Spatial patterns of maternal investment in *Strongylocentrotus franciscanus* along a marine–terrestrial gradient

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ABSTRACT: In near-shore marine ecosystems, organisms inhabit an interface between marine and terrestrial environments where both systems interact in complex ways. These interactions may occur along gradients in biophysical conditions that vary over spatial and temporal scales. These environmental conditions are known to drive many biological processes with resulting influence on a given species life history. In the present study we examined spatial variation in indices of reproductive fitness, namely fecundity, egg volume, and egg biochemistry in the red sea urchin *Strongylocentrotus franciscanus* along a spatial gradient of decreasing wave energy and increasing terrestrial influence in Barkley Sound, British Columbia, Canada. Stable carbon ($\delta^{13}C$) isotopic analysis was used as a bioindicator of (1) the influence of terrestrially derived carbon in egg production and (2) the concomitant effects of assimilation of terrestrial carbon on egg quality. Stable nitrogen ($\delta^{15}N$) isotopic analysis was used to assess trophic status among and within locations and relate this back to the strength of the gradients. We found that urchins in the lowest energy wave environment released the most eggs compared to all other locations. Egg volume was similar across all sites in the present study; however, within each site, mean egg volume varied among female urchins. Egg biochemistry (e.g. protein, carbohydrate and lipid levels), a proxy for egg quality, was the same at all sites, although analysis of percent carbon, a proxy for lipids, suggested a trend in increasing levels as distance from high wave energy environments increased. No obvious spatial pattern in $\delta^{13}C$ was observed, suggesting that input of terrestrial organic material is well diluted in Barkley Sound. With respect to $\delta^{15}N$, we found little variation in trophic level at which foraging takes place. In contrast there were different $\delta^{13}C$ and $\delta^{15}N$ levels when comparing egg to tube foot tissue, suggesting that temporally *S. franciscanus* was feeding on different food sources or that for these tissue types urchins differentially assimilate carbon and nitrogen isotopes.

KEY WORDS: Urchin · Stable isotopes · Barkley Sound · Terrestrial–marine interactions · Egg biochemistry

INTRODUCTION

In near-shore marine ecosystems, organisms inhabit an interface between marine and terrestrial environments where both systems interact in complex ways. For example, land-based runoff has long been thought to enhance demersal fishery production in various coastal areas where fish abundance follows river discharge variations (Cushing 1995, Lloret et al. 2001). This is believed to reflect a combination of terrestrial land-based nutrient runoff that affects phytoplankton production (see Darnaude 2005) and efficient exploi-

tation of river-borne particulate organic matter (POM) by some benthic organisms, which in turn contribute to the demersal fish web (Darnaude et al. 2004). The influences of terrestrial ecosystems on the near-shore are most pronounced during river floods (Darnaude 2005). Further, the interaction is enhanced in the absence of large estuaries that buffer terrestrial and/or anthropogenic input into the ocean.

The nature of this terrestrial–marine interaction is often expressed in the marine landscape as a gradient of conditions for variables including salinity, chlorophyll, light, terrestrially derived dissolved organic material (DOM) or particulate organic material (POM), and temperature (Longhurst 1998). These biogeochemical gradients provide interesting opportunities to examine their influence on life-history traits in marine benthic invertebrates (McEdward & Carson 1987, Berger et al. 2006). This is particularly important, as there are comparatively few studies of variability in maternal nutrition for benthic invertebrates along unidirectional gradients of environmental conditions. Maternal nutrition and resultant egg properties (e.g. number of eggs, egg size, and egg quality) are known to be closely tied to habitat-mediated differences in food availability and quality (George et al. 1990, Bertram & Strathmann 1998). Indeed, maternal nutritional stress has been found to produce eggs with lower organic content, protein concentrations, and lipid reserves (Bayne et al. 1978, George et al. 1990). Habitat differences that affect urchin nutrition include variation in macroalgal abundance and biochemical composition, which varies along a gradient of wave exposure (Harrold et al. 1988, Diez et al. 2003).

With an increase in storm events on the west coast of North America potentially due to global climate change (Graham & Diaz 2001, Bromirski et al. 2003), there may be an attendant increase in precipitation. Elevated precipitation over coastal terrestrial ecosystems will result in increased levels of terrestrial organic material flowing into nearby marine systems. Freshwater often enters the ocean environment through an estuary, whence it forms a plume (gradient) and becomes a local source of momentum and buoyancy. These waters typically act as an exporter of organic material into marine systems (Day et al. 1989). Therefore, a greater influx of terrestrial organic material into the marine environment occurs along a wave exposure gradient as distance from the open ocean increases. In the context of global climate change, it is necessary to better understand the role of terrestrial–marine interactions given the potential for change to coastal climatology.

