Sex differences in biochemical composition, energy content and allocation to reproductive effort in the brooding sea star *Leptasterias polaris*

Jean-François Raymond, John H. Himmelman*, Helga E. Guderley

Département de Biologie, Université Laval, Québec City G1K 7P4, Canada

ABSTRACT: We assessed sex differences in reproductive investment of the brooding sea star *Leptasterias polaris* (in the northern Gulf of St. Lawrence, eastern Canada) by examining the biochemical composition and energetic content of the principal body components in 3 contrasting periods: just before spawning, after spawning and after brooding. The reproductive effort for a standard sea star with an underwater mass of 10 g (equivalent to 8.2 cm in radius) was similar for both sexes (15.1 kJ in males and 12.2 kJ in females). However, the sexes differed in how energy was allocated for reproduction. The energy loss due to gamete release was almost 4-fold greater for males (15.1 kJ) than females (4.1 kJ). The major reproductive expenditure in females came from maintenance costs during the prolonged brooding period (8.1 kJ). For the body wall and stomach, lipid, protein and energy contents were similar for males and females over the spawning and brooding periods. The ratio of males to females in the population was 1:1 in smaller size classes but was about 2:1 for individuals measuring >11 cm in radius. This is possibly explained by slower somatic growth of females due to the repeated use of energetic reserves and lack of feeding during the 5 to 6 mo brooding period. The replenishment of body wall carbohydrates in males during the winter (not observed in females) suggests that males feed during this period.

KEY WORDS: Sea star · *Leptasterias polaris* · Brooding · Proximate biochemical composition · Reproductive effort · Gulf of St. Lawrence

INTRODUCTION

Of the many costs of reproduction, the energetic costs of gonad maturation are best established, whereas less is known about the cost of parental investment, particularly in invertebrates (Lawrence & McClintock 1994). Parental investment is any contribution by a parent that increases the fitness of the offspring while decreasing the ability of the parent to produce future offspring. The reproductive strategies of marine invertebrates are often reflected in patterns of energy allocation to somatic growth, reproduction and maintenance (Giesel 1976, Todd 1979, Thompson 1983, Lawrence 1985, Barnes et al. 1988, Lawrence & McClintock 1994). As organisms cannot exceed a maximum rate of food ingestion, allocation of energy to one

physiological process will reduce the availability of energy for others. This is the basic rule of trade-offs in energy allocation (Calow & Townsend 1981). As the reproductive strategy adopted will influence compromises among different processes (Chia 1974), it is important to take all reproductive costs into account to understand these trade-offs.

In most asteroid echinoderms, reproduction is cyclical and the organs involved, particularly the gonads and pyloric caeca, vary in size according to the point in the cycle (Lawrence 1973). In many asteroids, nutritional reserves in the pyloric caeca support gametogenesis (Jangoux & Van Imp 1977, Barker 1979, Jangoux 1982). Most asteroids are iteroparous, making it important that reproductive investment be gauged so as to minimise decreases in future reproductive success.

Comparisons of energy reserves before and after reproduction will reflect the energetic costs of reproduction (Doughty & Shine 1997).

Asteroids show 3 types of larval development: pelagic planktotrophic larvae, pelagic lecithotrophic larvae, or lecithotrophic development of embryos brooded by the females (Thorson 1946, 1950, Mileikovsky 1971, Lawrence & Herrera 2000). Brooding is often considered a response to limited food resources and is more common in small asteroids: the small size may mean that they cannot produce sufficient gametes to ensure successful external fertilisation (Menge 1974). An alternative hypothesis is that the small size of brooders optimises brood number relative to female body size (Strathmann & Strathman 1982, Strathmann 1985). This suggests that brooding is an unlikely strategy for large animals because the relative surface area for brooding decreases with increasing body size. Brooding may reduce parental fitness by limiting mobility and increasing vulnerability to predators (Strathmann 1985, Bosch & Slattery 1999). This limited mobility may also prevent or reduce feeding, leading to the use of somatic reserves (Chia 1969, Nimitz 1971, Menge 1974, 1975, Harrold & Pearse 1980, Hendler & Franz 1982, Jangoux 1982, Doughty & Shine 1997). Whereas reproductive investment is limited to the period of gametogenesis in species with pelagic larvae (Thorson 1950, Mileikovsky 1971), brooding parents also pay energetic costs for maintenance during brooding.

Many polar and boreal asteroids are brooders (Verrill 1914, Fisher 1940, I. P. Emerson 1973, C. J. Emerson 1977, Himmelman et al. 1982). A well-known example is the genus *Leptasterias* in northern temperate to Arc-

tic regions (Fisher 1930, Chia 1969, Menge 1974, 1975, O'Brien 1976, Hendler & Franz 1982, Himmelman et al. 1982, Boivin et al. 1986, Chia & Walker 1991, Hamel & Mercier 1995). *Leptasterias* species brood during cold periods (Chia 1966, Himmelman et al. 1982), and most are small (e.g. *L. hexactis*, *L. littoralis*, *L. tenera*, *L. epichlora*). Surprisingly, *L. polaris* maintains the brooding habit even though it attains up to 50 cm in diameter and is among the largest sea stars. This species occurs in subtidal communities in the western North Atlantic (Grainger 1966). Female *L. polaris* brood for extensive periods and do not feed during this period (Emerson 1973). The lack of feeding during brooding is also reported for *L. hexactis* in the eastern North Pacific (Chia 1966, 1968). *L. polaris* spawns between mid-November and mid-December and then the females brood through the winter and spring (Emerson 1973, Himmelman et al. 1982, Boivin et al. 1986, Hamel & Mercier 1995). The embryos are attached to bedrock, boulders or cobbles and are covered by the body of the female, which forms a disc by curving the arms laterally in one direction (Fig. 1). In the St. Lawrence Estuary, eastern Canada, brooding female *L. polaris* produce only a small quantity of eggs annually (1000 to 3000 eggs; Himmelman et al. 1982), whereas males release a large quantity of spermatozoa, much like broadcast spawners (Boivin et al. 1986). The spermatozoa that are negatively buoyant fall to the bottom and form a sticky film; the females then release eggs onto this layer of sperm (Hamel & Mercier 1995). After spawning, the females brood the embryos while the males resume other activities (Hamel & Mercier 1995).

