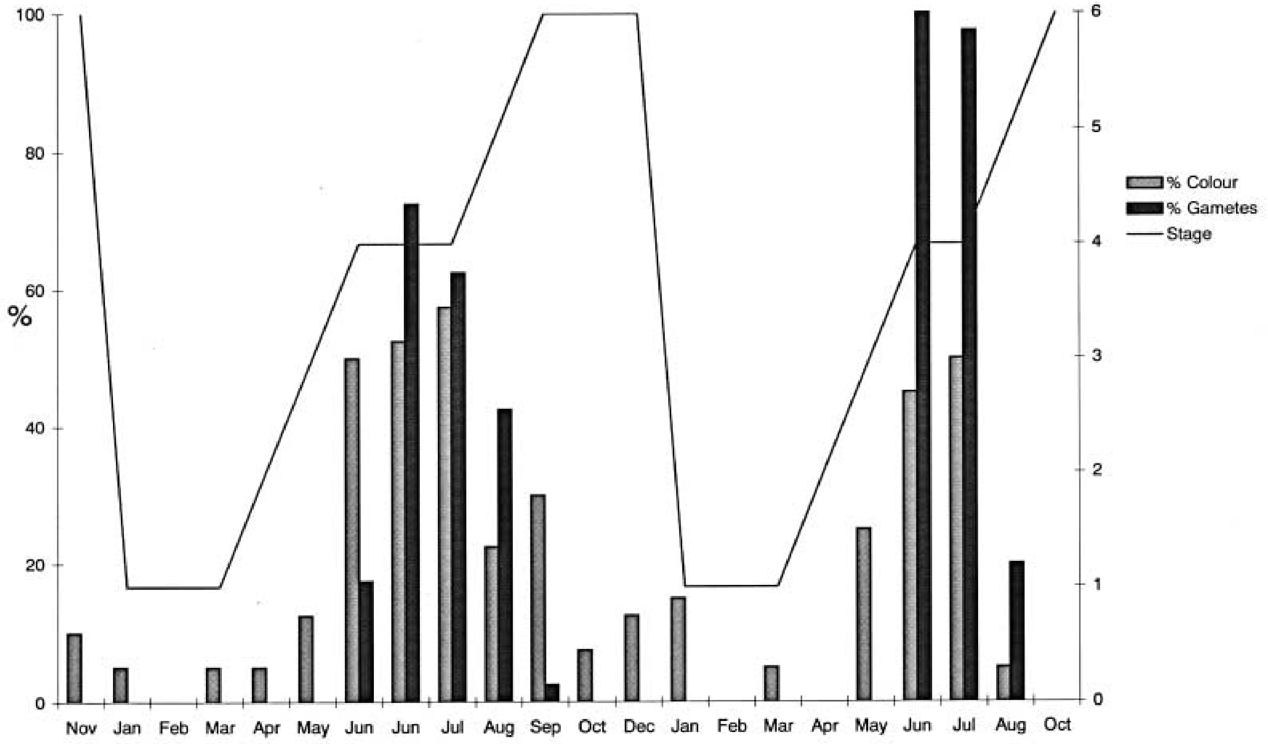

Figure 2. Seasonality of the reproductive status in *Psammechinus miliaris;* reproductive stage (I–VI) (N=12 for each data point), the percentage of urchins with gonads of a marketable colour and the percentage of urchins extruding gametes on dissection (N=40 for each data point) are illustrated.

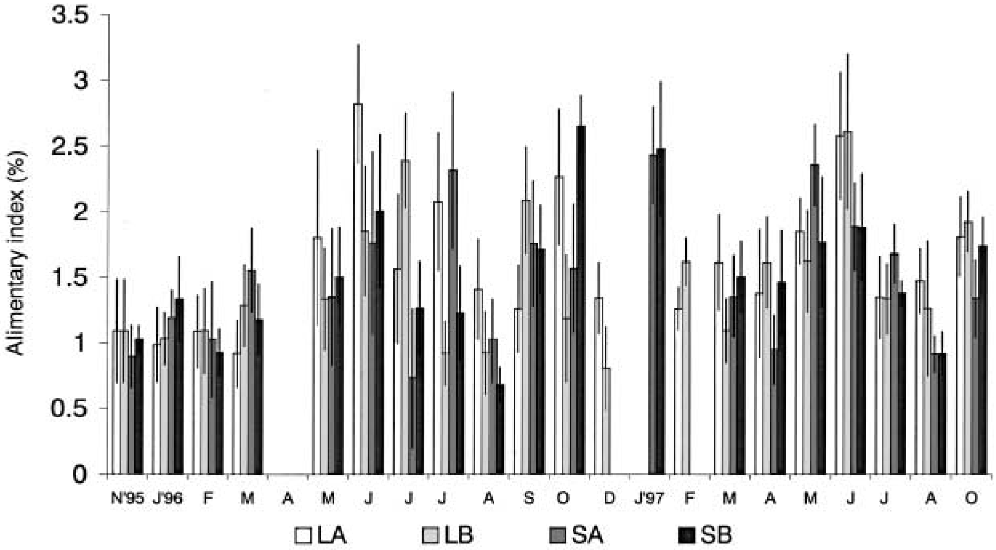

Figure 3. Alimentary indices (AI) (N=10) for four populations of *Psammechinus miliaris*. Error bars represent 95% confidence limits.

