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Field evidence of interpopulation variation in oocyte size of a marine invertebrate under contrasting temperature and food availability

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ABSTRACT: In marine invertebrates with planktotrophic larvae, modelling studies predict that water temperature and food availability are the primary factors influencing oocyte size, with large oocyte sizes being favoured by cold water and low food availability. We examined intraspecific variation in oocyte size in the polychaete *Spirobranchus cariniferus* in populations from the East and West Coasts of New Zealand's North Island, which differ in temperature and food availability during the reproductive season: the East Coast has consistently warmer seawater temperatures throughout the summer and higher phytoplankton abundance in late summer than the West Coast. A cross-fertilization experiment and DNA sequencing confirmed that *Spirobranchus* populations on both coasts belong to the same species. Then, analyses of *S. cariniferus* oocytes from 5 sites per coast revealed that the West Coast population produces oocytes that are on average 14% larger and contain up to 41% more triacylglycerols (TAGs) than the East Coast population. West Coast *S. cariniferus* oocytes also developed into larger larvae that reached competence 22% sooner than East Coast oocytes and larvae. These intraspecific comparisons of East and West Coast *S. cariniferus* populations reveal oocyte size to differ substantially even among populations that are probably not fully isolated, consistent with predictions of life history models. We estimate that genetic divergence may be responsible for up to 58% of the difference in oocyte size between East and West Coast populations, and that oocyte size may be subject to local adaptation to temperature and, to a lesser extent, food availability differences between coasts.

KEY WORDS: Offspring size · Life history strategy · Maternal provisioning · Egg size · Biogeography · Phenotypic plasticity · Environmental effects · Genetic effects

1. INTRODUCTION

Variation in offspring size has been widely documented in marine invertebrates (Strathmann & Vedder 1977, McEdward & Morgan 2001, Gosselin & Rehak 2007, Luttikhuizen et al. 2011). Inspired by a seminal paper by Vance (1973), studies seeking to elucidate the causes of this variation have largely focused on 2 central tenets of life-history theory (Smith & Fretwell 1974): the relationship between environmental conditions and offspring size, and the effect of offspring size on offspring performance. For species with planktonic larvae, water temperature and food availability have been identified as factors likely to have acted as major selective pressures on offspring size (Levitan 2000), primarily through their influence on offspring performance. Embryonic and larval development rates increase, and planktonic larval duration (PLD) decreases with increasing water temperature (Thorson 1950, Emlet 1995, O'Connor et al. 2007), and for species with planktotrophic larvae, PLD decreases with increasing food concen-

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tration (Thorson 1950, Olson & Olson 1989, Bos et al. 2006). Accordingly, modelling work by Levitan (2000) suggested cold temperature and low food availability select for larger offspring containing more energy reserves that shorten the PLD and thus reduce planktonic mortality, whereas warmer temperature and higher food availability select for relatively lower maternal provisioning per offspring, resulting in longer PLD but allowing greater fecundity. Numerous experimental studies have confirmed that temperature (Palmer 1994, Hoegh-Guldberg & Pearse 1995, Nomaguchi et al. 1997) and food availability (Paulay et al. 1985, Allison 1994, Fenaux et al. 1994) are major determinants of PLD, but few studies have examined whether offspring size or energy reserves vary among natural populations according to the predicted effects of water temperature and food availability.

An effective way to document the responsiveness of a life history trait in natural populations to a postulated selective pressure is to examine intraspecific variation in the trait, especially variation among populations. This approach avoids the confounding effects of phylogenetic constraints inherent to interspecific comparisons (Lessios 1990, Bernardo 1996, Räsänen et al. 2005, Collin & Salazar 2010). Surprisingly, empirical studies of interpopulation variation in offspring size are not common. In addition, those studies that have examined interpopulation variation have mostly compared only 2 locations (e.g. George 1994, Simonini & Prevedelli 2003, Collin & Salazar 2010) or compared a set of populations located along a latitudinal gradient (Hagström & Lönning 1967, Dugan et al. 1991, Lardies & Castilla 2001), which experience the confounding effects of environmental gradients associated with latitude such as temperature, day length and seasonality (Levitan 2000, Collin 2003) and intraspecific differences in phenology (e.g. Räsänen et al. 2005, Lindgren & Laurila 2010). Ideally, empirical field studies of variation in offspring size should aim to examine intraspecific variation, document small-scale (intrapopulation) as well as larger scale (interpopulation) variation (Bernardo 1996) and compare isolated populations located at the same latitude but experiencing different environmental conditions. Field settings satisfying all of these requirements do exist, such as on New Zealand's North Island.

Oceanographic studies of the East and West Coasts of the North Island have revealed contrasting surface seawater temperature (Chiswell 1994, Zeldis et al. 2004) and phytoplankton abundance (Chiswell & Booth 2008, Gall & Zeldis 2011). These studies report that nearshore surface seawater temperatures along the East Coast at latitudes north of East Cape (~37° 30′ S) are up to 2°C warmer and sustain higher phytoplankton abundance than at similar latitudes along the West Coast. If these differences between the East and West Coasts are persistent, and if the proposed influence of temperature and food availability on offspring size (Levitan 2000, Moran & McAlister 2009) is correct, then the conditions along the coasts of the North Island should favour offspring that are larger and have a shorter PLD on the West Coast than on the East Coast.

To further quantify and compare the environmental conditions prevailing on each coast of the North Island, we examined a time-series of satellite measurements of sea surface temperature (SST) and of chlorophyll *a* (chl *a*) for both coasts. We then examined intraspecific variation in offspring size (oocytes) on each coast in the serpulid polychaete *Spirobranchus cariniferus*; this species produces planktotrophic larvae (Gosselin & Sewell 2013) and is abundant on rocky intertidal shores along the coasts of the North Island (Morton 2004, Schiel 2006). Given the paucity of published information on *S. cariniferus*, we began by verifying that East and West Coast populations are of the same species, not cryptic species (e.g. McGovern & Hellberg 2003), using cross-fertilization and genetic similarity analyses. Following this, we studied populations located on opposite coasts but at similar latitudes to address the following goals: (1) determine if oocyte size and maternal provisioning of lipids varies among wild populations on the North Island's West and East Coasts in a way that is consistent with predicted effects of temperature and food availability; (2) determine if oocyte size and lipid content have consequences for offspring performance (larval size, larval development rate, and post-metamorphic size); and (3) assess whether oocyte size might be influenced by phenotypic responses of females by determining (3.1) if oocyte size varies with parental body size, (3.2) if populations with large females produce large oocytes, and (3.3) if a population's average oocyte size varies during the reproductive season.

2. MATERIALS AND METHODS

2.1. SST and chl *a* conditions on East and West Coasts

Measurements of SST and chl *a* made by the Moderate Resolution Imaging Spectroradiometer (MODIS)

on the Terra and Aqua satellites on both coasts of the North Island were obtained from NASA's Giovanni website (http://giovanni.gsfc.nasa.gov/giovanni/) for the years 2002 to 2017. West Coast measurements were obtained for an area ranging from the mouth of Manukau Harbour to Muriwai, within a rectangular area (bounding box) delimited by the longitudes 174.3935 to 174.4855° E and by the latitudes 36.8564 to 37.0651° S (Fig. 1). East Coast measurements were obtained for an area ranging from Auckland to Torbay, within a rectangular area bounded by the longitudes 174.7956 to 175.1307° E, and by the latitudes 36.6916 to 36.8454° S (Fig. 1). The shape, size and location of the bounding boxes were selected to (1) be positioned close to, and parallel to, each coast, where the planktonic larvae of *Spirobranchus* are most likely to develop; (2) be large enough to represent average conditions along each coast; and (3) be in the near vicinity of most study sites. The figures in Chiswell (1994) and Zeldis et al. (2004), although not finely detailed, show SST conditions along each coast as being quite homogenous along sections of each coast enclosing the 5 study sites; this suggests the conditions within the bounding boxes used here are representative of the broader area enclosing the 5 sites on each coast. The analysis of SST and chl *a* data focused on the months of November to April, the period when reproduction, larval development and settlement take place in *S. cariniferus* (Gosselin & Sewell 2013).