The objective of the present study was to investigate whether a gradient of wave energy and land-based

carbon might affect reproductive dynamics in *Strongylocentrotus franciscanus* (A. Agassiz, 1863). Our study area is a large embayment, Barkley Sound, British Columbia, Canada. A hypermaritime climate in the region results in large freshwater outflows from watersheds in the Sound, creating a strong salinity gradient in the channel used in the present study. In addition, heavy winter rains create a strong freshwater lens. The nature of the embayment is also structured such that there is a wave exposure gradient along an east–west axis (Salomon et al. 2006). Thus our study area included a strong terrestrial gradient that weakens with increasing exposure to the open ocean with its attendant gradient of wave energy.

The use of stable carbon isotope analysis can be a valuable tool to infer whether ingested food has a marine or terrestrial origin (Gannes et al. 1997). Lower carbon isotope ratios (more negative) indicate a terrestrial origin and higher values (more positive) a marine origin. Stable nitrogen isotope ratios are frequently used to determine trophic level (McCutchan et al. 2003). Consumption of higher trophic level food sources will result in higher (more positive) isotope ratios. An increase of 3 to 4‰ in nitrogen isotope ratio indicates a shift in trophic level feeding (McCutchan et al. 2003).

We coupled isotope analysis with measurements of life-history traits in urchins. Specifically, we addressed the following questions: (1) Does the number of eggs released vary along a horizontal wave exposure gradient? (2) Does egg volume vary along this gradient? (3) Does egg biochemistry (e.g. protein, carbohydrate, and lipid levels) vary along this gradient? (4) Do stable carbon and nitrogen isotopic signatures in reproductive (i.e. eggs) and somatic tissue (i.e. tube feet) vary along this gradient? A corollary to this last question is: What effect, if any, does carbon source (e.g. marine or terrestrial) have on egg quality?

MATERIALS AND METHODS

Study area. Barkley Sound is a large embayment (>550 km^2) on the west coast of Vancouver Island. It is divided into 3 channels; our study area was located in Trevor Channel, which is narrow and deep (150 m) and leads to the 350 m deep waters of Alberni Inlet. This channel is most heavily influenced by freshwater outflow from the 45 km long Alberni inlet (Tully 1949, Taylor & Haigh 1996), especially during the time an Aleutian Low dominates from late September through May, resulting in significant precipitation in coastal ecosystems. Thus, near-surface salinities show that freshest waters appear during winter, which coincides with rainfall maxima. For example, in the month of

May, Romanuk & Levings (2006) found surface salinities ranging from 4 to 33 (mean 25.8, SD 6.9). At the same time the winds are predominantly equatorward along the coast, resulting from the North Pacific high pressure system, which is favorable for upwelling. Intermediate waters exchange with the ocean in January through April; at other times of the year they stagnate and dissolved O_2 levels drop to as low as 1 ml l^{-1}. Tides are generally in-phase over the Sound and tidal mixing is weak.

Collection and sample preparation. *Strongylocentrotus franciscanus* were collected between 25 May to 1 June 2005 using SCUBA at 6 sites along a 14 km transect running in a southwest to northeast direction in Barkley Sound, British Columbia, Canada (48° 50.04′ N, 125° 08.21′ W). Approximately 50 urchins were collected subtidally at 8 m depth on the southeast side of islands in the Deer Group, specifically Bordelais, Edward King, Diana, Wizard, Fleming and Tzartus (Fig. 1, Table 1). Urchins were abundant at all sites. Substrate was algal covered rocks at all sites except Fleming, which was sand interspersed with boulders. During the sampling period, adult urchins were separated according to collection site and maintained in a seawater table at ambient temperature. Due to the time consuming process of collecting urchins at multiple sites, collecting and aliquoting egg samples, and collecting tube feet, urchins were held without access to food for a maximum of 17 d. Spawning was randomized by site to reduce bias. Short-term starvation is suggested to have a limited impact. For example, wet body mass of the urchin *Diadema antillarum* did not decrease over a 2 wk period of starvation (Levitan 1989). Additionally, Lares & Pomory (1998) have suggested that urchin gonad material is metabolized during long-term periods of starvation exceeding 4 wk.