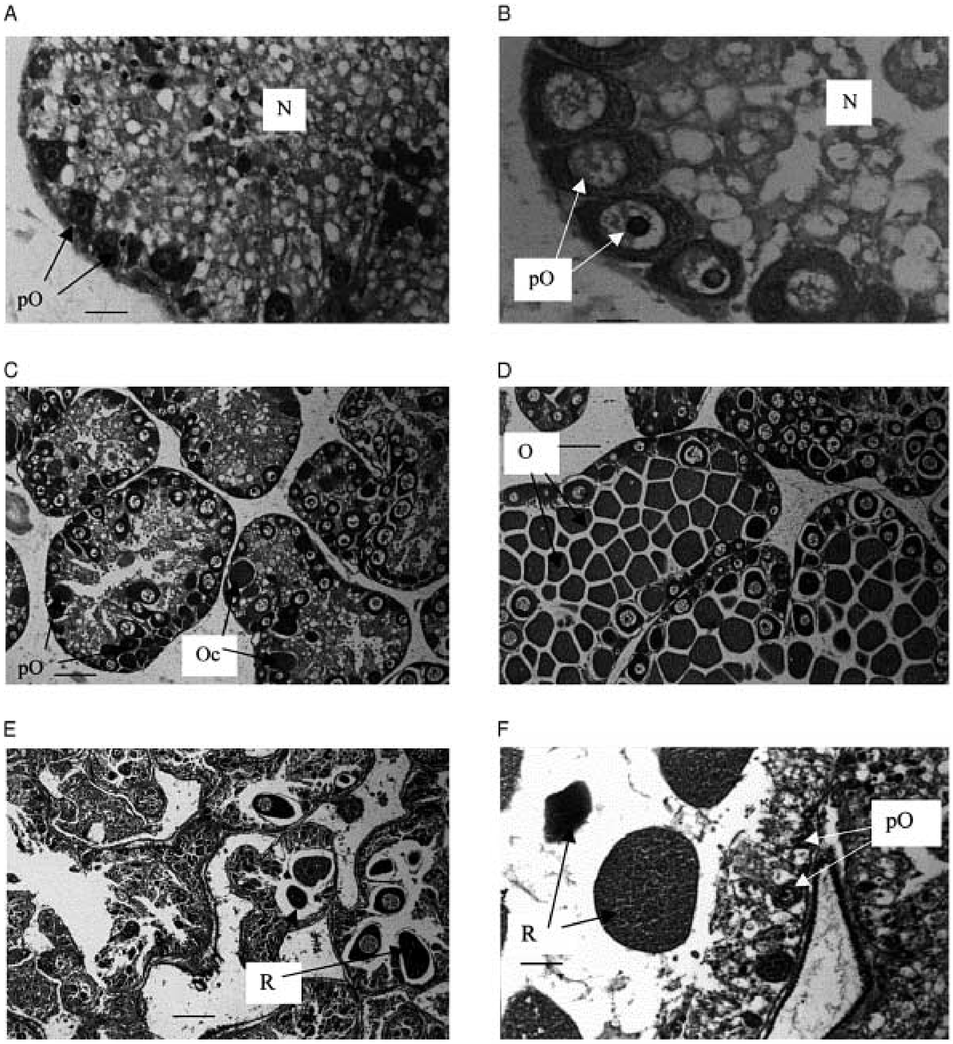

Figure 4. Histology of ovaries: (A) stage I, recovery phase, primary oocytes (pO) present along ascinal wall, nutritive phagocytes (N) fill lumen; (B) stage II, early growth phase, primary oocytes have increased in size, nutritive phagocytes still appear dense; (C) stage III, premature phase, premature ooyctes line ascini with some maturing ova (Oc) projecting towards the lumen; (D) stage IV, mature, lumen of ascini is packed with ova (O), premature oocytes remain around ascinal walls; (E) late stage V, very few ova remain, some relict ova are present (R); (F) stage VI, recovery phase, relict ova remain in lumen but nutritive layer containing some premature oocytes is reforming around ascinal wall.

(stage III) by May of 1996. All stages of vitellogenic oocytes were present except mature oocytes. As the oocytes matured they began to project towards the lumen of the ascini and the amount of eosinophilic nutritive phagocytes decreased (Figure 4C). In June and July the majority of females had ovaries classified as stage IV or mature. The lumen of the ascini were packed with large (eosinophilic) ova (85–105 μm diameter) and the nutritive phagocytes were restricted to a narrow band around the wall where there some premature oocytes also remained (Figure 4D). It is possible that these oocytes mature and move to the lumen to replace those that have been shed. By August all the females sampled were in the partially spawned state, (stage V). Some mature ova remained and there were spaces in the lumen left by recently shed ova. Oocytes of all stages of development were still present (Figure 4E). The females were categorized as spent (stage VI) from September to December. The ovaries were characterized by thin ascinal walls and empty appearance. A few relic ova were observed in a loose network of nutritive phagocytes (Figure 4F).

Testes

The pattern of growth for the testes was similarly divided into six stages. From January to March the testes were in the recovery stage (stage I; Figure 5A) the ascinal wall was lined by a thin layer (10–20 μm deep) of basophilic spermatogonia. The nutritive phagocytes were not completely filling the lumen of the ascini. By April, all males examined were either in stage I or stage II, the growing stage. The basophilic layer around the wall of the ascinus had increased in thickness (20–40 μm deep) and columns of spermatocytes had become visible projecting towards the centre of the lumen (Figure 5B). From June the males were categorized as stage III (premature) or stage IV (mature). In stage III the ascini were filled with intensely staining eosinophilic nutritive phagocytes. The spermatocyte columns lining the walls had increased in depth and spermatozoa were present in the lumen, where the nutritive phagocytes were no longer visible (Figure 5C). In stage IV the lumen of the ascini were packed with mature spermatozoa. The testes appeared largely devoid of eosinophilic material apart from a narrow band at the ascinal wall (Figure 5D). By