2.2. Do East and West Coast populations of *Spirobranchus* belong to the same species?

Two approaches were used: a cross-fertilization experiment and comparisons of sequenced DNA.

2.2.1. Cross-fertilization experiment

Clusters of adult worms were gently detached from the rock at one East Coast site (Glendowie, GL) and one West Coast site (Mill Bay, MB) (Fig. 1) on the morning of 17 March 2012 and transported to the laboratory. Spawning was induced within 24 h of collection in 14 worms from each site by gently cracking their tubes, as described by Gosselin & Sewell (2013). All oocytes from the same site were combined into one beaker (East: 5 females; West: 6 females), and all sperm from the same site were combined into a second beaker (East: 5 males; West: 4 males). Two separate cross-fertilization trials (East oocytes with West sperm, West oocytes with East sperm) were conducted by combining gametes for 10 min, then rinsing excess sperm from fertilized eggs, and finally placing eggs in beakers with 5 µm filtered seawater for 36 h (Gosselin & Sewell 2013). Large numbers of trochophores were obtained in both cross-fertilization cultures; for each culture, a haphazard sample of ~2000 larvae was then transferred to a beaker with 400 ml of seawater, and fed every 3 d with ~0.5 to 1 ×

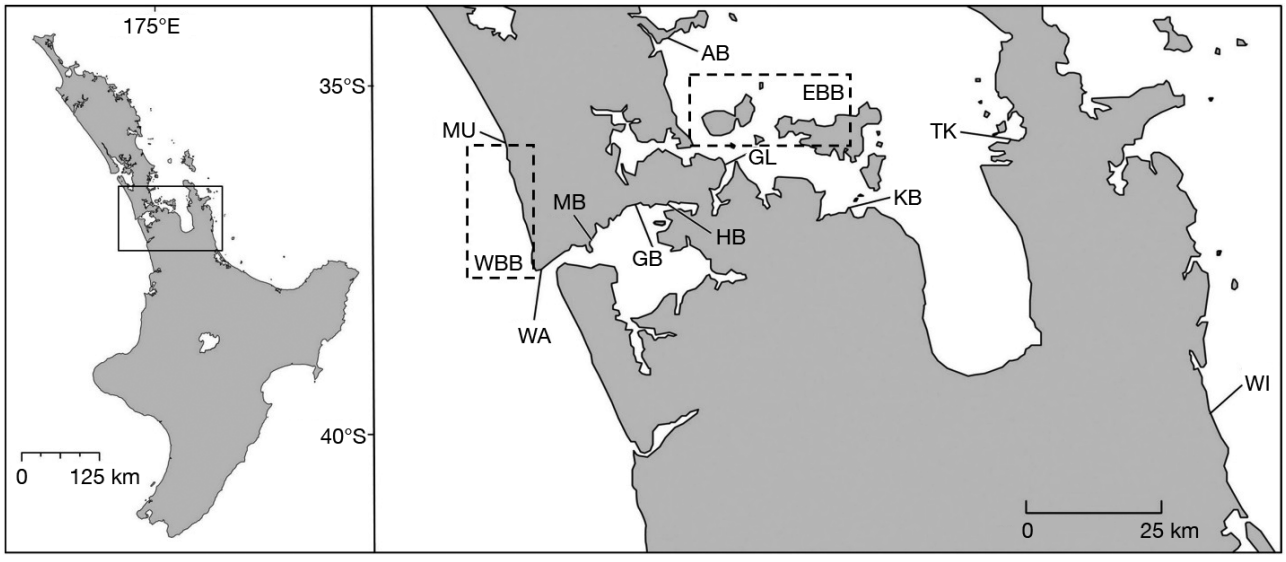

Fig. 1. The 10 study sites on New Zealand's North Island, with an enlarged view of the north-central area. East coast: AB: Arkles Bay; GL: Glendowie; KB: Kawakawa Bay; TK: Te Kouma; WI: Whiritoa. West coast: MU: Muriwai; WA: Whatipu; MB: Mill Bay; GB: Green Bay; HB: Hillsborough Bay. Dashed rectangles: west bounding box (WBB) and east bounding box (EBB) from which satellite measurements of sea surface temperature and chl *a* were obtained; pixels located on terrestrial habitats are not included in satellite measurements. Map modified from Gosselin & Sewell (2013)

10^6 cells ml^{-1} of mixed algae (equal parts *Isochrysis galbana*, *Chaetoceros muelleri* and *Dunaliella tertiolecta*). At this starting density (~5 larvae ml^{-1}), larvae survived well and developed normally to competence, as described in Section 3.2.1. Survivorship was estimated by monitoring larval densities in each of three 3 ml samples $culture^{-1}$ every 3 d up to Day 11 post-fertilization. Larval competence was tested on Day 11 by placing 10 larvae from each culture in 10 ml of 10^{-4} M 3-isobutyl-1-methylxanthine (IBMX) for 24 h (Gosselin & Sewell 2013).

2.2.2. Molecular comparisons

Genetic similarity between the East and West Coast populations was examined in worms collected at the GL and MB sites in May 2012. Sample sizes were 9 worms per population for 18S analysis, 8 worms $population^{-1}$ for 28S analysis, and for cytochrome *b* we examined 14 worms from GL and 9 from MB.

DNA extraction and PCR amplification. DNA was extracted using a combined Chelex and Proteinase K method (Heimeier et al. 2010) or a Proteinase K, phenol/chloroform method (Sambrook et al. 1989). PCR amplification targeted partial sequences from 2 nuclear markers (18S and 28S rDNA) and 1 mitochondrial marker (cytochrome *b*) using primers listed in Table S1 in the Supplement at www.int-res.com/articles/suppl/m619p069_supp.pdf. PCR regimes consisted of an initial denaturation at 95°C for 1 min, followed by 35 cycles of 95°C for 20 s, then 20 s annealing at different temperatures for each primer pair (60°C for 18S, 52°C for 28S and 50°C for cytochrome *b*) and 40 s extension at 72°C (2 min for 18S), with a final extension step of 3 min (10 min for 18S). PCR products were visualized on a 1.6% agarose gel, purified of carry-over deoxyribonucleotide triphosphates (dNTPs) and unused primers using Shrimp-Alkaline Phosphatase/Exonuclease (GE Healthcare), and di-deoxy sequenced using BigDye® Terminator v.5.1 chemistry (Applied Biosystems) and run on an ABI3130 automated capillary sequencer (Applied Biosystems). To get full coverage of the 18S locus, 2 internal primers were used for the sequencing reaction.

Alignment and species identification. Sequenced DNA reads were imported into Geneious v.5.6.4 (Drummond et al. 2011) where primers and bases with low quality Phred scores were trimmed. The resulting sequences were aligned against reference sequences downloaded from the NCBI website (Table S2 in the Supplement) using MAFFT (Katoh et al. 2002). These alignments were subsequently tested for nucleotide substitution saturation using the method of Xia et al. (2003) as implemented in DAMBE (Xia & Xie 2001), with the 3 coding positions of cytochrome *b* being analyzed independently. The best model of nucleotide substitution was selected using MODELGENERATOR (Keane et al. 2006), and the selected model was then used to construct maximum likelihood trees with PHYML (Guindon & Gascuel 2003) with 1000 bootstrap replicates. To search for cryptic species, we analysed the bootstrap support of each clade, the ratio of intra- to intergroup tree distances, and the probability of successful identification. These statistics were computed using the Species Delimitation plugin (Masters et al. 2011) available for Geneious.