Spawning was induced by intracoelomically injecting 5 ml of 0.55 M KCl through the oral disk; eggs were collected and washed in seawater. Total number of eggs per individual (i.e. fecundity) was estimated by determining the density of eggs in 6 replicate subsamples. To measure egg size, a sample of eggs was pipetted onto a glass slide and digitally photographed
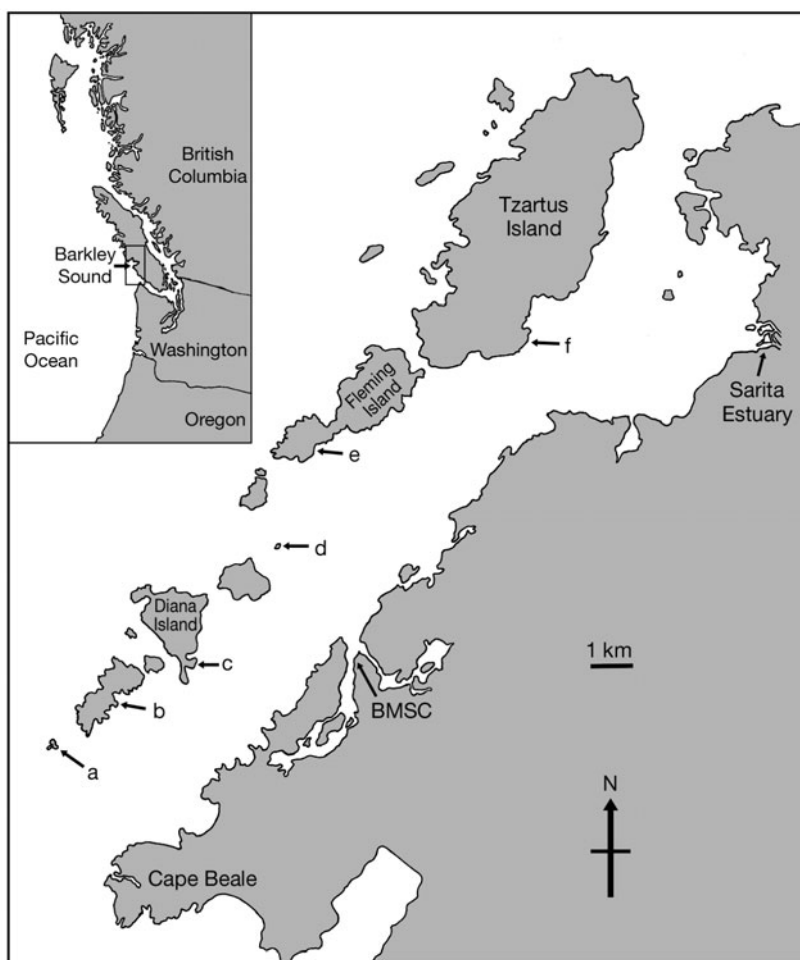

Fig. 1. Study sites along a southwest to northeast wave exposure gradient within Barkley Sound, British Columbia, Canada. See inset for approximate geographic location. The 6 study sites are labeled as (a) Bordelais, (b) Edward King, (c) Diana, (d) Wizard, (e) Fleming, and (f) Tzartus. Arrows indicate collection site on the southeast side of each island. Urchins were collected in the subtidal zones at all sites and were not shaded by trees, cliffs, or other island structures. The Bordelais Island site (a) experienced the highest wave energy. The Tzartus Island site experienced the lowest wave energy and was closest in proximity to freshwater discharge from the Sarita estuary. BMSC: Bamfield Marine Sciences Centre

Table 1. Study sites in Barkley Sound, British Columbia. Height of *Verrucaria* sp. refers to the mean (n = 3) upper distributional limit above mean lower low water (MLLW) ±95% CI (see 'Wave exposure'). Lowercase letters indicate significant difference based on a Tukey post hoc analysis (p < 0.05). See Fig. 1 for site locations

Site	Distance along transect (km)	Height of *Verrucaria* sp. above MLLW (m)
Bordelais	0	11.8 ± 0.28 a
Edward King	1.7	6.5 ± 0.52 b
Diana	3.1	5.1 ± 0.33 c
Wizard	6.6	4.1 ± 0.20 d
Fleming	8.8	4.6 ± 0.36 c,d
Tzartus	13.6	4.5 ± 0.57 c,d

through a compound microscope at 20× magnification. Maximum egg diameter of the first 200 eggs encountered was calculated digitally using ImageJ (version 1.33, NIH). Egg volume (*V*) was estimated as a sphere using $V = 4/3 \, \pi r^3$, where r is the radius. To quantify egg biochemistry and for stable isotope analysis, 30 000 eggs were estimated volumetrically from a solution of eggs suspended in seawater. Aliquots of 30 000 eggs were placed into replicate 1.7 ml microcentrifuge tubes and gently centrifuged at $100 \times g$ for 1 min. The remaining seawater was removed, and samples were frozen at –80°C for later analysis.