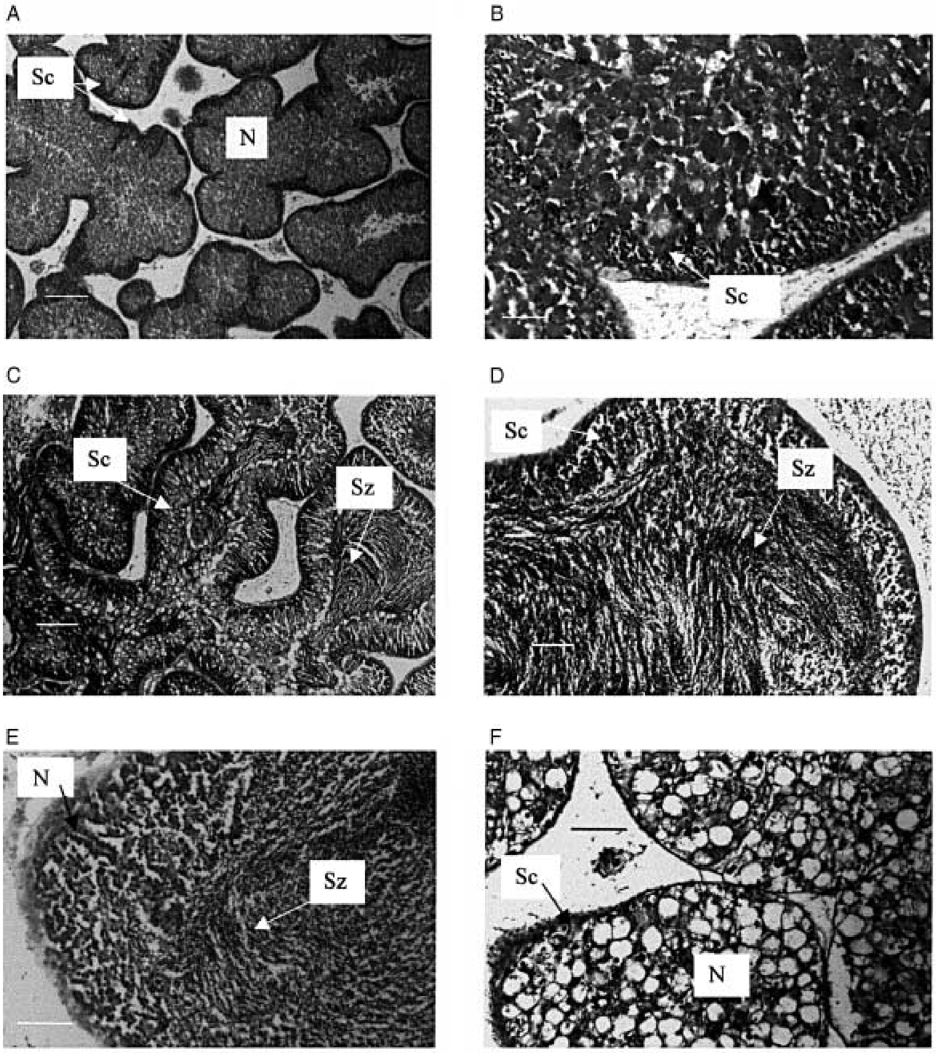

Figure 5. Histology of testes: (A) stage I, recovery phase, lumen is filled with nutritive phagocytes (N), spermatocytes (Sc) line the ascinal wall; (B) stage II, early growth phase, spermatocyte layer increases in thickness; (C) stage III, premature phase, spermatozoa (Sz) accumulate in lumen, replacing the nutritive phagocytes; (D) stage IV, mature, lumen is packed with mature spermatozoa; (E) stage V, partially spawned phase, spaces appear in lumen where spermatozoa have been shed; (F) stage VI, recovery phase, nutritive phagocytes appear as a loose, open network, spermatocytes form a narrow band along ascinal wall.

August the males were classified as stage IV or stage V, partly spawned. In stage V spaces had appeared in the ascinal lumen where spermatozoa had been shed. Some spermatozoa were observed free in the gonoduct (Figure 5E). The spent testis (stage VI) was largely devoid of contents, with thin ascinal walls and a loose network of nutritive phagocytes (Figure 5F).

Recruitment

There was evidence of recruitment to both sites LA and LB in 1996 and 1997. The total number of new recruits (individuals <5 mm test diameter) per site varied from ten (LA, December 1996) (Figure 6) to 92 (LB, January 1998) (Figure 7). The largest number of recruits collected (92, LB January 1998) represented 26% of the urchins found at that site, compared to the 24 (9.8%) urchins in this size-class (<5 mm) found at that site in the previous June. On every sample date some urchins of less than 5 mm test diameter were recorded, indicating that either a small number of recruits grow very slowly or there is a succession of late recruitment. The highest number of urchins recorded from one quadrat was 88 (LB, June 1999) equivalent to 352 ind m^{-2}; they ranged in size from 3.7 to 24.2 mm test diameter.