The sex differences in reproductive strategies in *Leptasterias polaris* suggest that males and females
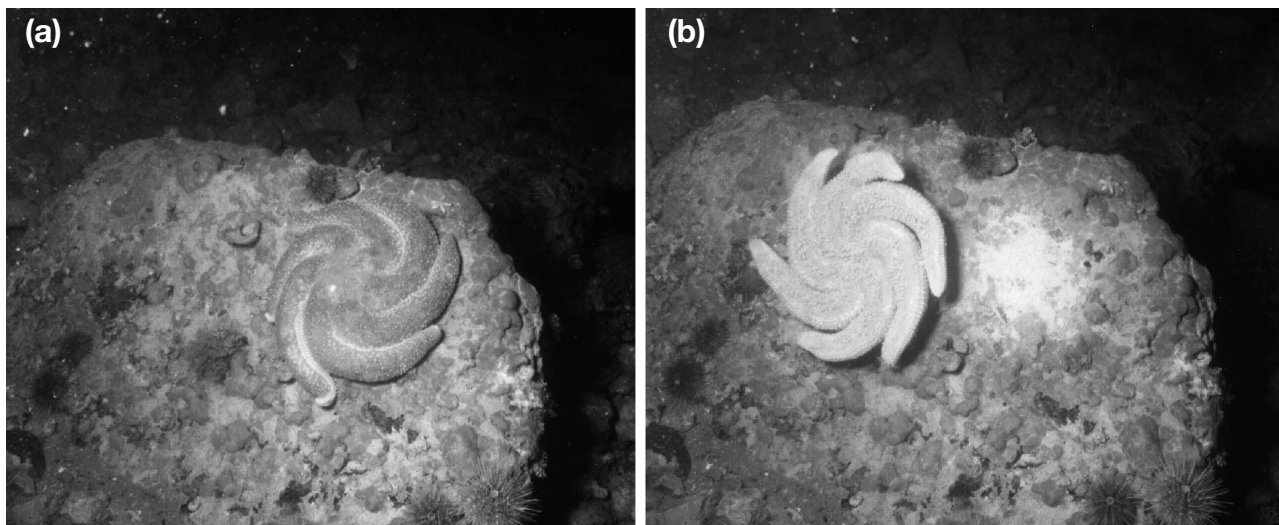

Fig. 1. *Leptasterias polaris.* (a,b) Adult female sea star brooding in the urchin barren habitat on bedrock (in b the sea star is turned so that the brood is visible)

may differ in their allocation of energy to somatic tissues. Although males seem to invest more in gamete production, only females brood. As food uptake is probably nil during the prolonged brooding period, brooding may impose a substantial energetic cost. If allocation of energy to reproduction were greater for females than males, females could have slower somatic growth. As a result, males may attain a larger size. If this is true, the proportion of males in a population should rise with increasing body size. Thus, males could accrue the increases in fecundity brought by large body size. The present study (1) compares the biochemical composition and energetic content of body components in male and female *L. polaris* in relation to their reproductive activities, (2) quantifies the reproductive effort of males and females and (3) examines the prediction that males outnumber females in the larger size classes.

MATERIALS AND METHODS

Sea star sampling. We used SCUBA diving to collect adult *Leptasterias polaris* (Fig. 1) measuring >2 cm in radius (distance from the centre of the oral opening to the extremity of an average-sized arm) 3 times during the reproductive cycle as documented by Boivin et al. (1986): (1) on 24 October 1998, just prior to spawning; (2) on 17 December 1998, when spawning was finished; and (3) on 18 May 1999, after 5 to 6 mo brooding (Fig. 2). These samples were taken to assess the biochemical composition and energy contents of body
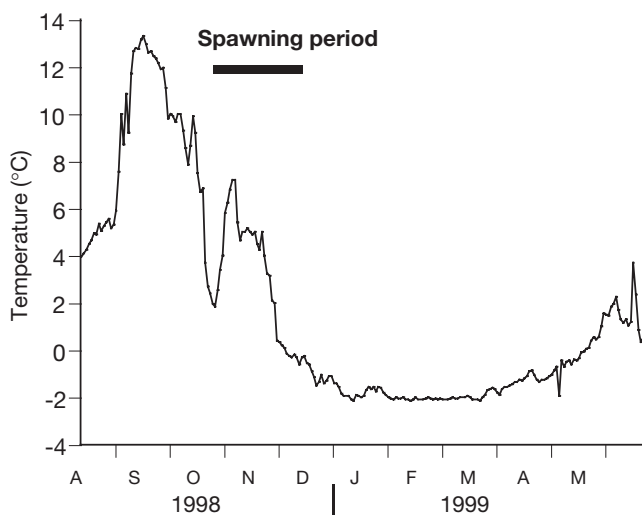

Fig. 2. Mean daily temperature at 8 m depth at Cap du Corbeau during the reproductive season of 1998-1999 of *Leptasterias polaris*. The horizontal bar indicates the approximate spawning period

components. An additional sample of 8 males and 8 females taken on 14 August 1998 served to establish the status of body components several months prior to the spawning period. The animals were selected haphazardly from 5 to 10 m depth from a bedrock bottom (urchin barrens) at Île aux Goélands, Mingan Islands, northern Gulf of St. Lawrence. In late October, no brooding individuals (females) were seen, whereas brooding females were frequently seen on 17 December. On 17 December, only females that were brooding were sampled. The females sampled on the third date were still brooding, and only those having young sea stars with visible arm buds were sampled. At this time, some females were brooding earlier stages of embryos, which may have represented embryos from a later spawning. Water temperature at a depth of 8 m at Cap du Corbeau, 1.8 km from Île aux Goélands, was measured from August 1998 to June 1999 with a thermograph.