Wave exposure. The intertidal lichen *Verrucaria* sp., which was observed at all study sites, was used as a biological indicator of wave energy. The upper distributional limit of intertidal lichens increases in areas of high wave energy and resulting wave splash (Ballantine 1961, Reimchen 1979). On islands adjacent to all collection sites, a sight level was used to measure the highest elevation above mean lower low water (MLLW) for *Verrucaria* sp.

Egg biochemistry. Protein levels were determined by homogenizing lyophilized aliquots of 30 000 eggs in 350 µl distilled water, centrifuging at $8000 \times g$ for 5 min at 4°C and removing the supernatant. Protein concentration was calculated spectrophotometrically at 595 nm using a Coomassie Blue assay (BioRad) based on a Bradford protein assay (Bradford 1976) with bovine serum albumin as a standard. Proteins were not extracted in trichloroacetic acid (TCA). A preliminary experiment indicated no difference in protein levels from egg samples with protein extracted in TCA compared to samples that did not have protein extracted.

Total lipids were extracted from lyophilized aliquots of 30 000 eggs following the charring method of Bligh & Dyer (1959) as modified by Holland & Gabbott (1971). Briefly, lyophilized aliquots of 30 000 eggs were extracted in water:methanol:chloroform (2:2:1) for 2 h. A total of 200 µl distilled water and 200 µl chloroform were then added for a final water:methanol:chloroform ratio (v:v:v) of 4:2:3 and then centrifuged at $8000 \times g$ for 10 min. The lower phase was removed and dried under nitrogen. Samples were re-dissolved in 350 µl chloroform, and a 50 µl subsample was dried under nitrogen followed by the addition of 500 µl sulfuric acid. Samples were heated at 140°C for 15 min, cooled, and diluted with 1.5 ml distilled water. Lipid concentration was calculated spectrophotometrically at 375 nm using tripalmitin as a standard.

Carbohydrate levels were determined using a ferricyanide reduction reaction following the methods of Holland & Gabbott (1971). Accordingly, lyophilized aliquots of 30 000 eggs were homogenized in 500 µl distilled water, protein was extracted in cold 5% TCA and the supernatant removed. The supernatant was hydrolyzed in 1 M HCl at 95°C for 2 h. Carbohydrate concentration was calculated spectrophotometrically at 420 nm using glucose as a standard.

Stable isotope analysis. Stable carbon and nitrogen isotope analyses were performed on *Strongylocentrotus franciscanus* eggs and somatic tissue (i.e. tube feet). Immediately following spawning, tube feet were removed and frozen at –80°C for later analysis. All samples were dried at 60°C to constant mass and then ground with a mortar and pestle to a fine powder after which 1 mg (±0.1 mg) of each sample was encapsulated in tin. Isotopic ratios of carbon and nitrogen were measured using an ANCA G/S/L sample preparation module coupled to a Tracer/20 mass spectrometer (Europa Scientific) at the Department of Soil Sciences, University of Saskatchewan. Ratios of carbon and nitrogen isotopes are expressed as:

$$\delta X \, (‰) = [(R_{\text{sample}}/R_{\text{reference}}) - 1] \times 10^3$$

where δ = the measure of heavy to light isotope in the sample, $X = {}^{13}\text{C}$ or ^{15}N and *R* is the corresponding ratio of $^{13}\text{C}{:}^{12}\text{C}$ or $^{15}\text{N}{:}^{14}\text{N}$ (Peterson & Fry 1987). We also measured percent carbon and nitrogen as proxies for lipid and protein levels, respectively (Holland 1978), for both eggs and tube feet tissue. International Standard references are Vienna Pee Dee Belemnite (VPDB) for carbon and atmospheric N_2 for nitrogen.

Data analysis. Data were analyzed with Systat (version 9.0, SPSS) and STATISTICA (version 6.0, StatSoft). To test the effect of location on fecundity, protein levels, carbohydrate levels and lipid levels, we used a 1-way ANOVA followed by a Tukey post hoc test to explore differences between sites. To test the effect of location on egg volume, a nested ANOVA was performed with female urchin (random factor) nested within location (fixed factor). To test the effect of location and tissue type on carbon and nitrogen stable isotope ratios, we used a 2-way factorial ANOVA for each isotope independently.

RESULTS

Using *Verrucaria* sp. as a biological indicator of relative wave energy, decreased wave energy was observed along the transect from Bordelais to Wizard Island (ANOVA, $F_{5,12} = 201$, p = 0.00; Table 1). No measurable difference was recorded for sites among the islands of Wizard, Fleming, or Tzartus.