2.3. Do East and West Coast populations differ in oocyte size and lipid content?

Variation in maternal provisioning within the population on each coast and between coasts was documented by measuring oocyte size in *S. cariniferus* at 5 sites per coast (Fig. 1) and oocyte lipid content at 1 site per coast; the 5 sites on each coast represent distinct replicate samples of the *S. cariniferus* population on each coast. Adult worms were collected on trips alternating between the East and West Coast sites from 21 December 2011 to 9 February 2012. Spawning was induced in 24 to 30 worms per site as described by Gosselin & Sewell (2013), and each worm was placed in a separate glass vial with 20 ml of 5 µm filtered seawater for 30 min. The diameter of 10 haphazardly chosen oocytes per female was measured under a compound microscope (400×, accuracy 1.25 µm) for the first 10 females to release an abundance of oocytes; oocyte volume was calculated using the equation for the volume of a sphere. All measurements of oocyte size and female live body mass (without tube) were made by a single observer.

To determine if oocyte size is an indicator of maternal provisioning, lipid content of oocytes was quantified for the sites with the smallest (GL, East) and largest (MB, West) average oocyte sizes. Adult worms were collected from GL (6 April 2012) and MB (9 April 2012) and induced to release gametes; 5 samples per site, each containing 1130 to 1350 oocytes, were then counted, placed in microcentrifuge vials and frozen at −80°C. Oocyte lipids were extracted using the chloroform/methanol extraction method of Holland & Gabbott (1971) as modified by Sewell (2005), with the minor difference that we used me-

thanol and chloroform from the LiChrosolv® Hypergrade for LC-MS range (Merck Millipore) and a lower concentration of ketone standard to estimate lipid recovery (10 µl of 1500 µg ml^{-1}). Lipid classes in *S. cariniferus* oocytes were quantified using the Iatroscan MK 6s Thin Layer Chromatography/Flame Ionization Detection system (TLC/FID) and silica gel S-III chromarods. Details of calibration, development and quantification are as described in Sewell (2005) with the exception that Iatroscan data was collected by Ulys 2 USB acquisition device (Datalys®) and chromatograms were recorded and measured using Datalys® AZUR 5.0 software.

2.4. What are the consequences of maternal provisioning for offspring performance?

Oocyte size and development rate, from fertilization to newly metamorphosed early juvenile, were compared among GL (East, smallest oocytes), Green Bay (GB; West, intermediate-sized oocytes), and MB (West, largest oocytes) sites. Spawning, fertilization and larval rearing were carried out at 18 to 19°C using natural seawater, under a light regime of 12 h light:12 h dark.

Adult worms were collected from all 3 sites on the same morning (17 March 2012). Later that same day, adult worms from each site were placed in finger-bowls with seawater, totalling 8 replicate bowls per site and 2 to 5 worms per bowl. Adults were then induced to release gametes as described by Gosselin & Sewell (2013). For each site, >20 females and >10 males released gametes. After 15 min, 20 oocytes per site (2 to 4 per bowl) were haphazardly collected and their diameter was measured at 400×. All gametes from a given site were combined in a large beaker and left for 10 min for fertilization; excess sperm was then removed and fertilized eggs transferred to a beaker with 2 l of filtered seawater to allow for embryonic development. The fertilization process for all 3 sites was carried out within a 1 h period. After 36 h, the trochus diameter of 30 newly hatched trochophore larvae from each site was measured at 400×. Approximately 10 000 larvae from each embryonic development beaker were then divided equally into 5 replicate larval rearing beakers per site, each containing 400 ml filtered seawater (i.e. ~2000 larvae per replicate). Water changes and feeding with microalgae were as described above for the cross-fertilization experiment.

Offspring performance at each site was assessed by measuring larval body length (Days 5, 8, 11; n = 6 larvae per beaker), survivorship (three 3 ml samples $beaker^{-1}$), and time to reach competence. Competence was determined by exposing larvae to a 10^{-4} M solution of IBMX on Days 7, 9 and 11; on each trial date, 10 larvae were haphazardly collected from each beaker, transferred to a 6-well cell culture plate containing 8 ml of IBMX solution and after 24 h were scored for metamorphosis as described by Gosselin & Sewell (2013). Finally, of the 10 larvae per culture beaker that were tested for competence in the Day 11 trial, we measured the body length of all newly metamorphosed juveniles (i.e. 3 to 9 juveniles per beaker).

2.5. Is oocyte size in *S. cariniferus* influenced by phenotypic responses of the female parent?

During the survey of oocyte size at our 10 study sites (Fig. 1), the live body mass of each female worm (without tube) was measured post-spawning after blotting external water with a paper towel, to an accuracy of 0.01 mg. This allowed for determination of the relationship between average oocyte size per female and female body mass. We also compared female body mass among the 10 study sites using the body mass of the 6 largest females collected from each site, an approach similar to that used by Collin (2003); this allowed us to determine if sites with the largest females also have the largest average oocyte size. Finally, we examined whether average oocyte size at a given site varies seasonally by comparing oocytes produced in early summer with oocytes produced in late summer for 3 sites: GL, GB and MB. This was accomplished by comparing the oocyte measurements for these sites, carried out in early summer (late December 2011 to early January 2012) during the survey of our 10 study sites, with oocyte measurements taken in late summer (17 March 2012) for the larval development experiment.

2.6. Statistical analyses

The experimental designs of all experiments involving *S. cariniferus* are summarized in Table S3 in the Supplement. All statistical analyses were carried out using Minitab v.17.3. The assumption of normality was tested using the Kolmogorov-Smirnov test or Bartlett's test, and homogeneity of variance was tested using Levene's test. Where necessary, data were analysed using non-parametric tests. Tukey post hoc tests used an $\alpha = 0.05$.

3. RESULTS

3.1. SST and chl *a* conditions on East and West Coasts

Satellite measurements from 2002 to 2017 revealed consistently warmer SST on the East Coast than on the West Coast. For the spring and summer months of November to April, the time of year when *Spirobranchus cariniferus* is known to release gametes or larvae, average monthly SST was significantly warmer on the East Coast for each month (Fig. 2A; paired *t*-tests, data paired by year). The difference in average monthly SST was greatest in February, with East Coast SST being 1.3 °C warmer than on the West Coast (Fig. 2A), whereas the greatest single-month difference occurred in February 2010 when East Coast SST was 2.27°C warmer than on the West Coast. There was also a significant trend (Fig. 2B) of increasing summer SST (pooled average SST for January, February and March) throughout the 2003 to 2017 period on the East (R^2 = 27.0 %, F = 4.80, n = 15, p = 0.047) and West (linear regression: R^2 = 28.1 %, F = 5.08, n = 15, p = 0.042) coasts; SST increased at rates of 0.066°C (West) to 0.083°C (East) yr^{-1}. The rate of change in summer SST did not differ between East and West Coasts (ANCOVA, comparison of slopes: $F_{1,26}$ = 0.12, p = 0.731).