Analysis of tissue size and biochemical composition. In the laboratory, we measured the underwater mass (the balance permitted measuring the increase in mass when the sea star was added to a basket suspended in seawater) and drained wet mass (after 2 min on paper towelling; 0.01 g precision) of each sea star, and then dissected the sea star to determine the drained wet mass of the gonads, pyloric caeca, stomach (cardiac and pyloric portions together) and body wall. For the samples taken in October, December and May, each of these body components was then frozen at –20°C for biochemical analysis (4 to 6 mo later). The small size of some of our tissues (gonads and stomach) limited the number of measurements that could be done accurately. Thus, ash content and dry mass were not measured on the tissues and we present values relative to wet mass. In a preliminary study, we estimated ash content and percentage dry mass as well as the biochemical composition of the gonads and pyloric caeca for 10 sea stars. This allowed us to evaluate the efficiency of our determinations of biochemical content. As the sum of the lipids, proteins and carbohydrates in the initial studies was between 80 and 90% of the ash free dry mass, our data are representative of the biochemical composition.

Lipid content was determined using the chloroform-methanol method as modified by Folch et al. (1957) and protein content using bicinchoninic acid (Smith et al. 1985). Protein concentrations were determined after extraction (1 g tissue per 10 ml buffer) in 50 mM KPhosphate, 0.1% Triton X-100, pH 7.0. Proteins were further solubilised by the addition of urea and acetic acid according to Somero & Childress (1990). This method yielded very small precipitates, suggesting a limited amount of insoluble material. To extract carbohydrates, we boiled the tissues in 5% trichloroacetic acid

for 10 min, and then determined the carbohydrate content in the supernatant using the phenol method of Dubois et al. (1956), with D-glucose as the standard.

Calculations and statistical analyses. As production is best expressed in energetic terms (Brody 1945), we estimated the energetic composition of each body component (gonads, pyloric caeca, stomach and body wall) from the biochemical components using the conversion factor of 39.5 kJ g^{-1} for lipids (Brody 1945), and 23.9 and 17.5 kJ g^{-1} for proteins and carbohydrates (Gnaiger & Bitterlich 1984). In comparing biochemical and energetic composition, we standardised values to that of a sea star with an underwater mass of 10 g (equivalent to 8.2 cm in radius), near the average size of the animals sampled. We statistically evaluated differences in biochemical composition and energetic composition between males and females on each sampling date using single factor ANOVAs (Steele & Torrie 1988), following the initial evaluation of homogeneity and normality, using Levene and Shapiro-Wilks tests, respectively. When needed, a log or arcsine transformation was applied. We also applied single factor ANOVAs to examine variations over time for each sex. When significant variations were detected, we followed with contrast tests to determine which sampling dates differed. All tests were carried out using the JMP statistical package (SAS Institute 1995).

For males, reproductive effort was calculated as the decrease in the mass or energetic content of the gonads from spawning as a percentage of total body mass (wet out-of-water mass). In females, reproductive effort includes the maintenance costs during brooding as well as the energetic decrease due to loss of gametes during spawning. Chia (1969) reported that the pyloric caecum is the main body component supplying energy for maintenance during brooding in female *Leptasterias hexactis*. Thus, we calculated reproductive effort of females as the decrease in the mass or energetic content of the gonads and pyloric caeca during the reproductive period (spawning and brooding) as a percentage of total body mass or energetic content.

Changes of sex ratio with sea star size. To determine if the sex ratio of *Leptasterias polaris* varied with size, we collected 150 individuals in 1997 (with a roughly even distribution over the various sizes from 2 to 16 cm in radius) and 305 in 1998 (100 or more individuals in each of 3 size classes: 2 to 7, 7 to 11, and >11 cm in radius), from 5 sites within a 3 km distance between Île au Marteau and Île du Havre in the Mingan Islands. The sex was determined from a smear of a small portion of gonad extracted from a 5 mm incision made on an arm near the central disc. We did not consider sea stars <2 cm in radius because of the difficulty in determining sex. We used chi-square tests to evaluate if sex ratios differed from a 1:1 ratio (Steele & Torrie 1988).

RESULTS

The body wall was by far the largest structure, weighing from 55 to 60 g for a standard *Leptasterias polaris* with an underwater mass of 10 g (76.1 g out-of-water mass), compared to 1.2 to 11.2 g for the gonads, 4 to 10 g for the pyloric caeca and 0.9 to 1.2 g for the stomach (Fig. 3). The body wall and stomach were stable body components in terms of mass, as no significant differences were detected between sexes or in different reproductive periods (Fig. 3). In contrast, the mass of the gonads and pyloric caeca showed marked variations in different periods and also differences between sexes in certain periods (before spawning for the gonads and after spawning for the pyloric caeca).

The seasonal changes in the size of the gonads and pyloric caeca of *Leptasterias polaris* collected at Île au Goélands in the Mingan Islands between August 1998 and May 1999 (Fig. 3) resembled the annual cycle described by Boivin et al. (1986) for a population in the St. Lawrence Estuary. The gonadal mass of both males and females was stable between August and October (gametogenic peak), leading up to the spawning period, and then decreased. For males the decrease was precipitous, from 9.16 to 2.72 g between 24 October and 17 December 1998 for a standard individual with an underwater mass of 10 g, indicating a massive release of spermatozoa. Testis mass remained unchanged between December and May. The mean ovarian mass tended to decrease, but not significantly, between 24 October and 17 December. It was clear that spawning had occurred because brooding females were abundant on 17 December (females represented 33% of the haphazardly collected non-brooding sea stars on this date, compared to 49% on 24 October). Ovarian mass showed no significant changes between December 1998 and May 1999.

The mass of the pyloric caeca for both males and females decreased in the months leading up to and during spawning (August to December), although the change was not significant for female (Fig. 3). Possibly reserves from this organ were being used for gamete production. Subsequently, the mass of the pyloric caeca decreased for both sexes. The major decrease was seen during spawning for males and during brooding for females.