Location as a variable had a significant effect on *Strongylocentrotus franciscanus* fecundity (ANOVA, $F_{5,51} = 3.46$, p = 0.009; Fig. 2). Fecundity at Tzartus was at least 3 times higher compared to Bordelais, Edward King, or Wizard (Tukey honestly significant difference test, HSD, df = 51, p < 0.05). However, fecundity at
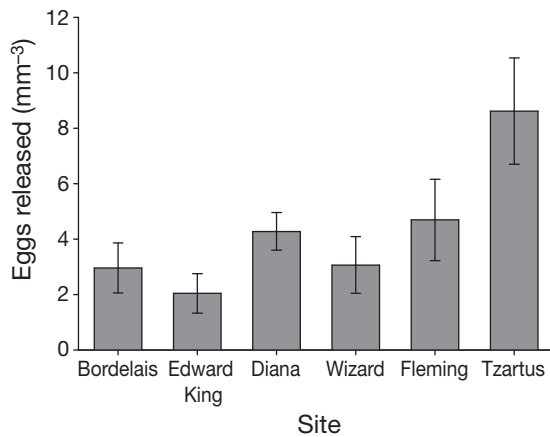
Fig. 2. *Strongylocentrotus franciscanus*. Fecundity of red sea urchins at 6 sites along a horizontal gradient in Barkley Sound. Number of eggs *S. franciscanus* released was normalized to test volume (test diameter^3). Each bar represents the mean of 10 urchins, with the exception of 7 at Edward King. Error bars are ±1 SE

Fig. 4. *Strongylocentrotus franciscanus*. Egg biochemistry at 6 sites along a horizontal gradient in Barkley Sound for (a) protein levels, (b) carbohydrate levels, and (c) total lipid levels. Bars represent the mean from 10 individual females, with the exception of 7 at Edward King. Error bars are ±1 SE

Tzartus was not significantly higher (p > 0.05) compared to Diana and Fleming. Although the number of eggs released increased with adult test volume (r = 0.33, p = 0.01), adult test volume was not significantly correlated with individual egg volume (r = 0.07, p = 0.61).

Across locations egg volume did not significantly vary (ANOVA, $F_{5,49}$ = 1.6, p = 0.27). However, egg volume did significantly vary between females within a site (ANOVA, $F_{49,11106}$ = 279, p < 0.001; Fig. 3).

Egg biochemistry, used as a proxy for egg quality, remained constant across all locations (Fig. 4). Protein levels (ANOVA, $F_{5,51}$ = 2.12, p = 0.08), carbohydrate levels (ANOVA, $F_{5,51}$ = 1.93, p = 0.1), and lipid levels (ANOVA, $F_{5,51}$ = 2.28, p = 0.06) did not significantly

vary across locations. There was no significant correlation between egg volume and levels of protein (r = 0.198, p = 0.14), carbohydrate (r = 0.03, p = 0.87), or lipid (r = 0.04, p = 0.77). Although mean percent carbon, a proxy for lipid level, was not significantly different between sites (ANOVA, $F_{5,48}$ = 1.04, p = 0.41), mean levels did increase as much as 4.4 % as distance from the open coast increased (Table 2).