Average monthly chl *a* concentrations were not significantly different between coasts in late spring, but there was a pattern throughout the summer and early fall (January to April) of average concentration values being higher on the East Coast than on the West Coast, with those differences being significant in late summer and early fall (March and April, Fig. 3A; paired *t*-tests, data paired by year). Average chl *a* concentrations differed most in April, being 0.69 mg m^{-3} (37 %) higher on the East Coast than on the West Coast; the greatest

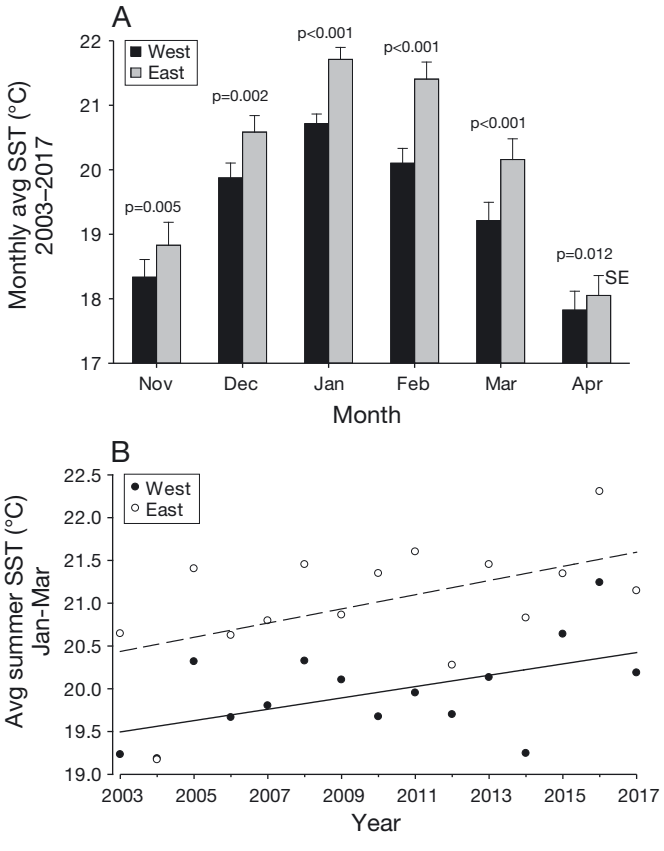

Fig. 2. Satellite measurements of sea surface temperature (SST) on the East and West Coasts of the North Island, New Zealand, made by the Moderate Resolution Imaging Spectroradiometer (MODIS) on the Terra and Aqua satellites from 2003 to 2017. (A) Monthly SST from late spring to early fall, averaged across years. (B) Trends in average summertime SST (January to March) over the period 2003 to 2017. Trend lines in (B) represent regression lines for East (solid) and West (dashed) SST values

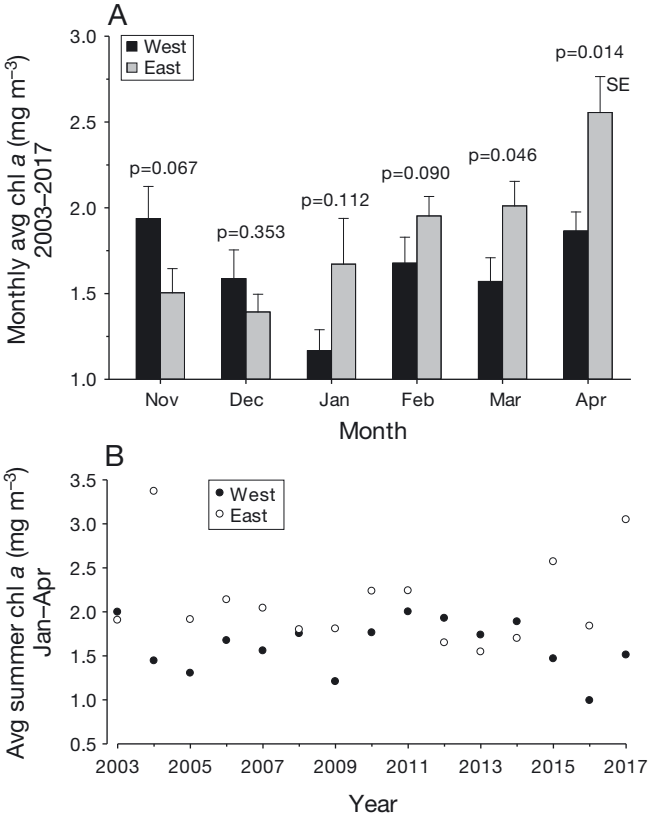

Fig. 3. Satellite measurements of sea surface chl *a* concentration on the East and West Coasts of the North Island, New Zealand, made by the Moderate Resolution Imaging Spectroradiometer (MODIS) on the Terra and Aqua satellites from 2003 to 2017. (A) Monthly chl *a* concentration from late spring to early fall, averaged across years. (B) Trends in average summertime chl *a* (January to March) over the period 2003 to 2017

single-month difference was in January 2004, when East Coast chl *a* was 4.18 mg m^{-3} (440%) higher than on the West Coast. There was no significant change in summer chl *a* (average of January to April) over the 2003 to 2017 period (Fig. 3B) on the West Coast (linear regression: $F = 0.29$, n = 15, p = 0.599) or on the East Coast ($F = 0.01$, n = 15, p = 0.911).

3.2. Do East and West Coast populations of *Spirobranchus* belong to the same species?

3.2.1. Cross-fertilization experiment

Hybrid zygotes derived from both male/female gamete combinations of East and West Coast populations were viable: tens of thousands of trochophore larvae were produced and these swam normally, had good survival and developed normally from fertilization through to 4 wk post-metamorphosis. The 2000 larvae monitored from each cross-fertilization had 86 to 88% survivorship to Day 11, at which time IBMX induced metamorphosis in 30% (3/10) of larvae from the MB × GL culture and in 50% (5/10) from the GL × MB culture. In addition, several hundred larvae naturally metamorphosed on the walls of the rearing beaker and produced healthy early juveniles with calcareous tubes that grew to tube lengths of 4 to 8 mm within the next 4 wk.

3.2.2. Molecular analyses

A total of 57 nucleotide sequences were analyzed (GenBank accession IDs MK775593 to MK775649), with no nucleotide differences detected in the 18S rDNA and 28S rDNA sequences (34 sequences, 1744 and 283 bp) in worms from East and West Coast populations. Among the 23 cytochrome *b* sequences, 13 different haplotypes were found; 10 were found only on one or the other coast, the remaining 3 were found on both coasts. The alignments revealed no substitution saturation (Xia et al. 2003) and therefore could be used for subsequent analysis.

None of the 3 phylogenetic trees that were generated (Figs. S1–S3 in the Supplement) revealed phylogenetic differentiation between populations from East and West Coasts of the North Island; a single clade that included all *S. cariniferus* sequences from this study and all those available in GenBank was supported by a 100% bootstrap confidence score. Furthermore, intraspecific distances within that clade were 2 orders of magnitude smaller than interspecific distances, and the calculated probability of successful identification was 1.00 or 0.99. Detailed statistics are summarized in Table S4 in the Supplement.