Biochemical and energetic composition

Whereas the body wall represented the heaviest body component, the percentage content of biochemical components (lipids, proteins and carbohydrates) per wet gram of tissue was much lower than for other
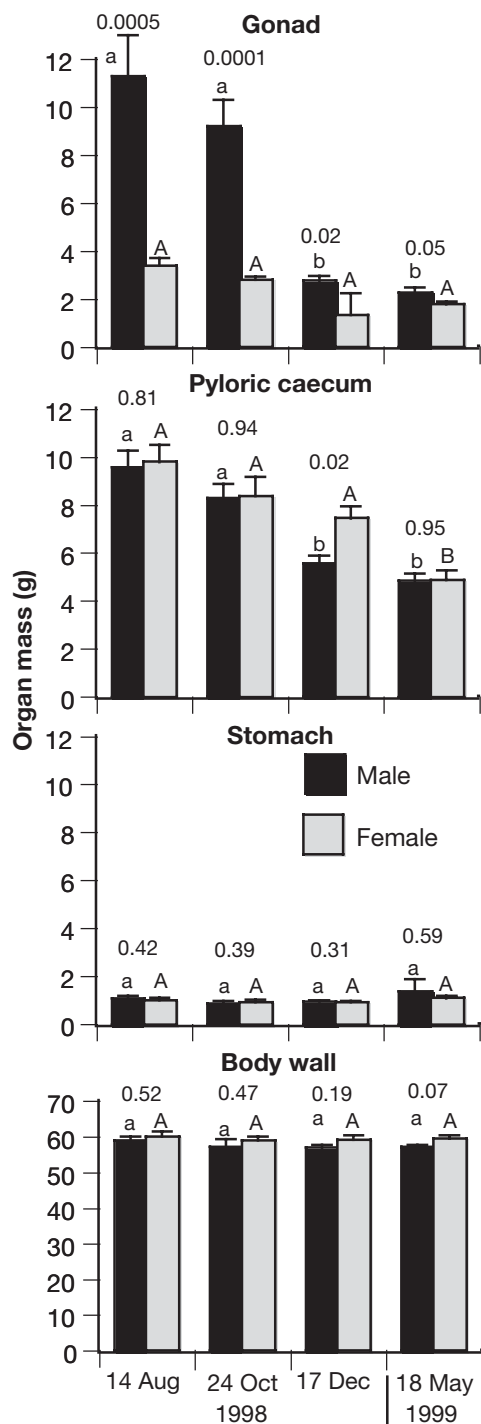
Fig. 3. *Leptasterias polaris*. Mean wet mass of different body components for a standard male and female sea star (underwater mass = 10 g) on 14 August (several months prior to spawning), 24 October (just prior to spawning), and 17 December 1998 (just after spawning), and on 18 May 1999 (after 5 mo of brooding in the case of females). Error bars are +SE. Values above the bars represent probabilities associated with comparisons of the sexes on the same dates. Different letters above bars of the same colour indicate changes within a sex between sampling dates (contrast tests, p < 0.05)

body parts (Fig. 4). This was due to the body wall's high content of inorganic matter (69.91% of dry mass, SE = 0.27; from analysis of 30 sea stars collected in July 1997). The lipid and protein levels of the body wall (% wet mass and g per standard animal) were very stable (Figs. 4 & 5). Carbohydrate levels (g per standard animal) appeared to decrease after spawning in males and after brooding in females (Fig. 4). The composition of the stomach changed little, as the contents of lipids, proteins or carbohydrates did not vary between sexes or in different periods (Figs. 4 & 5).

The gonads and pyloric caeca showed the greatest seasonal variation in biochemical components (% content, Fig. 4; and g per standard animal, Fig. 5). In the gonads, these changes parallel those observed in gonadal mass (Figs. 3 & 5). For males, the total mass of lipids, proteins and carbohydrates in the gonads decreased markedly during the spawning period (between October and December 1998) and remained stable over the winter (until May 1999). In females, a similar but less pronounced pattern was observed. Thus, ovarian lipids and carbohydrates (g per standard animal) decreased during spawning and remained stable between December and May (Fig. 5). The ovarian tissue remaining after spawning tended to have a higher percentage of lipids and proteins than before spawning (Fig. 4).

Two major changes were detected for the pyloric caeca (Fig. 5). First, the carbohydrate content (g per standard animal) decreased by 40 to 60% during spawning for both males and females, and then low levels were maintained during brooding (significantly lower for females than males). Second, lipid levels decreased by almost half during the winter for both sexes. A surprising change was that the protein content of the pyloric caecum of females increased between October and December and decreased during the winter, whereas it did not vary over time in males.

