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Original Article

Maternal legacy: Unraveling the crucial role of the parental population against larval nutrition and its profound impact on developmental trajectories and morphological outcomes in *Pseudechinus huttoni* (Echinoidea: Temnopleuridae) larvae

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Abstract: This study examined the relative contribution of parental population and larval diet on development and larval characteristics in *Pseudechinus huttoni*. We further investigated the effects of parental population on egg traits. Two populations of sea urchins were selected—shallow and deep—with a known difference in nutritional history, where the deep population showed poor nutritional condition and lower egg size. However, eggs from both populations had the same nutritional content. However, larvae from the lower-nutrition population were more advanced in growth and development, and their body shape was more flexible. More rapid growth and development and flexibility in body shape were seen in the larvae reared with high-plankton food. More importantly, the parental population contributed more towards variations in larval growth and shape than to the diet received by larvae. Additionally, larvae from the low-nutrition parental population were nutritionally increased, which could indicate that this parental effect is more than just a simple change in egg composition.

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Introduction

Planktotrophic larvae use two sources of energy for growth and development: an exogenous source, dissolved and particulate organic matter, and an endogenous source, the egg yolk. Planktotrophs have small eggs with small reserves; thus, the exogenous source is the larvae's major energy supply (Strathmann, 1985). Contrastingly, lecithotrophs have larger eggs and larvae highly depend on the maternal source of food invested in eggs, namely yolk (McEdward and Janies, 1997). Therefore, lecithotrophy and planktorophy specify the degree of dependence on endogenous or exogenous food sources (Havenhand, 1995).

The egg reserves, as the endogenous food source for the larvae, have mainly been linked to the egg size and/or the nutritional status of the parents. However, factors like maternal habitat and body size may influence egg size and quality (George, 1999). It is believed that in better parental nutritional conditions, eggs with greater amounts of biochemical reserves can be produced, subsequently leading to larvae with greater growth, faster development, and higher survival and metamorphosis rates (George et al., 1990; George et al., 1991). The strong dependence of planktotrophic larvae on the exogenous diet has already been demonstrated in several studies (Boidron-Metairon, 1988; Strathmann et al., 1992; Hart and Strathmann, 1994; Sewell et al., 2004). In addition to exogenous food sources, it has been suggested that endogenous sources (the egg reserves) may significantly affect the characteristics of planktotrophic larvae (George et al., 1990; George et al., 1991). Therefore, the importance of parental nutritional status on characteristics of planktotrophic larval remains equivocal.

The sea urchin *Pseudechinus huttoni* (Echinoidea: Temnopleuridae) is distributed on the continental shelf of New Zealand (Kirby et al., 2006) and has planktotrophic pluteus larvae (McEdward and Miner,

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2003). This species lives mostly on coarse-grained sediments (McClary and Barker, 1998) but can be found on rocky substrates in the fiords of southern New Zealand. The studies about the importance of parents on characteristics of sea urchin larvae have mainly focused on egg size or its energy content (Emlet et al., 1987; McEdward, 1986a; McEdward, 1986b). In this respect, it has been found that the egg size or energy content may not be the only vectors by which parents influence the characteristics of planktotrophic larvae (Poorbagher et al., 2010). Therefore, we aimed to examine (1) the importance of *P. huttoni* parental population from contrasting habitats (defined mainly by nutritional status) versus exogenous planktonic diet on characteristics of sea urchin larvae and (2) how egg size and/or energy can explain larval characteristics influenced by two *P. huttoni* populations from different habitats and possibly with different nutritional status (see Materials and methods). Whilst an *in vitro* study allows controlling intervening environmental variables, it may not be able to simulate the optimal conditions for parents. In particular, there is not much information about the biology of *P. huttoni*, e.g., the feeding requirements. We were aware of confounding geographic and/or genetic factors, but the type of environmental variables the parents experienced were not important to the aims of the present study. We chose *P. huttoni* as a southern hemisphere species to do the current research as studies on the relative importance of parental habitat (mainly nutritional status) on sea urchin larvae have mostly been limited to the northern hemisphere.