3.3. Do East and West Coast populations differ in oocyte size and lipid content?

3.3.1. Oocyte size

Average (±SE) oocyte diameter per site ranged from 55.83 ± 0.29 to 60.55 ± 0.33 µm, corresponding to average oocyte volumes of 91.2 ± 1.4 × 10^{-6} to 116.3 ± 1.9 × 10^{-6} µl. Average oocyte volume differed significantly between the 2 coasts (nested ANOVA, with females nested within sites and sites nested within coasts: $F_{1,8} = 16.17$, n = 10 sites, p = 0.004). Average oocyte volume on the West Coast was 13.9% larger than on the East Coast (Fig. 4A). In

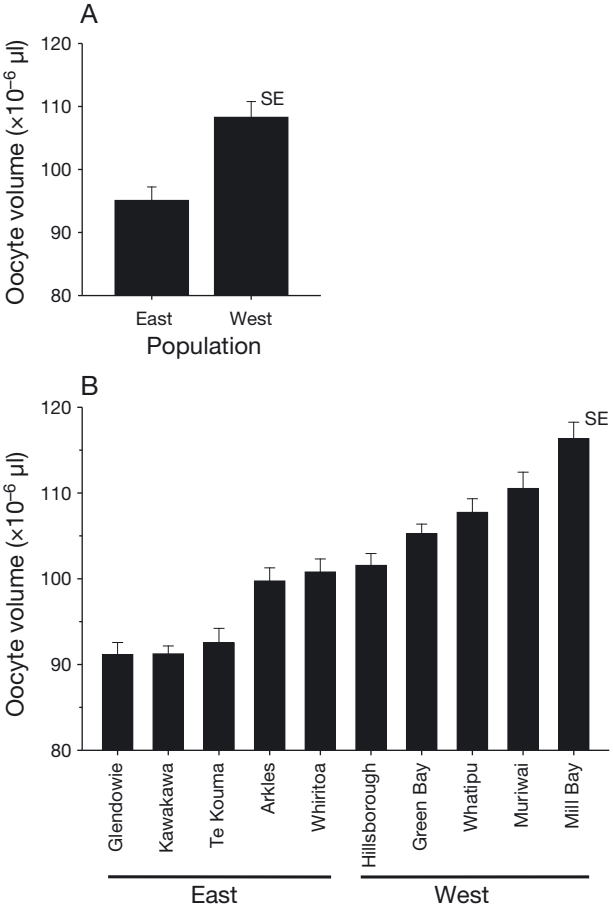

Fig. 4. Volume of *Spirobranchus cariniferus* oocytes from New Zealand's North Island. (A) Overall average volume of oocytes from the East and West Coasts of the North Island; (B) average oocyte volume per site for the 5 East Coast sites and 5 West Coast sites; n = 10 females per site. Sites are ranked from smallest to largest average oocyte volume

addition, when ranked from smallest to largest average oocyte volume, the 5 East Coast sites all ranked as the smallest oocyte volumes and the 5 West Coast sites ranked as the largest oocyte volumes (Fig. 4B). The range among sites in average oocyte volume was substantial, with a 27.6% difference between the sites with the smallest (GL, East) and largest (MB, West) average volumes.

3.3.2. Oocyte lipid content

A total of 6 lipid classes were quantified in *S. cariniferus* oocytes: 3 used by marine invertebrates as energy reserves (aliphatic hydrocarbons [AH], triacylglycerols [TAG], free fatty acids [FFA]), and 3 used primarily in the structure of cell membranes (sterols [ST], acetone-mobile polar lipids [AMPL], and phospholipids [PL]). West Coast (MB) oocytes contained significantly more TAG (41%) than East Coast (GL) oocytes (*t*-test: $t = 2.660$, df = 8, n = 10, p = 0.029; Fig. 5A), but did not differ significantly in content of other energetic lipids (AH, FFA) (for each of these 2 *t*-tests: $t \leq 0.850$, $p \geq 0.420$). For the 3 structural lipid classes (Fig. 5B), differences between the 2 sites were not quite significant (for the 3 *t*-tests: $t \leq 2.159$, $p \geq 0.063$).

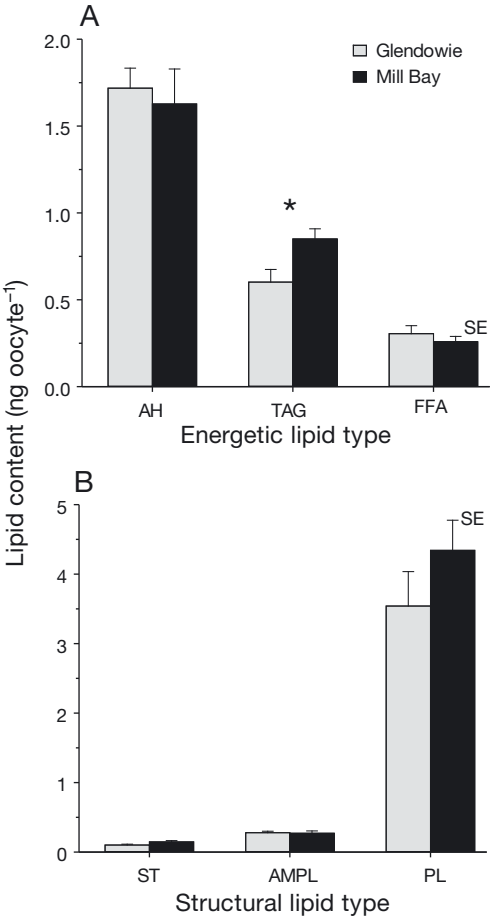

Fig. 5. Lipid content of oocytes spawned by *Spirobranchus cariniferus* females from Glendowie (East) and Mill Bay (West). (A) Energetic lipids: aliphatic hydrocarbons (AH), triacylglycerols (TAG), and free fatty acids (FFA). (B) Structural lipids: sterols (ST), acetone-mobile polar lipids (AMPL), and phospholipids (PL). *Significant difference between sites

3.4. What are the consequences of maternal provisioning for offspring performance?

On Day 11 of the experiment, average survivorship of *S. cariniferus* larvae ranged from 71 to 85% and did not differ significantly between the West Coast (MB, GB) and East Coast (GL) sites (1-way ANOVA: $F_{2,12} = 2.787$, n = 15, p = 0.101). Oocyte measurements on Day 0 in this experiment confirmed that oocyte volume differed among the 3 sites (ANOVA: $F_{2,57} = 34.200$, n = 60, $p < 0.001$) as observed in the earlier field survey. Tukey post hoc tests revealed that oocytes produced by GL (East) females had a significantly smaller volume ($96.43 \pm 1.24 \times 10^{-6}$ µl, mean ± SE) than those produced by West Coast females from GB ($110.50 \pm 1.86 \times 10^{-6}$ µl) and MB ($114.69 \pm 1.77 \times 10^{-6}$ µl), which were not significantly different.

The average diameter of newly hatched trochophores, 36 h after fertilization, also differed significantly among sites (ANOVA: $F_{2,87} = 33.287$, n = 90, $p < 0.001$), with all sites differing significantly from each other (Tukey post hoc tests). The GL (East) trochophores were smallest (82.58 ± 0.52 µm, mean ± SE) and MB (West) trochophores were largest (90.50 ± 0.73 µm); GB (West) trochophores had an intermediate size (86.00 ± 0.75 µm). As trochophores are nearly spherical, the corresponding volume of MB trochophores was ~31% greater than GL trochophores.

Size differences persisted during larval development, with East Coast larvae remaining smaller than those from the 2 West Coast sites (Fig. 6A). Larval body length differed significantly among sites through Days 5 and 8 (ANOVA, repeated measures design: $F_{2,12} = 7.48$, n = 30, p = 0.008). The body length of MB and GB larvae increased very little after Day 8, likely because most had already reached final size at competence, whereas the smaller GL larvae continued to grow until larval size was no longer different among sites on Day 11 (ANOVA: $F_{2,12} = 0.46$, n = 15, p = 0.641).