Calculations of energetic content again demonstrated the stability of the body wall and stomach (Fig. 6). The energetic content of the testes decreased by 75.7% after spawning compared to a 34.7% decrease for the ovaries. Differences between sexes were also apparent during the winter, when the energetic content of the testes increased slightly but not significantly (p = 0.097), and that of the ovaries remained constant. The energetic content of the pyloric caeca varied in a similar pattern for the 2 sexes, with no significant change during spawning (although energetic content tended to increase for females) but a marked decrease during the following 5 mo. As the energetic content of the pyloric caeca did not change during spawning, the observed (but not significant) decrease in mass (Fig. 3) must have been due to a decrease in water content.
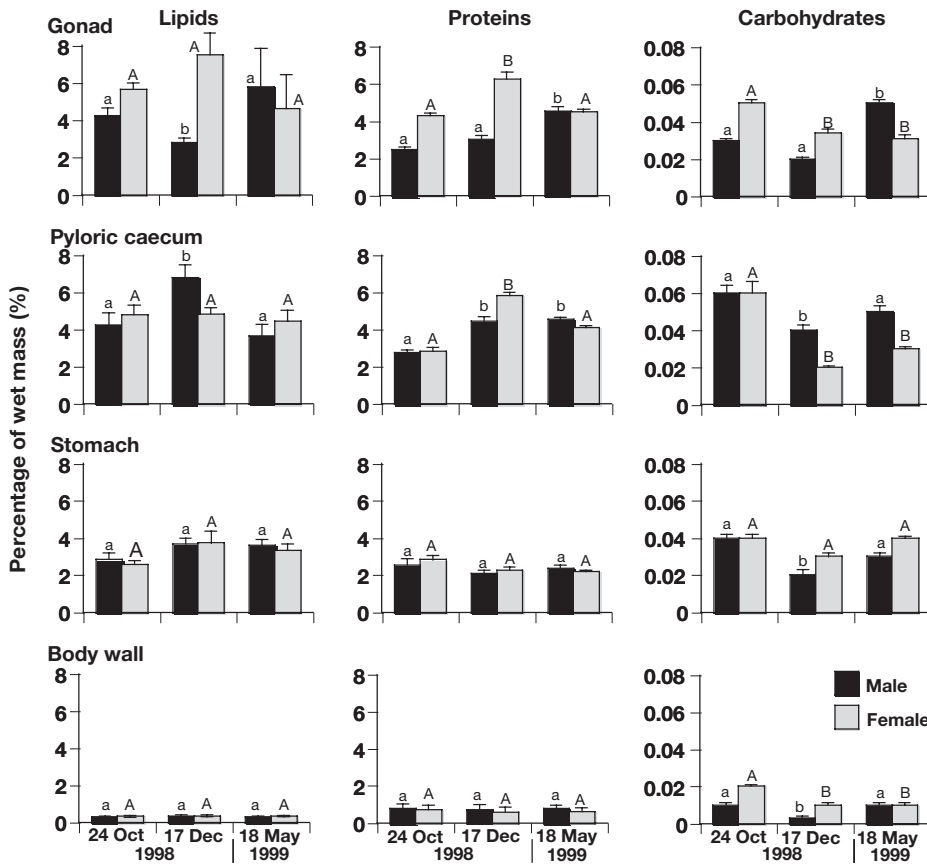
Fig. 4. *Leptasterias polaris.* Percentage mass of lipids, proteins and carbohydrates to total mass for different body components for a standard male and female sea star (underwater mass = 10 g) on 24 October and 17 December 1998 (just before and after spawning, respectively) and on 18 May 1999 (after 5 mo of brooding in the case of females). Error bars are +SE. Different letters above bars of the same colour indicate changes within a sex between sampling dates (contrast tests, p < 0.05)

Allocation to reproduction

For males, the loss in wet gonadal mass due to spawning as a percentage of total live body mass was 7.99%. For females, reproductive effort involved both the loss in mass by the ovary during spawning (1.99%) and the loss by the pyloric caecum due to maintenance costs during brooding (4.55%). Thus, total reproductive effort (as a % of body mass) was 6.54% for a standard female, which is only slightly less than for a standard male.

After energetic conversions were made, reproductive effort for males (from gonadal loss) was 15.05 kJ. For females, reproductive effort (from losses in the gonads and pyloric caeca) was estimated at 12.24 kJ, of which only 4.13 kJ was due to gonadal loss. Thus, at spawning, males lost approximately 3 times more energy than females. The energetic loss from the pyloric ceaca during brooding by females (8.11 kJ) likely represents maintenance costs over 5 mo. Our calculations of total reproductive effort, based on loss in mass or energetic content, did not indicate major differences between males and females. Nevertheless, a major difference between the sexes was that the main

cost of reproduction was gamete production for males and brooding for females.

Sex ratio

In 1997 and 1998 the sex ratio favoured males at greater sizes (Table 1). Thus, in both years, sex ratios were similar to 1 for small (2 to 7 cm) and medium-sized (7 to 11 cm) individuals, whereas males were more abundant than females for large sea stars (>11 cm), with ratios of 2.33 in 1997 and 1.86 in 1998.

DISCUSSION

Males invest considerably more effort in gamete production than females in populations of *Leptasterias polaris* from both the Mingan Islands (present study) and the St. Lawrence Estuary (Boivin et al. 1986). Males show a substantial increase in testicular size in the 6 mo prior to spawning and a marked decrease during spawning. In contrast, ovarian size varies by much less over the year, and the decrease during
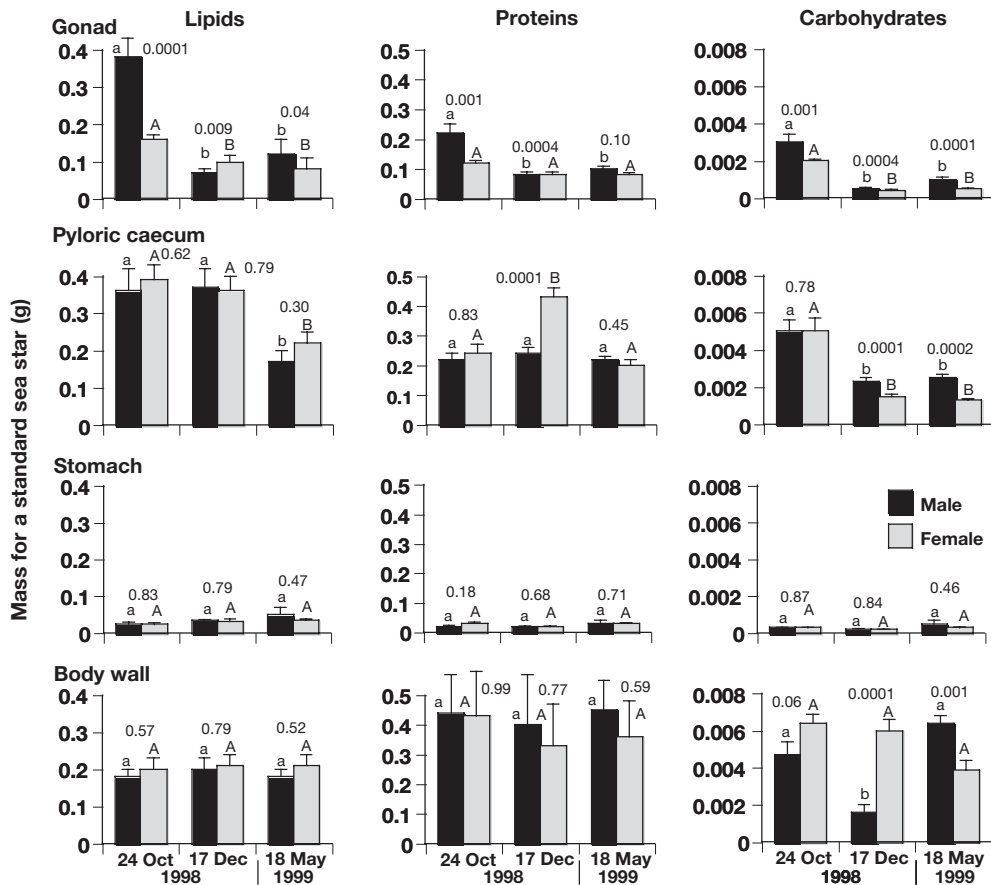
Fig. 5 *Leptasterias polaris*. Mass of lipids, proteins and carbohydrates for different body components for a standard male and female sea star (underwater mass = 10 g) on 24 October and 17 December 1998 (just before and after spawning, respectively) and on 18 May 1999 (after 5 mo of brooding in the case of females). Error bars are +SE. Values above the bars represent probabilities associated with comparisons of the sexes on the same dates. Different letters above bars of the same colour indicate changes within a sex between sampling dates (contrast tests, $p < 0.05$)