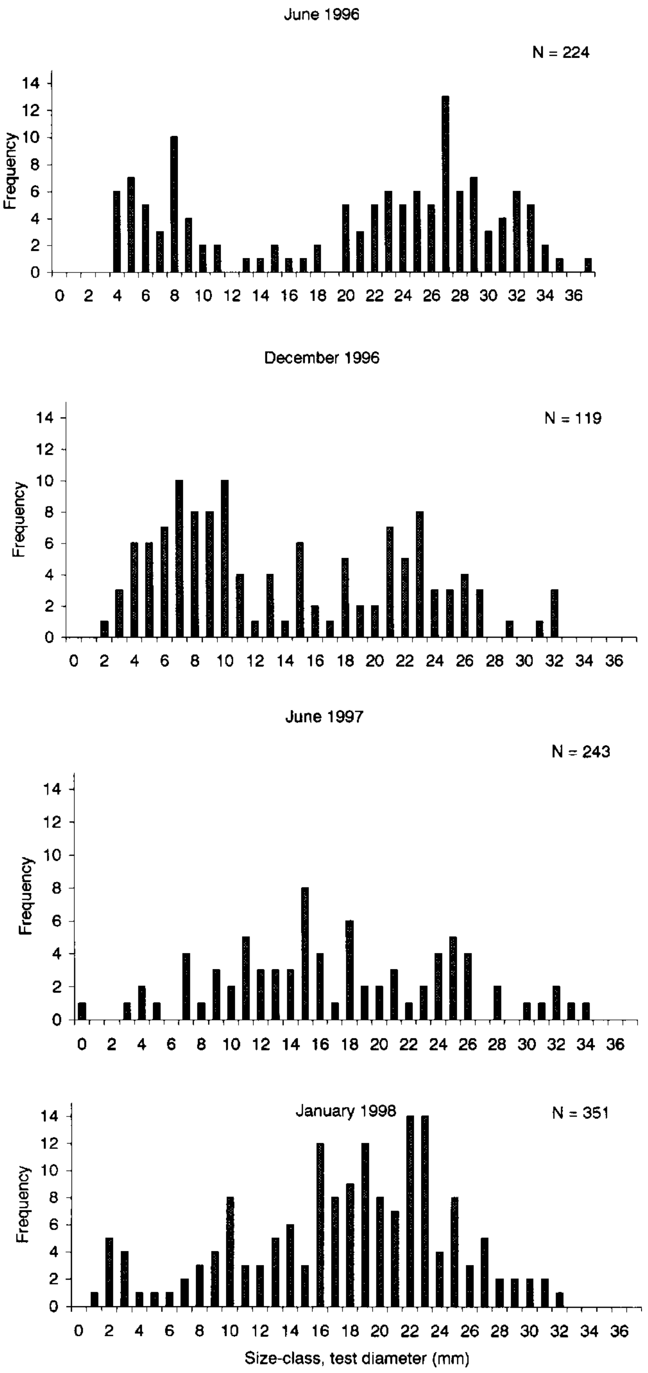

Figure 6. Size–frequency histograms for urchin population site LA monitored over two reproductive cycles. (N=sample size on each date.)

The population structure also varied between the two sites, site LA having a higher proportion of larger urchins (22–38 mm test diameter). No urchins of test diameter >30 mm were recorded at site LB perhaps reflecting the smaller boulder cover at this site, offering fewer larger

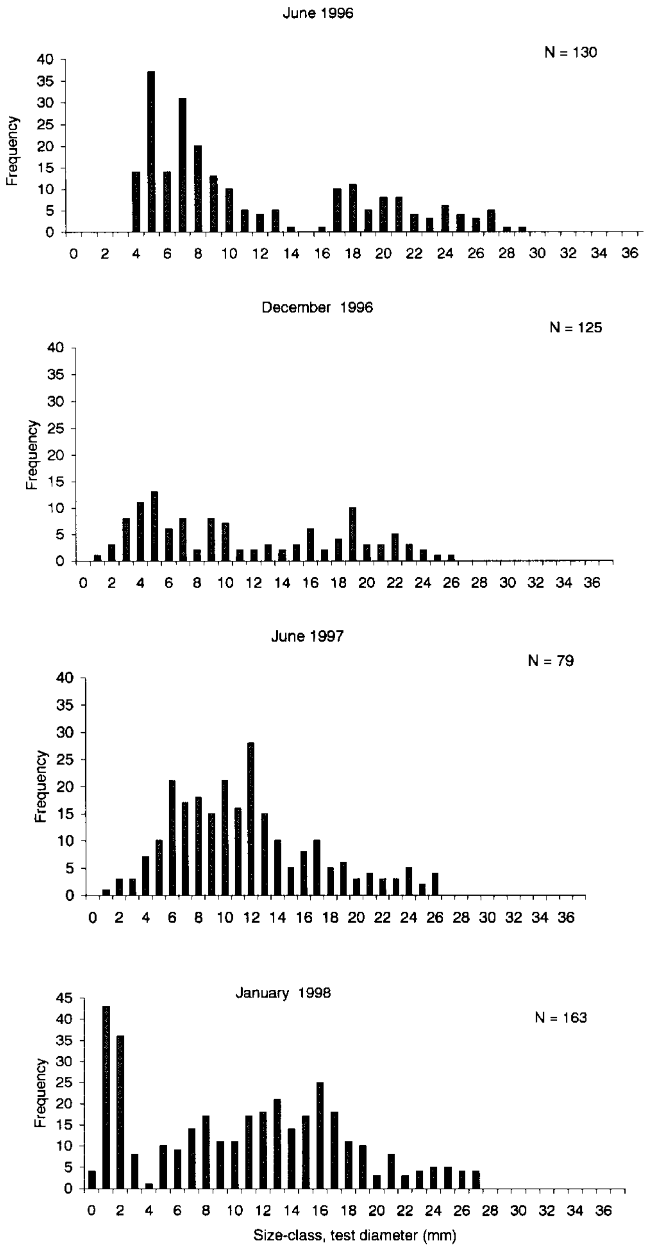

Figure 7. Size–frequency histograms for urchin population site LB monitored over two reproductive cycles. (N=sample size on each date.)

niches. There was evidence of bi-modality in site LA in June and December 1996 and in site LB in June 1996, with proportionally low representation of the 12–17 mm size-classes.

DISCUSSION

Psammechinus miliaris has a defined annual cycle of gametogenesis and a single spawning period. The annual cycle is similar to that described for other sympatric species e.g. *Echinus esculentus* and *Paracentrotus lividus* (Comely & Ansell, 1989; Byrne, 1990). There is no evidence of secondary spawning events as described for *P. lividus* and Mediterranean *Spherechinus granularis* (Crapp & Willis, 1975; Guillou & Michel, 1993).

The use of data from urchins collected from replicate sites at each location should permit the legitimate interpretation of variation in GI as being attributable to the different habitat types found at littoral and subtidal locations. However, in this study it also served to emphasize the extent of the variation in GI found between urchins sampled from different sites at the same location. Sites LA and LB are only 200 m apart and in the same bay, however urchins from LA had higher GI than urchins from LB in both seasons studied. By contrasting LA and SA alone we would have assumed that littoral populations vastly outperform subtidal populations in terms of summer GI. Similarly a comparison of sites LB and SA would have led to the conclusion there is little variation in GI between littoral and subtidal populations. There is a full discussion of the pitfalls of pseudoreplication in Hurlbert (1984). Previous comparisons of urchin populations from different habitat types (Gonor, 1973; Regis, 1979; Byrne, 1990; Lozano et al., 1995; Guillou & Lumingas, 1999) have contrasted urchins collected from a single site in each habitat type and have not quantified variation within habitat types. One can not therefore legitimately state that differences in biological indices are due to habitat differences. The present study indicates that the use of two replicates per habitat type may also be insufficient for defining trends in GI.