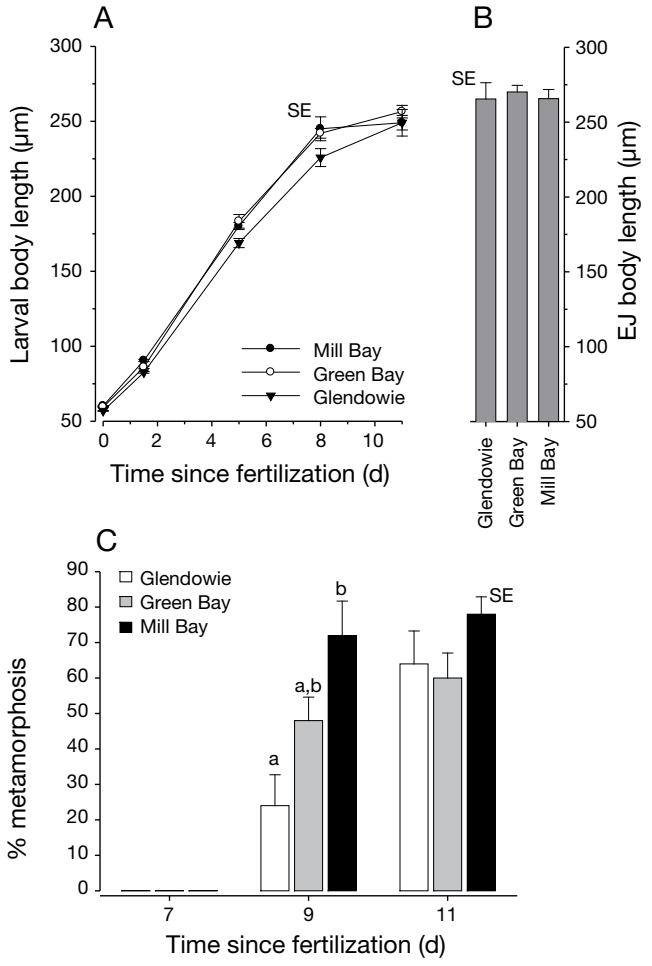

Fig. 6. Growth and metamorphosis of *Spirobranchus cariniferus* larvae originating from Glendowie (East), Green Bay (West) and Mill Bay (West). (A) Larval body length, from fertilization to 11 d after fertilization; (B) body length of newly metamorphosed juveniles after exposure to 3-isobutyl-1-methylxanthine (IBMX); (C) percentage of larvae undergoing metamorphosis, when exposed to IBMX, on Days 7, 9 and 11 after fertilization. For Day 9, values with the same letter are not significantly different, as determined by Tukey's HSD test

MB (West) larvae reached competence sooner than GL (East) larvae (Fig. 6C). No larva had reached competence on Day 7 after fertilization, but by Day 9 the number of larvae initiating metamorphosis in response to IBMX differed significantly among sites (ANOVA: $F_{2,12} = 8.07$, n = 15, p = 0.006). Most MB larvae had reached competence on Day 9, whereas only 24% of GL larvae and about half of GB larvae had reached competence by that age. By Day 11, larval response to IBMX (Fig. 6C) was high and no longer significantly different among sites (ANOVA: $F_{2,12} = 1.67$, n = 15, p = 0.228). Finally, the body length of newly metamorphosed early juveniles (Fig. 6B) was not significantly different among sites (nested ANOVA, juveniles nested within replicate culture beakers: $F_{2,12} = 0.12$, n = 15, p = 0.885).

3.5. Is oocyte size in *S. cariniferus* influenced by phenotypic responses of the female parent?

The relationship between the average volume of oocytes produced by a female and female body mass (Fig. 7A) was significant, but female body size explained less than 5% of the variation in oocyte volume (linear regression: $R^2 = 0.048$, $F = 8.817$, n = 82, p = 0.048). In addition, an analysis of the 6 largest females collected from each site revealed that the maximum body mass reached by *S. cariniferus* varied considerably among the 10 study sites (Fig. 7B), but these differences were unrelated to average oocyte volume (linear regression: $F = 0.654$, n = 10, p = 0.442) and average female body mass did not dif-

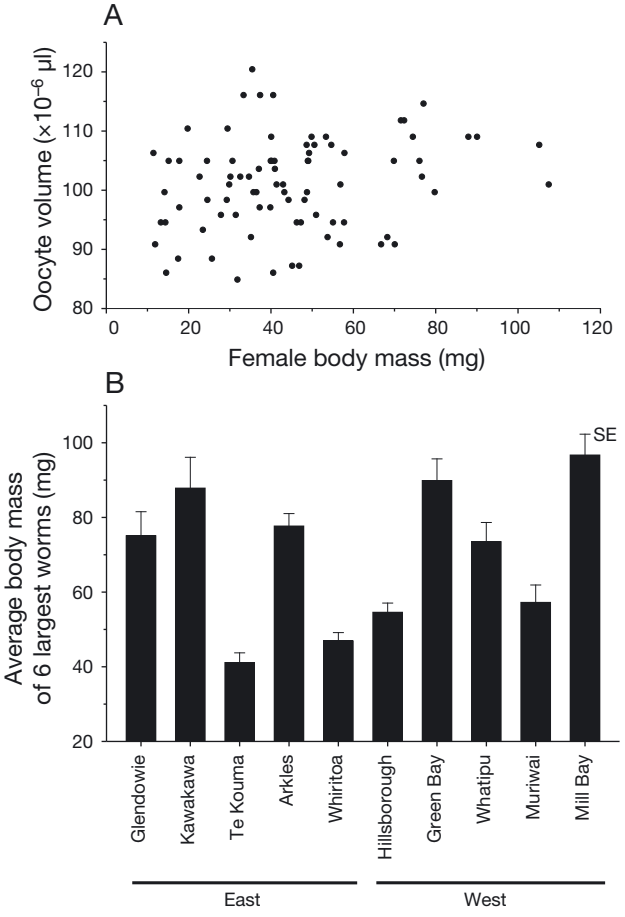

Fig. 7. Variation in *Spirobranchus cariniferus* female body mass, as measured by blotted wet weight, without the shell. (A) Female body mass vs. average oocyte size; (B) average body mass of the 6 largest females collected from each of 10 field sites

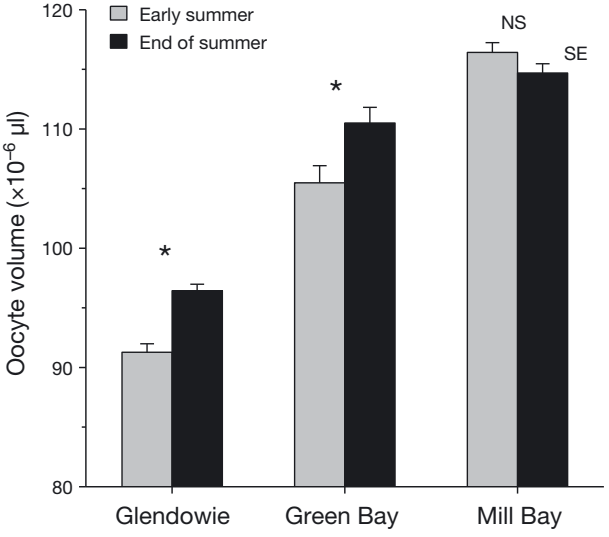

Fig. 8. Comparison of average *Spirobranchus cariniferus* oocyte volume between early and late summer for one East Coast population (Glendowie) and 2 West Coast populations (Green Bay and Mill Bay)

fer between coasts (nested ANOVA, females nested within sites and sites nested within coasts: $F_{1,8} = 0.48$, n = 10 sites, p = 0.507).