The δ^{13}C values in *Strongylocentrotus franciscanus* eggs and tube feet showed variation along a horizontal gradient in Barkley Sound (ANOVA, $F_{5,48}$ = 23.17, p = 0.000). Lower carbon isotope ratios (more negative) indicate a terrestrial origin and higher values (more positive) a marine origin. At both ends of the gradient (i.e. Bordelais and Tzartus), δ^{13}C values were more negative compared to all other locations (Tukey HSD, df = 48, p < 0.05; Fig. 5a). Across all locations δ^{13}C
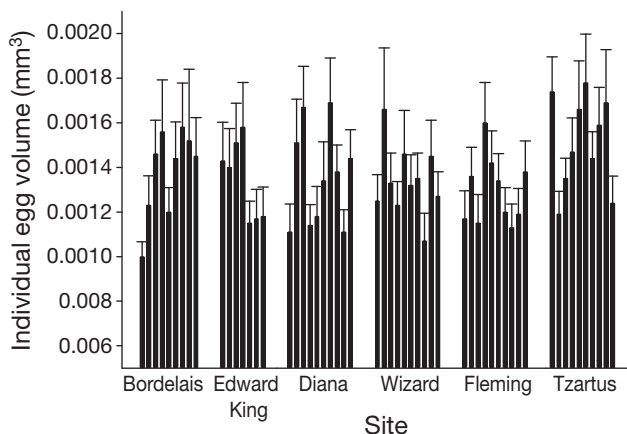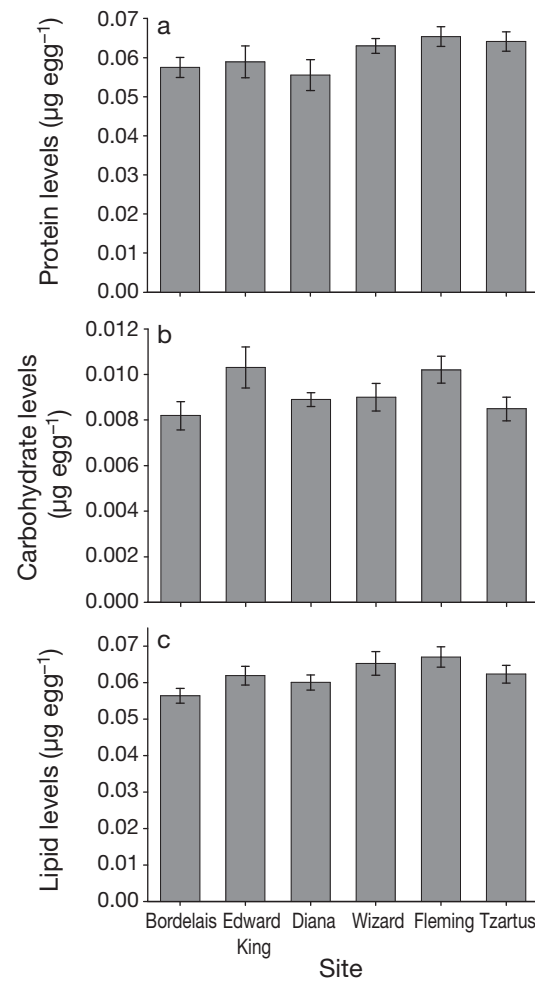

Fig. 3. *Strongylocentrotus franciscanus*. Egg volume from individual females at 6 sites along a horizontal gradient in Barkley Sound. Each bar represents the mean of 200 eggs measured from 1 female; error bars are +1 SE

Table 2. *Strongylocentrotus franciscanus*. Mean percent carbon (C) and percent nitrogen (N) in egg and tube foot tissue at 6 sites in Barkley Sound, British Columbia. Values in parentheses represent 1 SE

Site	Egg % C	Tube foot % C	Egg % N	Tube foot % N
Bordelais	43.67 (2.37)	37.53 (2.03)	8.47 (0.45)	8.89 (0.53)
Edward King	44.43 (2.75)	31.26 (1.95)	8.69 (0.50)	6.97 (0.50)
Diana	44.78 (2.28)	36.08 (1.43)	8.74 (0.47)	8.68 (0.56)
Wizard	45.99 (1.95)	37.65 (1.82)	9.42 (0.50)	9.16 (0.67)
Fleming	46.82 (2.53)	36.94 (1.58)	9.37 (0.54)	8.23 (0.35)
Tzartus	48.04 (2.86)	34.94 (0.74)	9.29 (0.57)	8.10 (0.21)

values were significantly more negative in eggs compared to tube feet (ANOVA, $F_{1,48}$ = 410.94, p = 0.000; Fig. 5a) with combined mean values (SE) for eggs and tube feet of –17.03 (0.19) and –14.03 (0.17), respectively.

The $\delta^{15}N$ values for eggs and tube feet also varied spatially (ANOVA, $F_{5,48}$ = 5.97, p = 0.000) with the highest values observed at Wizard and Fleming for eggs and Diana and Wizard for tube feet (Fig. 5b). The
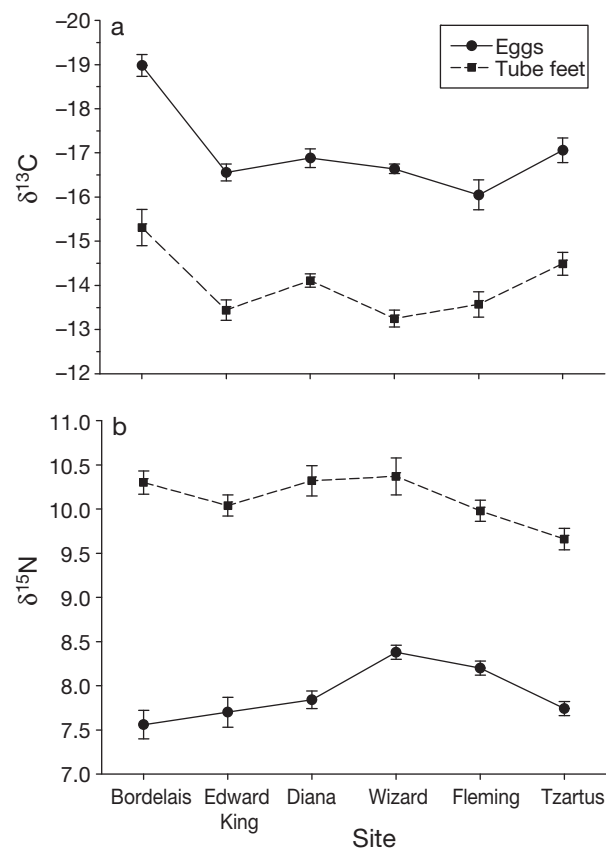
$\delta^{15}N$ values were ca. 2 to 3‰ lower in egg samples compared to tube feet across all locations (ANOVA, $F_{1,48}$ = 806.15, p = 0.000; Fig. 5b).