The relationship between GI, food availability and reproductive output in echinoids is well documented (Thompson, 1982; Andrew, 1986; Guillou & Lumingas, 1999). Urchins from site LA had higher summer GI than those at the other three sites in both seasons examined suggesting greater, seasonal, food availability at this site. Urchins from both littoral sites always had short, blunt spines in contrast to urchins from the subtidal site that had long pointed spines. This breakage of spines was attributed to greater wave action in the more exposed littoral location, and also to wave impact as these urchins were exposed on low spring tides and therefore also had waves actually breaking over them. Ebert (1968) demonstrated that urchins whose spines were broken showed a resulting decrease in test growth as energy was channelled to spine repair. In addition the urchins at the littoral site presumably do not feed when exposed to air and may expend more energy in remaining physically attached to the substrate. Taking these factors which have a negative impact on growth into consideration, the food supply or quality at site LA may be considerably greater than that at the subtidal location. *Psammechinus miliaris* is known to be omnivorous and has been reported, either by analysis of gut contents or by direct observation, to feed upon hydroids, worms, echinoderms, crustaceans, diatoms, molluscs, bryozoans, sponges, *Spirorbis* and *Mytilus* as well as plant material (see authors cited in Lawrence, 1975). Kelly et al. (1998a,b) and Cook et al. (1998) reported rapid increases in the GI of *P. miliaris* which have access to salmon food pellets rich in fish oils, demonstrating the ability of this species to utilize food of animal origin. As there is apparently an abundance of macroalgae throughout the spring and summer at both the littoral and subtidal locations, the hypothesis proposed here to explain higher GIs at littoral site LA is the seasonal availability of larger numbers of encrusting intertidal organisms e.g. mussel or barnacle spat.

Studies on the influence of food availability and the degree of exposure to wave action on gonad growth in wild echinoid populations (Ebert, 1968; Gonor, 1973) concluded that more exposed or food-limited populations had smaller gonads. Similarly Byrne (1990) found that *Paracentrotus lividus* from intertidal rock pools which were exposed by the tide on most days had lower GI than those from a sheltered subtidal location. In contrast, Lozano et al. (1995) reported that *P. lividus* from an 'unstable' environment, with high wave activity and fluctuations in salinity and temperature had a higher GI than populations from a 'stable' habitat. They suggested that this investment in reproductive effort was occurring at the expense of somatic growth, resulting in a population of urchins of smaller test diameter at maturity. The unstable habitat they described was, however, a shallow environment (0.2–0.5 m depth) and although the algal cover was less abundant than in the stable habitat they record that sponges, ascidians and serpulids were present. As *P. lividus* has been reported to feed on animal material (Mortensen, 1927; Regis, 1979), it is possible that the higher GI of these urchins is a result of their grazing encrusting intertidal organisms, as proposed for the *Psammechinus miliaris* populations in this study. The capacity of urchin grazing activity to alter the composition of benthic algal assemblages has been well studied (Paine & Vadas, 1969; Palacin et al., 1998). The omnivorous grazing habits of several species are also well documented (Nestle & Harris, 1994; Briscoe & Sebens, 1988; Emson & Moore, 1988). However the role of urchins in regulating invertebrate encrusting communities has yet to be fully quantified. Cook (1999) demonstrated that fatty acid biomarkers can be effectively used to distinguish diets of plant and animal origin in echinoids. Such techniques, in addition to gut content analysis, may prove useful in determining diet sources and assessing the impact of urchin grazing activity on invertebrate diversity and distribution.

Attempts to contrast studies of reproductive strategies of urchins from varying habitat types are not only limited by a lack of information on the within-habitat variation of echinoid biological parameters but by the number of environmental parameters at the selected sites which vary. Gonor (1973) attempted to minimise these differences by selecting sites at the same depth but which differed in the degree of wave exposure. However, contrasting what are perceived to be harsh and favourable sites has more usually meant that the degree of wave action, current strength, water depth, bottom type, echinoid density, food quality, food quantity and its seasonal abundance also varied (Lozano, 1995; Byrne, 1990; Meidel & Scheibling, 1998; Guillou & Lumingas, 1999; this study). The use of current meter data as in Lozano et al. (1999) would help define parameters such as 'exposure'. Similarly the degree of severity between the habitat types contrasted has varied, the intertidal site used by Byrne (1990), where urchins were exposed to air almost daily, is a more extreme environment than the intertidal location used in this study where the urchins were only exposed to air on low spring tides, once or twice a month.

When provided with excess food reserves *P. miliaris* uses its gonad as a storage organ. This is evidenced by high GI maintained out of the reproductive season and gonads which contain a predominance of nutritive phagocytes rather than developed gametes (Cook et al., 1998; Kelly et al., 1998b). Their observations of rapid gonad growth were made on *P. miliaris* which originated from wild populations. The wild populations described here, would therefore appear to be food-limited, demonstrated by the very low over-winter GI and gonads that contain a loose network of empty nutritive phagocytes. It is likely therefore that these food-limited populations channel energy to reproductive effort at the expense of somatic growth during the breeding season as postulated for *Paracentrotus lividus* and *Sphaerechinus granularis* (Lozano et al., 1995; Guillou & Lumingas, 1999). For such food-limited populations, of this species, it is therefore possible to equate GI and reproductive state.

The role of photoperiod in regulating gametogenesis has been recorded for temperate water echinoids (Pearse et al., 1986). McClintock & Watts (1990) reported that short days/long nights entrain gametic development in the tropical *Eucidaris tribuloides*. As the reproductive cycle of *Psammechinus miliaris* appeared synchronous between populations where food availability varied, it is likely the periodicity of the reproductive cycle in this species is also controlled by environmental parameters. Gametogenesis commences in *P. miliaris* as daylength increases, but may also require a concomitant increase in seawater temperatures. From the data presented here it is not possible to separate or predict the effects of temperature and photoperiod.