spawning may be difficult to detect. Thus, males adopt a strategy characteristic of broadcast spawners, whereas females spawn only a small number of eggs as characteristic of brooders. Our comparisons are based on values calculated for standard animals with an underwater mass of 10 g. The sexual size dimorphism revealed by the sex ratio data will accentuate these differences in reproductive investment. If females invest so little effort in gamete production, why are they less likely to become larger (i.e. grow more slowly) than males? Conversely, as males invest so heavily, how can they attain such large sizes? The costs of brooding may provide an answer.

The pyloric caeca have both a digestive and a storage function. In many sea stars, the pyloric caeca show an inverse cycle to that of the gonads (Giese 1966, Crump 1971, Jangoux & Vloebergh 1973, Barker 1979, Barker & Nichols 1983, McClintock et al. 1995). We did not observe this pattern. Rather, the mass of the pyloric caeca attained its maximum in the months leading up to the late fall spawning, and decreased during the following winter months. Interestingly, for both males and females, the energetic and biochemical contents of the pyloric caeca only decreased during over-winter-

ing. As females cannot feed during brooding, these reserves in the pyloric caeca probably provide energetic support during brooding.

Biochemical and energetic components

Allocation of energy to the gonads and the pyloric caeca reflected the different reproductive strategies of males and females. The energetic content of the body wall and stomach showed virtually no variation between sexes and in different periods. The most striking difference between males and females was the massive decrease in energy in the testes with spawning, compared to the slight decrease for the ovaries. During the 5 mo following spawning, the energetic content of the ovaries and testes showed no significant changes, although that in the testes tended to increase. Between October and December, the energy content of the pyloric caeca was stable in males and increased slightly (20%) in females. Both sexes showed a pronounced drop in pyloric caeca energy content (by 40 to 45%) during the winter. In females, this decrease was likely due to maintenance costs during brooding. In
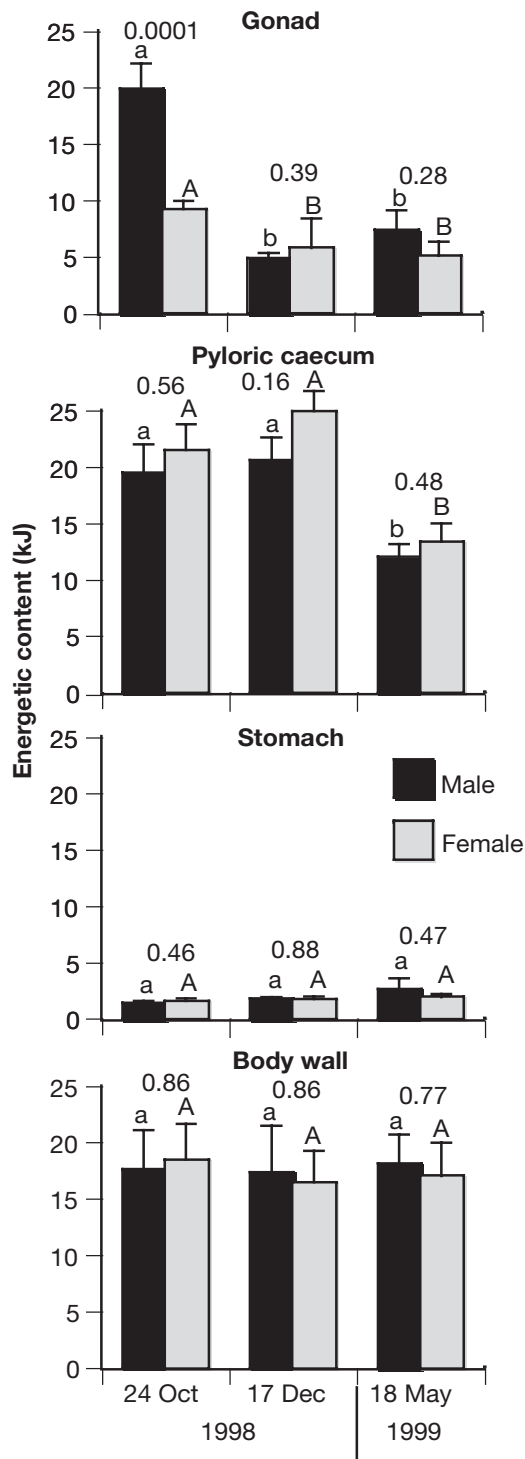
Fig. 6. *Leptasterias polaris*. Mean energetic content of different body components of a standard male and female sea star (underwater mass = 10 g) on 24 October and 17 December 1998 (just before and after spawning, respectively) and on 18 May 1999 (after 5 mo of brooding in the case of females). Error bars are +SE. Values above the bars represent probabilities associated with comparisons of the sexes on the same dates. Different letters above bars of the same colour indicate changes within a sex between sampling dates (contrast tests, p < 0.05)

males, this decrease may reflect renewed testicular development but suggests that males feed little during the winter (perhaps due to subzero temperatures). Periods of reduced feeding at temperature extremes have been noted for numerous sea stars (Mauzey 1966, MacKenzie 1969, Brun 1972).