Finally, oocyte size comparisons between early and late summer were carried out using Mann-Whitney tests, with n = 100 oocytes for early summer and n = 20 for late summer for each of 3 sites; this 2-sample test was used because oocytes in the larval development experiment (late summer data) were not measured separately for each female, precluding the use of a preferred nested analysis with oocytes nested within females. Average oocyte size in late summer differed significantly from early summer (Fig. 8) for the GL ($W = 5637.5$, n = 120, p = 0.001) and GB sites ($W = 5687.5$, n = 120, p = 0.004), but not for the MB site ($W = 6139.0$, n = 120, p = 0.438). Oocyte volume in mid-March 2012 at the GL and GB sites was 5.7 and 4.8% larger, respectively, than in late December 2011 (GL) and early January 2012 (GB).

4. DISCUSSION

4.1. SST and chl *a* conditions on East and West Coasts

Satellite measurements of sea surface conditions for the years 2002 to 2017 revealed warmer SST during the spring and summer months on the East Coast of the North Island than on the West Coast, with an average differential of 1.3°C and single month differences of as much as 2.3°C. These findings are consistent with earlier oceanographic studies that were based on measurements made in the late 1980s (Chiswell 1994) and late 1990s (Zeldis et al. 2004), showing SST during summer months that were up to 2°C warmer on the East Coast than on the West Coast. In addition, the 15 yr data set reported herein reveals parallel trajectories for SST on both coasts, a further indication that the differences between coasts in SST have been persistent over time. Chl *a* concentrations were also highest on the East Coast in late summer; differences in chl *a* were at times substantial (up to 440%) but were not consistent through spring and early summer months or across all years. Thus, during the time of year when planktonic larvae of many coastal organisms are developing in the water column along each coast, the waters on the East Coast are consistently warmer, and support levels of phytoplankton biomass that are equal to or at times higher than at the same latitude on the West Coast.

4.2. Do East and West Coast populations of *Spirobranchus* belong to the same species?

Populations of *Spirobranchus cariniferus* on the East and West Coasts of New Zealand's North Island were confirmed as belonging to the same species by the cross-fertilization experiment as well as by DNA sequencing. *S. cariniferus* populations from East and West Coasts comprise a single species, with no evidence of a cryptic *Spirobranchus* species complex. These results, along with previous DNA analyses comparing tubeworm samples from the East Coasts of North and South Islands (Smith et al. 2012), confirm that *S. cariniferus* is the only intertidal *Spirobranchus* species throughout New Zealand.

4.3. Does oocyte size vary among natural populations according to the predicted effects of temperature and food availability?

S. cariniferus oocytes were largest on the West Coast where waters are coldest and at times have lower food availability. Females from the West Coast sites produced average oocyte volumes that were 13.9% larger than at the East Coast sites. Lipid analyses revealed that the large MB (West) oocytes were provisioned with 41% more TAG lipids than the small GL (East) oocytes, indicating a link between oocyte size and maternal provisioning. This

difference in TAG content is particularly important because maternally provided TAG are the primary source of energy fuelling the development and growth of embryos and larvae in benthic invertebrates (Sewell 2005, Prowse et al. 2008, Whitehill & Moran 2012). Our study therefore demonstrates an East–West difference in oocyte size and TAG content, a pattern that is consistent with predictions that cooler water and lower food availability favour offspring that are larger and supplied with greater energy reserves. Given the consistent differences in SST between the coasts throughout the reproductive season, and the lack of consistent differences in chl *a* during early summer, SST seems a more likely cause of the difference in maternal provisioning between coasts than food availability.

Factors other than temperature and food availability, however, have been proposed to explain variation in oocyte size. One such factor is fertilization rate: in free-spawning marine invertebrates, larger oocytes have higher fertilization rates as they are larger targets for sperm, a selective pressure that may be important in environments with low sperm densities (Levitan 2006, Luttikhuizen et al. 2011). Given the gregarious nature and high population densities of *S. cariniferus* on both coasts (L. A. Gosselin pers. obs.), the 50:50 sex ratio and the high proportion (75 to 100%) of worms bearing ripe gametes at any time during the summer (Gosselin & Sewell 2013), it is unlikely that sperm densities would be limiting or, more importantly, that the West Coast population would experience substantially lower sperm densities than the East Coast population. Two other factors to consider are (1) lower pelagic mortality experienced by larger embryos and larvae due to predation (Allen 2008) and (2) patchiness of suitable benthic habitats, which may select for a shorter PLD and thus for larger offspring size (Burgess et al. 2013). Limited information is available on the abundance of planktonic predators in northern New Zealand, but it is unlikely that the food web on the West Coast would support a larger guild of planktonic predators than on the East Coast during the summer given the similar or, in some months, lower phytoplankton abundance on the West Coast. Patchiness of habitats, however, cannot be ruled out, as much of the shoreline along the West Coast, particularly in the far north of the North Island, is sandy and interspersed by only a few small rocky headlands. This factor might therefore also be selecting for larger offspring of rocky intertidal species on the West Coast.

4.4. What are the consequences of maternal provisioning for offspring performance?

Under controlled laboratory conditions, rates of larval development differed significantly among the 3 source populations in a way that was consistent with differences in oocyte size and lipid content. MB larvae, developing from oocytes that were the largest and containing the most TAG, maintained a larger body size and had a shorter larval development time than larvae from the other 2 sites. GL larvae, developing from the smallest oocytes with the least TAG, remained smaller as larvae and were last to reach competence. The higher TAG content of MB oocytes provided sufficient energy reserves to reduce larval development time by ~2 d, or 22%, relative to GL larvae. This intraspecific relationship between oocyte size and larval development time is consistent with previous intraspecific (George et al. 1990) and interspecific (Levitan 2000, Allen 2012) comparisons showing a negative relationship between oocyte size and larval development time.

Maternal provisioning, in the form of oocyte size and lipid content, therefore does influence larval development in *S. cariniferus* and, accordingly, selective pressures on larval performance can be addressed by changes in maternal provisioning, a requirement of models linking maternal provisioning to the temperature and food conditions experienced by larvae. Interestingly, both the size at competence (~250 µm body length) and post-metamorphic juvenile size (~260 µm) were the same for all 3 sites and were thus independent of oocyte size. These findings are consistent with the hypothesis that, in species producing small oocytes such as *S. cariniferus*, maternally provided energy reserves serve almost exclusively to support larval development (Levitan 2000, Alcorn & Allen 2009, Allen 2012), establishing a strong link between maternal provisioning and larval performance.

4.5. Mechanisms controlling variation in oocyte size

Intraspecific variation in offspring size can result from 3 mechanisms: (1) developmental instability (Moran & McAlister 2009), (2) phenotypic plasticity of maternal provisioning (McGinley et al. 1987, Atkinson et al. 2001) or (3) inherited genetic differences (Moran & McAlister 2009, Sanford & Kelly 2011). When offspring traits are influenced by developmental instability, the outcome is increased ran-

dom variation among females and among offspring (Lajus & Alekseev 2004). In our study, however, there was minimal variation in *S. cariniferus* oocyte size among females from a given site; within-site coefficients of variation in oocyte size were only 3.2 to 5.7% among *S. cariniferus* females. This very low within-site variation suggests developmental instability contributes little, if at all, to the observed variation among sites.