Byrne (1990) showed that the onset of spawning activity varied between seasons studied in *Paracentrotus lividus* and was therefore unlikely to be under photoperiod control. Increasing phytoplankton levels have been associated with the onset of spawning in *Strongylocentrotus droebachiensis* (Starr et al., 1993) but spawning in *Psammechinus miliaris* occurs well after the Loch Creran phytoplankton bloom typically commences. Although spawning occurs when daylength is maximal, other factors such as the intensity of illumination may also influence spawning (Pearse et al., 1988) and it is not possible to comment on the separate effects of these parameters on *P. miliaris* from the data presented here.

Both the occasional low numbers of small (<5 mm test diameter) urchins (assumed to be less than six months old) and the lack of some older age-classes, as evidenced by bimodality, may indicate recruitment to the littoral *P. miliaris* populations is sporadic and of variable magnitude. Trials using 15 spat collector units each constructed from three corrugated PVC plates (20×25 cm) suspended at the subtidal location in 1996 produced a small number of newly settled urchins ($N < 50$). For commercial exploitation of this species, spat collection may not therefore be a feasible alternative to the production of juveniles in a controlled environment. The parameters for successful production of relatively large numbers of juveniles have been described (Kelly et al., 2000). In this study relatively few large urchins (test diameter >30 mm) were collected. For marketing purposes urchins of at least 35 mm test diameter would be required; further investigation of the maximum grow out sizes obtainable for specimens in culture are required.

Some *P. miliaris* collected had the bright orange coloured gonads desirable for a marketable product. Gonad colour is the result of the assimilation and conversion of carotenoid pigments obtained from the diet. Tsushima & Matsuno (1990) found the primary pigments in 11 species of sea urchins were echinenone and β-carotene, however small amounts of the more highly oxidized pigments canthaxanthin and astaxanthin have been described in the gonads of some species (Tsushima & Matsuno, 1990; Goebel & Barker, 1998). As dietary pigments influence gonad colour (Goebel & Barker, 1998) the observed seasonal change in gonad colour described in this study may reflect seasonal variation in the abundance of macroalgae rich in carotenoids. *Psammechinus miliaris* is frequently found grazing the macroalgae *Laminaria saccharina*. The major carotenoid (primary xanthophyll) in this algae is fucoxanthin and beta, β-carotene the only carotene present (Haugan & Liaaenjensen, 1994). It was noted at both study locations that the standing *L. saccharina* crop was depleted by wave action during the winter months. However, the presence of mature gametes in the gonad also influences gonad colour; Griffiths & Perrott (1976) described seasonal changes in the carotenoid content of *S. droebachiensis* ovaries and the sex of ripe *P. miliaris* can often be distinguished on the basis of colour.

The gut wall has been postulated as a storage organ (Fuji, 1961; Lawrence et al., 1966). Annual cycles in the size of the gut have been found in *Strongylocentrotus purpuratus* (Lawrence et al., 1965; Pearse et al., 1970) and these have been assumed to reflect the nutritional condition of the echinoid. Klinger et al. (1988) showed that the gut tissue of *Lytechinus variegatus* functioned as a short-term nutrient storage organ and changes in cell number and cell size reflected the sequestration and mobilization of nutrients. Kelly et al. (1998b) showed *P. miliaris* with access to salmon feed pellets, a nutritious food resource, had higher AIs than local wild populations suggesting the use of the gut as a storage organ. A similar pattern of AIs was found in this study in that values decreased over the spawning period. The seasonal reduction in AI seen in these populations may reflect the mobilization of nutrients from the gut wall to help meet the energetic demands of the reproductive season.

The maximum gonad yields recorded from *P. miliaris* (~ 2.5 g per urchin) are too low to be considered marketable quantities. In addition maximum GIs were obtained when the gonads contained ripe gametes and were therefore of a soft texture and unsuitable for marketing. Allain (1975) reports gonad yields of 5 g per urchin as marketable quantities for *Paracentrotus lividus*. Cultivated *Psammechinus miliaris* are capable of producing gonad in this quantity (Kelly et al., 1998). Additional feeding outside the reproductive season prompts a proliferation of nutritive phagocytes resulting in the firm gonads the market desires. There is no evidence, from the populations studied here, that gonad yields of wild *P. miliaris* are sufficient to support a fishery.

This study was funded by the Highland Council, Highlands and Islands Enterprise and a LINK Aquaculture award SHL01 (to J.D. McKenzie and M.S. Kelly). I thank Dr G.B. Shimmield for the use of the facilities at the Scottish Association for Marine Science (SAMS) and Dr Liz Cook, Mr Tom Wilding, Miss Claire Scholfield and Miss Amanda Hunter for their invaluable assistance.

REFERENCES

Allain, J.Y., 1975. Structures des populations de *Paracentrotus lividus* (Lamarck) (Echinodermata; Echinoidea) sousimes à la pêche sur les côtes de Bretagne. *Revue des Travaux de l'Institute (Scientifique et Technique) des Pêches Maritimes. Paris*, **39**, 171–209.

Andrew, N.L., 1986. The interaction between diet and density in influencing reproductive output in the echinoid *Evechinus chloroticus* (Val.). *Journal of Experimental Marine Biology and Ecology*, **97**, 63–79.

Bedford, A.P. & Moore, P.G., 1985. Macrofaunal involvement in the sublittoral decay of kelp debris: the detritivore community and species interactions. *Estuarine, Coastal and Shelf Science*, **18**, 97–111.

Briscoe, C.S. & Sebens, K.P., 1988. Omnivory in *Strongylocentrotus droebachiensis* (Müller) (Echinoidea: Echinodermata): predation on subtidal mussels. *Journal of Experimental Marine Biology and Ecology*, **115**, 1–24.