As lipids are rich in energy and easily metabolised, they are typically the first energetic reserve used during prolonged periods of low food availability (Pearse & Giese 1966, Bosch & Slattery 1999). The stomach and the body wall showed no significant variations in lipid content between sexes and during the reproductive cycle. Barker & Xu (1991) also report little variation in the lipid content of the body wall over the annual reproductive cycle for 2 populations of *Sclerasterias mollis*. In contrast, lipid content of the gonads and pyloric caeca varied considerably. Testicular lipid content dropped markedly during spawning, due to the massive loss of spermatozoa (Boivin et al. 1986; their Fig. 2). A slight increase in lipids during the winter may reflect renewed testicular development. Ovarian lipid content showed a small but significant decrease during spawning and a slight decrease during brooding. The pyloric caeca of both males and females showed major and parallel decreases in lipids during the winter.

Proteins play important functional and structural roles during the reproductive cycle, slowing the mobilisation for energetic purposes in both sexes. Accordingly, significant changes in protein content were limited, occurring only in the testes of males and the pyloric caeca of females. Changes in the protein content of the gonad are expected during gamete production and spawning. Proteins in the pyloric caeca may reflect food intake as well as structural components. An annual cycle in epithelium thickness and in the quantity of spermatozoa occurs in the lumen of the testes of *Leptasterias polaris* (Boivin et al. 1986). Given that the epithelium and spermatozoa are largely made of lipids and proteins, major changes in lipid and protein levels in the testes were expected. The stability of ovarian protein content during spawning is related to the small number of oocytes released. After spawning, females showed an intriguing peak in the protein content of the pyloric caeca. Females lay their eggs on a layer of sperm previously deposited by the males (Hamel & Mercier 1995) and begin brooding their eggs. Given the limited egg production by females, only a small proportion of the sperm released by the males is likely needed for successful fertilisation. Possibly, the increase in proteins of the pyloric caeca of females reflects ingestion of sperm by brooding females. Ingestion of the excess sperm could decrease the negative impact of brooding on the future reproductive success of females.

Table 1. *Leptasterias polaris*. Ratio of males to females obtained for different size classes of sea stars collected in the Mingan Islands in the northern Gulf of St. Lawrence, eastern Canada, in 1997 and 1998. χ^2 tests identified departures from a 1:1 ratio. ns: not significant

Size class (cm)	No. males	No. females	Male/female ratio	χ^2
1997				
2–7	25	25	1.00	ns
7–11	27	23	1.17	ns
>11	35	15	2.33	<0.05
1998				
2–7	48	52	0.92	ns
7–11	50	50	1.00	ns
>11	65	35	1.86	<0.05

In contrast to lipids, carbohydrate levels are low. They likely represent storage of energy from food for short-term use (Pearse & Giese 1966). Thus, changes in carbohydrate levels should reflect recent energy expenditures or feeding activity. In males, carbohydrate levels in the gonads, pyloric caeca and body wall decreased markedly during spawning (October to December). This may reflect glycogen use during the formation of spawning aggregations and during gamete release. By May, males had shown considerable recuperation of carbohydrate levels in the body wall. This increase indicates recent feeding and suggests that males allocate energy to growth as well as to maintenance during over-wintering. For females, carbohydrate levels in the pyloric caecum and gonads decreased at spawning and no recovery was seen by May. This was likely due to a lack of feeding.

Winter brooding and reduced metabolic costs

In *Leptasterias polaris*, brooding occurs during the coldest months of the year (Himmelman et al. 1982), limiting the energetic costs of this maternal investment (Boivin et al. 1986). As both females and males showed a marked drop in energy reserves during over-wintering, brooding occurs when subzero temperatures may limit feeding. Tamplin & Stickle (1998) reported a significant influence of temperature on the metabolic rate of *Leptasterias epichlora*. If organisms with low basal metabolic rates are frequently associated with low-energy systems (e.g. polar environments) (Parson 1993, Lawrence & McClintock 1994), it should be advantageous for females to brood during the winter. Yet as temperature has a direct influence on most physiological rates in ectothermic species (Klinger et al. 1986, Roller & Stickle 1993), embryonic development may also be slowed, extending the period during which

brooding is required. The increased oxygen content of cold waters may favour brooding at cold temperatures, increasing oxygen availability for the embryos. However, females that brood in shallow waters may be exposed to stress (winter storms and ice scouring) related to the brooding location (Himmelman et al. 1982). The wave action may enhance oxygen availability for the embryos, but it and the ice scouring could prove damaging for the mothers. Thus, the choice of shallow sites for brooding could bring an increased risk of mortality. Over time, such mortality would lead to fewer females reaching large sizes and thus change the sex ratio. Such mortality would not occur in offshore populations. A rise in mortality could also explain why there is no increase in females at <11 cm radius.

In females, the basal metabolic rate should be lowered by starvation, reducing energy expenditures during brooding. *Leptasterias epichlora* reduces basal metabolic rate (lowered oxygen consumption) with starvation and can breakdown tissues and decrease body mass to cover maintenance requirements (Tamplin & Stickle 1998). Thus, despite occurring under the 'least expensive' conditions, brooding may lead female *L. polaris* to lose body mass. As we compared different individuals sampled from the natural environment, and as we expressed our data as values obtained for a standard animal, our analysis cannot assess the energetic contribution of changes in body mass during long-term brooding. For example, we would not have been able to detect a shrinking in size (of all body components including calcareous structures), as described for a number of echinoderms under conditions of low food availability (Ebert 1968). Thus, our analysis may have underestimated the energetic cost of brooding by females.