Eggs had a higher percent carbon compared to tube feet (ANOVA, $F_{1,48}$ = 66.43, p = 0.000), but percent nitrogen was similar between the 2 tissue types (ANOVA, $F_{1,48}$ = 3.46, p = 0.069; Table 2).

DISCUSSION

We hypothesized that reproductive traits in *Strongylocentrotus franciscanus* would vary along a spatial gradient where environmental conditions in Barkley Sound were influenced by wave exposure and terrestrial runoff. Over the range of conditions that occurs in Barkley Sound, we found that a decrease in wave exposure concomitant with an increase in terrestrial influence resulted in only site-specific variation in fecundity. We did not find change in egg volume or egg biochemistry between sites.

Life-history models suggest that a tradeoff between number and size of eggs produced should result in an optimal egg size (Smith & Fretwell 1974, Stearns 1992). Larger eggs have been shown to confer higher larval and post-metamorphic fitness (Emlet & Hoegh-Guldberg 1997, Bertram & Strathmann 1998) or increase the probability of fertilization success (Levitan 1996, but see Styan et al. 2005). However, intrapopulation variation in egg size is common in many species (Turner & Lawrence 1979, McEdward & Carson 1987, Jones et al. 1996). We show this to be the case for *Strongylocentrotus franciscanus* in the present study, where within a population that experiences similar environmental conditions, significant variation in egg volume occurred between mothers.

In terms of egg size between populations arrayed along a gradient of wave energy, we found no effect. Site-specific changes in marine invertebrate egg size have been shown to occur along depth gradients (Bertram & Strathmann 1998), wave exposure gradients (George et al. 1990; however, see McEdward & Carson 1987), and among other environmental gradients (Jones et al. 1996). Food limitation, temperature, and salinity, which all could vary along our sampled transect, have been proposed for between-population differences in egg size (Emlet et al. 1987, Garrido & Barber 2001). Our data suggests that strong environmental gradients do not necessarily result in site-specific changes in size and composition of eggs.

Stronglyocentrotus franciscanus, in a low wave energy environment and in close proximity to an estu-

Fig. 5. *Strongylocentrotus franciscanus*. Stable (a) $\delta^{13}C$ and (b) $\delta^{15}N$ isotope values for egg and tube feet tissue collected along a horizontal gradient in Barkley Sound, British Columbia. Each data point represents the mean of 3 individuals; error bars are ±1 SE

arine environment, released the greatest number of eggs. Food limitation may decrease the number of off-spring produced (Keats et al. 1984, Meidel & Scheibling 1999). We did not measure the quality and quantity of available food at the study sites and can only infer from nitrogen isotope ratios that urchins were obtaining food from different sources. This is not surprising because *S. franciscanus* is an opportunistic herbivore known to forage on attached and adrift macroalgae, encrusting organisms, and diatoms (reviewed in Lawrence 1975). *S. franciscanus* has also been observed to opportunistically scavenge on salp tests (Duggins 1981). One explanation for an increase in fecundity at the site furthest from the open coast could be an increase in the quality or quantity of available food. Exposure to increased levels of wave energy can either increase energy expenditures by forcing urchins to migrate to refugia or decrease available feeding time while the urchin remains strongly attached to the substrate (Kawamata 1998, Siddon & Witman 2003). Additionally, urchins in high wave energy sites often experience spine damage (Ebert 1968), which has been to shown to allocate resources to spine, test, and lantern calcification (Edwards & Ebert 1991). An alternative explanation is that increased energy expenditures at wave-exposed sites resulted in reduced urchin fecundity.