Byrne, M., 1990. Annual reproductive cycles of the commercial sea urchin *Paracentrotus lividus* from an exposed intertidal and a sheltered subtidal habitat on the west coast of Ireland. *Marine Biology*, **104**, 275–289.

Chatlynne, L.G., 1969. A histochemical study of oogenesis in the sea urchin *Strongylocentrotus purpuratus*. *Biological Bulletin. Marine Biological Laboratory, Woods Hole*, **13**, 167–184.

Comely, C.A., 1979. Observation on two Scottish west coast populations of *Psammechinus miliaris*. *Internal Report. Scottish Marine Biological Association, Oban.*

Comely, C.A. & Ansell, A.D., 1989. The reproductive cycle of *Echinus esculentus* L. on the Scottish west coast. *Estuarine, Coastal and Shelf Science*, **29**, 385–407.

Cook, E.J., 1999. Psammechinus miliaris *(Gmelin) (Echinodermata: Echinoidea): factors effecting its somatic growth and gonadal growth and development*. PhD thesis, Napier University, Edinburgh.

Cook, E.J., Kelly, M.S. & McKenzie, J.D., 1998. Gonad development and somatic growth in the green sea urchin, *Psammechinus miliaris* fed artificial and natural diets. *Journal of Shellfish Research*, **17**, 1549–1555.

Crapp, G.B. & Willis, M.E., 1975. Age determination in the sea urchin, *Paracentrotus lividus* (Lamarck), with notes on the reproductive cycle. *Journal of Experimental Marine Biology and Ecology*, **20**, 157–178.

Davies, M., 1989. Surveys of Scottish sealochs. Lochs A'Chairn Bhain, Glendhu and Glencoul. *Report. Marine Nature Conservation Review*, no. 983.

Ebert, T.A., 1968. Growth rates of the sea urchin *Strongylocentrotus purpuratus* related to food availability and spine abrasion. *Ecology*, **49**, 1075–1091.

Edwards, A. & Sharples, F., 1986. Scottish sea lochs: a catalogue. *Internal Report. Scottish Marine Biological Association*, no. 134.

Emson, R.H. & Moore, P.G., 1998. Diet and gonad size in three populations of *Echinus esculentus*. In *Echinoderms. Proceedings of the Ninth International Echinoderm Conference, San Francisco, 1996* (ed. R. Mooi and M. Telford), pp. 641–644. Rotterdam: A.A. Balkema.

Faller-Fritsch, R. & Emson, R., 1972. On the relationships between marine plants and sea urchins. *Oceanography and Marine Biology. Annual Review*, **13**, 213–286. [From Lawrence (1975).]

Fuji, A., 1960. Studies on the biology of the sea urchin. I. Superficial and histological changes in gametogenic processes of two sea urchins *Strongylocentrotus nudus* and *S. intermedius*. *Bulletin of the Faculty of Fisheries, Hokkaido University*, **11**, 1–14.

Fuji, A., 1961. Studies on the biology of the sea urchin IV Histological observation of the food canal of *Strongylocentrotus intermedius*. *Bulletin of the Faculty of Fisheries, Hokkaido University*, **11**, 195–202.

Goebel, N. & Barker, M.F., 1998. Artificial diets supplemented with carotenoid pigments as feeds for sea urchins. In *Echinoderms. Proceedings of the Ninth International Echinoderm Conference, San Francisco, 1996* (ed. R. Mooi and M. Telford), pp. 667–672. Rotterdam: A.A. Balkema.

Gonor, J., 1972. Gonad growth in the sea urchin *Strongylocentrotus purpuratus* (Stimpson) (Echinodermata: Echinoidea) and the assumptions of gonad index methods. *Journal of Experimental Marine Biology and Ecology*, **10**, 89–103.

Gonor, J., 1973. Reproductive cycles in Oregon populations of the echinoid, *Strongylocentrotus purpuratus*. (1) Annual gonad growth and ovarian gametogenic cycles. *Journal of Experimental Marine Biology and Ecology*, **12**, 45–64.

Griffiths, M. & Perrott, P., 1976. Seasonal changes in the carotenoids of the sea urchin *Strongylocentrotus droebachiensis*. *Comparative Biochemistry and Physiology*, **55B**, 435–441.

Guillou, M. & Lumingas, L.J.L., 1999. Variation in the reproductive strategy of the sea urchin *Sphaerechinus granularis* (Echinodermata: Echinoidea) related to food availability. *Journal of the Marine Biological Association of the United Kingdom*, **79**, 131–136.

Guillou, M. & Michel, C., 1993. Reproduction and growth of *Sphaerechinus granularis* (Echinodermata: Echinoidea) in southern Brittany. *Journal of the Marine Biological Association of the United Kingdom*, **73**, 179–192.

Haugan, J.A. & Liaaenjensen, S., 1984. Algal carotenoids 54. Carotenoids of brown algae (Phaeophyceae). *Biochemical Systematics and Ecology*, **22**, 31–41.

Holt, R., 1991. Surveys of Scottish sealochs. Lochs Laxford, Inchard, Broom and Little Loch Broom. *Report. Marine Nature Conservation Review*, no. 16.

Hurlbert, S.H., 1984. Pseudoreplication and the design of ecological field experiments. *Ecological Monographs*, **54**, 187–211.

Jensen, M., 1969. Breeding and growth of *Psammechinus miliaris*. *Ophelia*, **7**, 65–78.

Kelly, M.S., Brodie, C.C. & McKenzie, J.D., 1998a. Sea urchins in polyculture: the way to enhanced gonad growth. In *Echinoderms. Proceedings of the Ninth International Echinoderm Conference, San Francisco, 1996* (ed. R. Mooi and M. Telford), pp. 707–711. Rotterdam: A.A. Balkema.

Kelly, M.S., Brodie, C.C. & McKenzie, J.D., 1998b. Somatic and gonadal growth of the sea urchin *Psammechinus miliaris* maintained in polyculture with the Atlantic salmon. *Journal of Shellfish Research*, **17**, 1557–1562.