Egg reserve has widely been considered to explain larval characteristics, even though other materials in the egg, e.g., thyroxin (Heyland et al., 2004; Saito et al., 1998), have also been found to have effects on larvae similar to those of egg reserves or energy. If egg biochemical reserves are responsible for exerting parental effects on larvae, we think there should be a correlation between egg and larval biochemical contents. To examine this hypothesis, we measured the biochemical contents of the larvae in the early larval stage.

Materials and Methods

Collection of adult *Pseudechinus huttoni*: It was essential to find two sea urchin populations with different nutritional status. The lantern index was used to infer the nutritional condition of the two populations. A greater lantern index indicates a lower nutritional status in sea urchins (Ebert, 1980). The lantern index was measured as the percent ratio of the lantern length to the horizontal test diameter. Two populations were sampled, one from the continental shelf of Otago (45º47´218˝S; 170º51´717˝E), at a depth of 60 and a second from Doubtful Sound (45º25´770˝S; 167º06´884˝E), at a depth of 10-14 m. The *P. huttoni* from the Otago Shelf had significantly greater lantern indices than those from Doubtful Sound, indicating poor nutritional status (Otago Shelf: n = 86, mean±SE = 25.50±0.25 %, Doubtful Sound: n = 97, 23.54±0.27 %; t-test: df = 181, t = 5.132, $P<0.001$). Adult sea urchin populations were sampled when they were at the peak of the gonad index (see below). The population from the Otago shelf was collected using Agassiz bottom trawl, and the population from Doubtful Sound was collected using SCUBA.

Egg studies: During the breeding season (May-September), five female urchins were randomly collected from each population, and a piece of gonad (3-4 mm) was biopsied from each specimen. The gonad samples were wet mounted on slides, and the oocytes were examined by smearing them onto a slide to inspect the released oocytes under a compound microscope. When the oocytes' diameters were 100 µm (diameter of mature ova: 70-100 µm, McClary and Barker (1998)) or more and the germinal vesicle and the nucleolus were invisible or difficult to find in the cytoplasm, a further nine to ten females were spawned from each population by intracoelomic injection of 1.5-2 mL 0.5 M KCl per animal. Eggs of each female, collected in 200 ml seawater, were sampled (10-20 mg final dry weight), briefly rinsed with distilled water to remove salt (Jong-Westman et al., 1995), and frozen at -80ºC for later biochemical analysis.

To reduce genetic variation, the sperm of only one male sea urchin (from the same population as the eggs)

was used to fertilize the eggs (Bertram and Strathmann, 1998). After KCl injection, the animal was put on a cooled dry surface so that gonopores were positioned on the top to avoid immersing the sperm in water collecting from the animal's surface. Diluted sperm was obtained by rinsing the gonopores of a male urchin that had finished spawning with 5-10 ml filtered seawater. The motility of sperm was checked under a compound microscope, and non-motile or sluggish animals were discarded. The sperm of one male from the same diet treatment as the females was used. To fertilize eggs, 2-4 drops of diluted sperm were added to the egg solution (= 200 ml, see above).

The following features of the egg were measured for each parental population: (1) Egg diameter: 30 eggs per female measured using an ocular micrometer installed on the eyepiece of a compound microscope (when eggs were not spherical, the average of the major and minor axes was calculated as the egg diameter). (2) Egg dry weight: A known number of eggs (15000-180000) were put in a dry pre-weighed eppendorf tube. The sample was placed in a freeze-dryer for 48 hours and re-weighed. The weight of the empty eppendorf tube was subtracted from the weight of the eppendorf tube. The weight of the dry sample was divided by the egg number in the eppendorf tube. (3) Fecundity: The average number of eggs in 10 subsamples (which were well-mixed) per female were measured. (4) Fertilization percentage: For each female, fertilization percentage of 100 randomly-selected eggs was measured. Inflation of the fertilization envelope and forming a perivitelline space were used to indicate successful fertilization.

Unfertilized eggs were placed in a freeze-drier for 48 hours, then the protein concentration was measured by the Bradford protein assay. We applied Bradford protein assay which has been used widely (Bradford, 1976; Bryan, 2004; Moran and Manahan, 2004). This method may not detect all protein components in the eggs of the two populations (Moran and McAlister, 2009) and/or underestimate the total protein content (Chu and Casey, 1986). However, the present study was a comparative investigation, and the absolute protein amount was unimportant. Lipids and carbohydrates were determined using the colorimetric methods (Mann and Gallager, 1985). The only difference between our method and Mann and Gallager's was that we applied lyophilized samples, but they used dry or wet samples. The egg energy content was estimated using the energy equivalent for protein, lipid, and carbohydrate: 2.40×10^{-2}, 3.95×10^{-2}, 1.75×10^{-2} J μg^{-1} (Gnaiger, 1983), respectively, and remainder: 2.70×10^{-2} J μg^{-1} (Jaeckle, 1995). The remaining eggs were used for the cultivation of larvae.