The second mechanism, phenotypic plasticity in maternal provisioning (McGinley et al. 1987, Perrin 1988, Bernardo 1996), occurs when offspring size varies as a function of the size (Emlet 1989, George 1994, Bingham et al. 2004) or age (Qian & Chia 1992) of the female parent, or in response to environmental conditions experienced by the parent prior to offspring release (e.g. temperature, Atkinson et al. 2001, Simonini & Prevedelli 2003, Collin & Salazar 2010; food, Thompson 1983, George et al. 1990, Bertram & Strathmann 1998). Maternal size and age appear to be of minor importance in determining *S. cariniferus* oocyte size, as female body mass explained <5% of the variation in oocyte volume, despite the inclusion of a 10-fold range in body masses, corresponding to a broad age range (Riedi & Smith 2015). Similar conclusions were reached for the bivalve *Mytilus trossulus* (Phillips 2007), for urchin species in the genus *Echinometra* (Lessios 1987) and for calyptraeid gastropods (Collin 2003, Collin & Salazar 2010). The comparison of early and late summer oocyte sizes at 3 sites also suggests a modest degree of phenotypic plasticity in maternal provisioning. No seasonal differences in oocyte volume were detected at the MB site, and late summer (March) oocyte volume at the GL and GB sites was 4.7 to 5.8% larger than in early summer (late December to early January), changes that may have been maternal responses to the cooling SST over the December to March interval (Fig. 2B). This phenotypic variation revealed by seasonal differences in maternal provisioning at 2 of the 3 sites could account for up to 21% of the 27.6% difference between the sites with the smallest (GL) and largest (MB) oocytes, or up to 42% of the 13.9% difference in oocyte size between coasts. It is also interesting to note that the observed seasonal variation at the MB (no variation), GB (4.7%) and GL (5.8%) sites approximates the level of phenotypic variation in average oocyte size among females within a given site (3.2 to 5.7%, early summer survey). These low levels of among-female and across-season variation in oocyte size imply that either (1) variation in oocyte size represents plastic responses of the females to an environment with modest temporal (i.e. late spring vs. late summer) variation in conditions or (2) there is limited scope for phenotypic plasticity in oocyte size in this species. The first hypothesis is not well supported by SST data (Fig. 2B), as both coasts do experience substantial variation in temperature throughout the spring and summer.

The third mechanism, genetic divergence, would be responsible for the portion of oocyte size difference that is not explained by phenotypic plasticity, i.e. up to, but not exceeding, 58% of the difference between coasts. Oocyte size has been shown to have a high heritability component in the serpulid polychaete *Hydroides elegans* (Miles et al. 2007), a species that is closely related to *S. cariniferus*, and also in the spionid polychaete *Streblospio benedicti* (Levin et al. 1991). In addition, *H. elegans* and *S. benedicti* both contain significant genetic variation for oocyte size, and oocyte size in *H. elegans* was shown to evolve rapidly in response to selective pressures, diverging significantly within only a few generations in the laboratory (Miles et al. 2007). Also, our estimate of up to 58% of oocyte size variation in *S. cariniferus* being genetically determined closely matches the finding by Miles et al. (2007) that nearly half of their observed variation in oocyte size was due to additive genetic variation. Local adaptation can occur even in the presence of some gene flow between populations (Sanford & Kelly 2011), but the likelihood of genetic divergence between populations is enhanced if migration and gene flow are limited, which is likely the case on the North Island. The dispersal distance and physical conditions along the northern coasts of the North Island do not favour gene flow between coasts, because (1) for sites at mid-island latitudes (36° 40′ to 37° 17′ S), the dispersal distance between coasts is ~700 km by way of Cape Reinga to the north, or ~1500 km by way of Cook Strait to the south; (2) there are extensive sections at the northern end of the North Island, up to 80 km long, of continuous sandy coastline preventing the establishment of settlers seeking rocky substrata; (3) surface currents provide little opportunity for the exchange of larvae between coasts at the northern tip of the North Island: the persistently southeastward-flowing East Auckland Current on the East Coast (Heath 1985, Chiswell & Booth 2008) directs planktonic larvae southward (Chiswell & Rickard 2011), and the weak and variable currents on the West Coast result in either no net along-coast displacement or a slight northward movement (Chiswell & Rickard 2011, Sutton & Bowen 2011), resulting in a cul-de-sac situation for passively drifting particles on

the northern West Coast (Sutton & Bowen 2011); and (4) upwelling that occurs at the northern end of the North Island might carry larvae offshore and thus further impede gene flow between coasts (Veale & Lavery 2011). Finally, significant genetic differentiation between East and West Coast populations has been revealed in 5 other benthic species: the amphipod *Paracorophium lucasi* (Stevens & Hogg 2004); the chiton *Sypharochiton pelliserpentis* (Veale & Lavery 2011); the estuarine clam *Austrovenus stutchburyi* (Ross et al. 2012); the urchin *Evechinus chloroticus* (Nagel et al. 2015); and the seagrass *Zostera muelleri* (Jones et al. 2008). Those findings confirm that the conditions prevailing on the island are favourable to genetic differentiation between coasts. Thus, (1) oocyte size is heritable and responsive to selective pressures, (2) temperature and to a lesser extent food availability differ between coasts and (3) the geographic and oceanographic conditions along the North Island act to minimize gene flow between East and West Coast populations, all of which are consistent with local adaptation and genetic variation as a mechanism responsible for an important portion of the variation in oocyte size between coasts.

Although phenotypic plasticity is intuitively expected to be the main cause of within-coast variation because connectivity and gene flow are assumed to be relatively high among sites of the same coast, recent evidence suggests significant genetic variation can also exist among sites of the same coast. A study of the urchin *E. chloroticus* (Nagel et al. 2015) found significant genetic structure among sites along the East Coast of the North Island, including genetic differences among sites located within the Hauraki Gulf, where 4 of our 5 East Coast sampling sites were located. These genetic differences in *E. chloroticus* are surprising given the proximity of the sites and the relatively long larval development time of 30 d in this species. By comparison, larval development time in *S. cariniferus* is only 8 to 15 d (Gosselin & Sewell 2013, this study). Thus, genetic divergence may also be responsible for some of the variation in oocyte size among sites on the same coast. Further research examining the large and small-scale genetic structure of *S. cariniferus* on the East and West Coasts of the North Island and the plasticity of oocyte size and lipid content are needed to fully resolve the relative roles of local adaptation and phenotypic plasticity in determining variation in maternal provisioning and offspring traits at different scales.

How responsive, then, is oocyte size to environmental conditions in nature? Interspecific comparisons of geminate species pairs of echinoderms (Lessios 1990) and bivalves (Moran 2004) have shown species isolated on the Atlantic and Pacific coasts of Panama for 3 million years to have diverged in average oocyte size, a divergence that has been attributed to the different environmental conditions prevailing on Pacific and Atlantic coasts. The intraspecific comparisons of East and West Coast populations in the present study reveal oocyte size to differ substantially even among populations of the same species that are probably not fully isolated, suggesting a trait that is sufficiently responsive to regional conditions to cause divergence among populations of the same species.

In closing, New Zealand's North Island is an example of a natural setting that is well-suited to studying the responsiveness of life history traits to environmental conditions. In the mid-island region, populations on the East and West Coasts are positioned at the same latitude, yet are isolated by distance, suitable substrata, and currents that minimize gene flow. In addition, the 2 coasts experience distinct environmental conditions with the potential to favour divergent evolution of life history traits. From a logistic perspective, field locations such as the mid-island region of the North Island offer the added advantage of very close proximity between coasts and easy access to many sections of both coasts by vehicle; the closest of our study sites on opposite coasts were at a driving distance of <1 h, such that a sampling trip to 2 to 4 sites spanning both coasts could readily be completed on the same day. The North Island, as well as islands with comparable features elsewhere in the world, constitute unique natural experiments where empirical data can readily be obtained to explore hypotheses regarding the role of environmental conditions in shaping the life history of marine species.

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