Reproductive effort

Although we detected little difference between males and females in overall reproductive effort, we did document differences between the sexes in the use of energy resources during the reproductive cycle. Male *Leptasterias polaris* display the typical pattern for echinoderms, i.e. production of a massive quantity of gametes which are released during spawning (Boivin et al. 1986, present study). Reproductive effort in males is represented by the energetic loss during sperm release. The males can likely feed throughout the year, as spawning is not followed by a period of parental care. In contrast, female *L. polaris* invest only a small amount of energy into egg production each year (less than 30% of that invested by males), but then invest a substantial amount into maintenance while brooding. Calculation of the reproductive effort from the organ indices reported by Boivin et al. (1986) for *L. polaris* in

the St. Lawrence estuary showed values equivalent to 8% of wet body mass for males (gonadal decrease), and 7% of wet body mass for females (decrease of gonad and pyloric caecum), thus similar to the values we obtained (8.0 and 6.5%, respectively). Menge (1974) reported similar results for *Leptasterias hexactis* (9 to 10% of their mass is allocated to reproduction). Nonetheless, as noted above, since our estimates are based on values expressed for a standard animal, we may be underestimating the cost of brooding since prolonged food limitation, even at cold temperatures, typically leads to a loss of body mass. On the other hand, not all of the energetic loss of females during brooding may be considered as reproductive effort, since males, which were not brooding, also lost energy during the winter.

Sex ratios

In structuring our study, we predicted that the energetic costs of brooding would limit somatic growth in females, leading males to attain greater sizes, so that the sex ratio in a population would increase in favour of males in larger size classes. This was indeed shown with our sampling of sea stars in 1997 and 1998: the proportion of males was almost twice that of females in the largest (>11 cm) size class (Table 1). However, increased long-term growth of males compared to females did not appear to be related to differences in reproductive effort, as overall reproductive effort was similar for both sexes (the mean value was only slightly less for females). Thus, we return to the question 'what causes the change of the sex ratio with increasing size?' The most likely possibility is that males grow more rapidly because they have at least 6 mo longer per year for foraging to acquire resources for growth. Although Himmelman et al. (1982) and Boivin et al. (1986) indicate that brooding is finished in the spring (April or May), we have frequently seen brooding *Leptasterias polaris* in July (particularly large individuals). The feeding rate of *L. polaris* does not change significantly between 2.5 and 12°C (Gaymer et al. 2001) and extrapolation of the relationship to –2°C indicates at most a 10% decrease in feeding at winter temperatures. Thus, males can probably allocate more energy into somatic growth than females because they can feed during the period when females are brooding. The slightly higher (although not significantly) energy content of the body wall and stomach of males than females during the brooding period (Fig. 6) is consistent with greater allocation to somatic tissues. This difference between males and females would pertain to both shallow and deeper water habitats.

The reproductive strategy of male *Leptasterias polaris* may favour an increase in size to increase fecundity as indicated for broadcast spawners (Strathmann & Strathmann 1982). As brooding success may not increase with size in females, fast growth rates would not be favoured. Two costs of brooding that we could not quantify are decreases in size due to starvation and the increased risk of mortality. The lack of mobility of females during the long brooding period could increase vulnerability to predators, such as the sea star *Crossaster papposus* (Himmelman 1991), or to mortality from ice abrasion (for individuals brooding in shallow water). Enhanced mortality of brooding females may limit the occurrence of females in larger size classes. Thus, mortality during brooding as well as the sex-specific reproductive strategies and their effects on energy acquisition and allocation could explain why male *L. polaris* in the Mingan Islands are more frequent in larger size classes.

We conducted a parallel study that examined biochemical and energetic content of the broadcast spawning sea star *Asterias vulgaris* during the summer spawning period in the Mingan Islands (Raymond 2002). Surprisingly, the reproductive effort (in this case represented by the energetic loss in kJ during gamete release for both sexes) was 6.3 times greater for females than for males. Neither sex invests in parental care. Thus, broadcast spawners can also show marked differences between sexes in investment in gametes. However, this difference in reproductive investment did not result in slower growth of female *A. vulgaris* as the sex ratio was 1 in all size classes. We suggested that females might compensate by feeding more to be able to support both gonadal and somatic investment. This hypothesis is supported by laboratory feeding trials conducted in July 1999 (*A. vulgaris* maintained with an abundant supply of mussels), in which females consumed 3.6 mussels d^{-1} and males 2.4 mussels d^{-1} (J. F. Raymond unpubl. data). This contrasts with *Leptasterias polaris*, for which the energetic loss during spawning was much greater for males. *A. vulgaris* showed decreases in the energetic content of the pyloric caecum during their abrupt spawning, which may have represented the costs of forming spawning aggregations and in releasing gametes. We saw no evidence of a decrease in the pyloric caecum of *L. polaris* during spawning (mean values actually increased, particularly in females). Spawning in *A. vulgaris* differs from that of *L. polaris* because it occurs at higher temperatures (8 to 10°C), and because synchronisation among individuals during spawning is critical, as in all broadcast spawners. These factors could explain the differences observed between the 2 species in their needs for expenditures from the caecum during reproduction.

Future studies

Further studies are needed to explain how brooding may lead to differences between males and females in size structure and population dynamics. Knowledge of the basal metabolic rates of both sexes at different periods of their reproductive cycles, of the feeding rates of males and females, of behaviour during the winter and of mortality rates could indicate how the mode of reproduction affects the size structure in a population of sea stars. Finally, as our analysis of standardised females before and after the brooding period may have underestimated the cost of brooding, studies are required to determine whether females reduce their size and tissue mass over the brooding period.

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