Kelly, M.S., Hunter, A.J., Scholfield, C. & McKenzie, J.D., 2000. Morphology and survivorship of larval *Psammechinus miliaris* (Gmelin) (Echinoidea: Echinodermata) in response to varying food quantity and quality. *Aquaculture*, **183**, 223–240.

King, C.K., Hoegh-Guldberg, O. & Byrne, M., 1994. Reproductive cycle of *Centrostephanus rodgersii* (Echinoidea), with recommendations for the establishment of a sea urchin fishery in New South Wales. *Marine Biology*, **120**, 95–106.

Klinger, T.S., Watts, S.A. & Forcucci, D., 1988. Effect of short-term feeding and starvation on storage and synthetic capacities of gut tissues of *Lytechinus variegatus* (Lamarck) (Echinodermata: Echinoidea). *Journal of Experimental Marine Biology and Ecology*, **117**, 187–195.

Lawrence, J., 1975. On the relationships between marine plants and sea urchins. *Oceanography and Marine Biology. Annual Review*, **13**, 213–286.

Lawrence, J.M., Lawrence, A.L. & Giese, A.C., 1966. Role of the gut as a nutrient storage organ in the purple sea urchin *Strongylocentrotus purpuratus*. *Physiological Zoology*, **39**, 281–290.

Lawrence, J.M., Lawrence, A.L & Holland, N.D., 1965. An annual cycle in the gut of *Strongylocentrotus purpuratus*. *Nature, London*, **205**, 1238–1239.

Le Gall, P., 1989. *L'echinoculture. Technique et documentation*, pp. 467–491. Paris: Lavoisier Publishing.

Laedsgaard, P., Byrne, M. & Anderson, D.T., 1991. Reproduction of sympatric populations of *Heliocidaris erythrogramma* and *H. tuberculata* (Echinoidea) in New South Wales. *Marine Biology*, **110**, 359–374.

Lozano, J., Galera, J., Lopez, S., Turon, X., Palacin, C. & Morera, G., 1995. Biological cycles and recruitment of *Paracentrotus lividus* (Lamarck) (Echinodermata: Echinoidea) in two contrasting habitats. *Marine Ecology Progress Series*, **122**, 179–191.

McClintock, J.B. & Watts, S.A., 1990. The effects of photoperiod on gametogenesis in the tropical sea urchin *Eucidaris tribuloides* (Lamarck) (Echinodermata: Echinoidea). *Journal of Experimental Marine Biology and Ecology*, **139**, 175–184.

Meidel, S. & Scheibling, R.E., 1998. Annual reproductive cycle of the green sea urchin, *Strongylocentrotus droebachiensis*, in differing habitats in Nova Scotia, Canada. *Marine Biology*, **131**, 461–478.

Mortensen, T.H., 1927. *Echinoderms of the British Isles*. Oxford: Oxford University Press.

Nestler, E.C. & Harris, L.G., 1994. The importance of omnivory in *Strongylocentrotus droebachiensis* (Müller) in the Gulf of Maine. In *Echinoderms through time* (ed. David, Feral and Roux), pp. 813–818. Rotterdam: A.A. Balkema.

Paine, R.T. & Vadas, R.L., 1969. The effects of grazing by sea urchins, *Strongylocentrotus* spp. on benthic algal populations. *Limnology and Oceanography*, **14**, 710–719.

Palacin, C.G., Giribet, G., Garner, S., Dantart, L. & Turon, X., 1998. Low densities of sea urchins influence the structure of algal assemblages in the western Mediterranean. *Journal of Sea Research*, **39**, 281–290.

Pearse, J.S., 1970. Reproductive periodicities of IndoPacific invertebrates in the Gulf of Suez. III. The echinoid *Diadema setosum* (Leske). *Bulletin of Marine Sciences*, **20**, 697–720.

Pearse, J.S., McClary, D.J., Sewell, M.A., Austin, W.C., Perez-Rufaza, A. & Byrne, M., 1988. Simultaneous spawning of six species of echinoderms in Barkley Sound, British Columbia. *Invertebrate Reproduction and Development*, **14**, 279–288.

Pearse, J.S., Pearse, V.B. & Davis, K.K., 1986. Photoperiodic regulation of gametogenesis and growth in the sea urchin *Strongylocentrotus purpuratus*. *Journal of Experimental Zoology*, **237**, 107–118.

Picton, B.E., 1993. *A field guide to the shallow-water echinoderms of the British Isles*. London: Immel Publishing Ltd.

Read, S., 1994. *European marketing project report*. Diploma, Napier University, Edinburgh.

Regis, M.B., 1979. Analyse des fluctuations des indices physiologiques chez deux echinoides (*Paracentrotus lividus* (Lamarck) et *Arbacia lixula* L.) du Golfe de Marseille. *Tethys*, **9**, 167–181.

Sokal, R. & Rohlf, J., 1995. *Biometry. The principles and practices of statistics in biological research*, 3rd ed. New York: W.H. Freeman & Co.

Starr, M., Himmelman, J.H. & Therriault, J.C., 1993. Environmental control of green sea urchin *Strongylocentrotus droebachiensis* spawning in the St Lawrence Estuary. *Canadian Journal of Fisheries and Aquatic Sciences*, **50**, 894–901.

Thompson, R.J., 1982. The relationship between food ration and reproductive effort in the green sea urchin *Strongylocentrotus droebachiensis*. *Acta Ecologicia Sinica*, **56**, 50–57.

Tsushima, M. & Matsuno, T., 1990. Comparative biochemical studies of carotenoids in sea-urchins. I. *Comparative Biochemistry and Physiology*, **96B**, 801–810.

Vadas, R.L., 1977. Preferential feeding: an optimisation strategy in sea urchins. *Ecological Monographs*, **47**, 337–371.

Submitted 13 April 2000. Accepted 11 May 2000.