Environmental conditions have been shown to affect egg biochemistry in marine invertebrates (Bayne et al. 1978, George et al. 1990). In the present study, lipid, carbohydrate, and protein levels did not vary significantly between sites. However, a trend for an increase in percent carbon in eggs with distance from the open coast did occur, suggesting that sites with less wave exposure and/or an increased terrestrial input had eggs with higher lipid levels. Lipid levels have been shown to correlate with carbon content (Petersen & Anger 1997). Examining percent carbon may be a more precise measure of lipid levels than the charring method of Bligh & Dyer (1959) used in the present study. Relatively high levels of phospholipids may have masked site-specific differences in lipid levels analyzed with the charring method.

We hypothesized that terrestrial carbon may influence egg gametogenesis and that this relationship is expressed along a gradient of decreasing distance from estuarine sources. However, and contrary to our hypothesis, we found little variation in carbon isotope signatures and, paradoxically, the site farthest from estuarine sources had the strongest terrestrial signal. Our best explanation for this is that high intensity waves 'wash' locally produced carbon from Bordelais Island into the adjacent subtidal zone in a concentration greater than that transported long distance from distant upland and estuarine sources. In nearby Clay-oquot Sound, Mulkins et al. (2002) mapped the carbon isotopic signature of mysids in a bay and found the strongest terrestrial signal at the most marine-dominated site in the study. They attributed the lack of an obvious spatial pattern to hydrodynamic conditions 'diluting' terrestrial organic matter in complex ways (Mulkins et al. 2002).

Although there were statistically different $\delta^{15}N$ values across sites, those differences were always less than 1‰. A change of ca. 3 to 4‰ in $\delta^{15}N$ indicates a shift in trophic level (DeNiro & Epstein 1981, McCutchan et al. 2003). Therefore, small site-specific differences of $\delta^{15}N$ in the present study do not reflect any obvious trophic level shift in feeding but rather reflect site-specific feeding opportunities.

Of interest are the tissue-specific differences in carbon and nitrogen isotopic signatures. A ca. 3‰ decrease in $\delta^{13}C$ was observed in urchin eggs compared to tube feet. Urchin eggs are high in lipid compared to the muscle tissue of tube feet (Van Dover et al. 1992). Since lipids tend to be depleted in ^{13}C (DeNiro & Epstein 1978, Focken & Becker 1998), a more negative $\delta^{13}C$ value in egg samples is explained by relatively high lipid levels. McCutchan et al. (2003) found a change of ca. 1‰ in $\delta^{13}C$ values when lipid-free muscle tissue was compared to muscle tissue containing lipids. Our differences were greater than 1‰ between tissue types; high levels of lipids in egg samples might not account for the total difference. Therefore, we suggest that a shift in $\delta^{13}C$ value between eggs and tube feet was in part an artifact of tissue type, but also may be due to the uptake of DOM by tube feet (discussed below).

Two potential scenarios may explain why $\delta^{15}N$ levels were ca. 2 to 3‰ higher in tube feet compared to eggs. First, eggs and tube feet may be formed at different times when red urchins are feeding on different food sources at different times of the year, suggesting that a temporal shift in trophic feeding level may have occurred. As pointed out previously, *Strongylocentrotus franciscanus* are opportunistic with a variable diet (Duggins 1981). We know that $\delta^{15}N$ derived from food can vary over temporal scales and be integrated over time (Hesslein et al. 1992, Jennings et al. 1997). *S. franciscanus* oocyte growth occurs during late summer and fall with maximal development during October in southern British Columbia (Bernard 1977, Pearse 1981), and the signatures in tube feet may represent integration over a longer time period than oocyte formation. Second, the difference in $\delta^{15}N$ values between tissue types might instead be due to uptake of DOM by external tissues such as tube feet. Echinoderms take up DOM in external epidermal tissue, including tube feet, without taking up DOM into internal tissues, such as gonads (Fontaine & Chia 1968, Ferguson 1982).

To conclude, our findings suggest that despite the strong gradients of land-based runoff and wave exposure in Barkley Sound, size, and composition of eggs in urchins are surprisingly consistent between sites. This suggests that urchins have significant phenotypic plasticity with respect to resource allocation and fitness-related proxies. Except for fecundity at the extreme end of the gradient, urchin egg size and composition are not impacted by increased runoff in the near-shore coastal zone. This finding is intriguing insofar as possible consequences of climate change and increased freshwater runoff of the near-shore coastal zone. We posit that in environments such as Barkley Sound, urchins will be largely unaffected by an enhanced winter estuarine pump.

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