Larval studies: larvae from the two parental populations were reared at 12ºC, at a density of 2 mL^{-1} (Jong-Westman et al., 1995; George et al., 1997) in six three-litre glass jars. Larvae were fed *Isochrysis galbana* every other day at densities of 5000 or 20000 cells mL^{-1} for standard and high-concentration planktonic diets, respectively. The study's experimental design was full factorial (2 parental populations: Otago Shelf and Doubtful Sound × 2 planktonic diet concentrations × 3 replicates). A paddle system was used to keep larvae suspended in the water column with 10 paddle strokes min^{-1} (Strathmann, 1987). A 90% water change was done every four days; fresh seawater was filtered through a 1 μm pore filter before being added. Jars were scrubbed and rinsed with freshwater.

Every eight days, the five larvae from each jar were randomly sampled and classified as 2, 4, 6, and 8-arms (Meidel et al., 1999) or competent larvae. Larvae were considered competent when they had a well-formed rudiment (> 200 μm) with the presence of some spines on the rudiment. Total larval length, postoral, anterolateral, posterodorsal, preoral arm lengths, body width, stomach length, and rudiment length were measured using an ocular micrometer (Fig. 1). The larval period of each jar was considered complete when more than 50% of the larvae were competent. When the larval cultures fed the high-concentration planktonic diet reached competency, larval cultures fed the standard-concentration planktonic diet were terminated.

In addition to rearing larvae in three-litre glass jars, larvae from parents collected from the two field

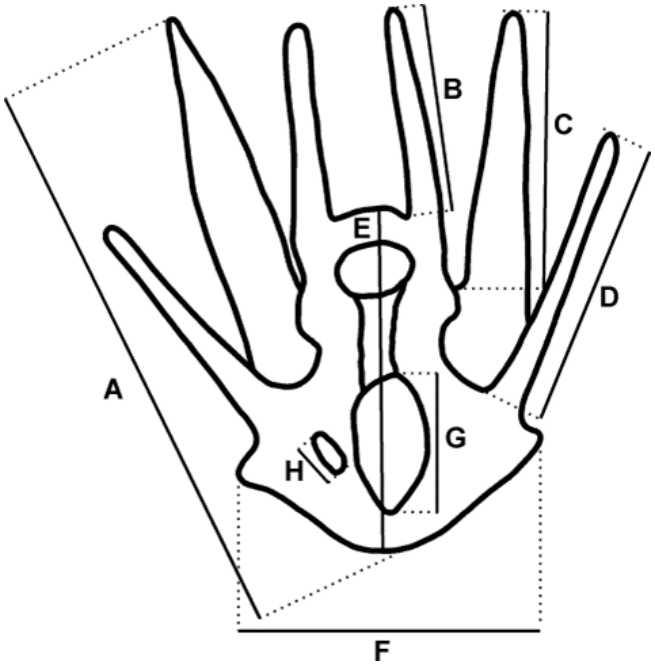

Figure 1. Morphometric features were measured in a 6 arm *Pseudechinus huttoni* larva. A. total length; B. anterolateral arm length; C. postoral arm length; D. posterodorsal arm length; E. midline length; F. body width; G. stomach length and H. rudiment length.

populations were reared at a density of 2 mL^{-1} in two 67-litre plastic tanks (0.53 m height × 0.51 m diameter) at 12-14ºC for biochemical analyses. Tanks were equipped with paddles that were connected serially to a gearbox motor (1/8 HP), generating 12.5 strokes $minute^{-1}$. A 50-µm mesh round filter (10 cm diameter) was installed on the outlet at the top of each tank to stop larvae from leaving the outlet. The algal diets (*I. galbana*) were either 5000 or 20000 cells mL^{-1} for the standard and high larval diets, respectively. One tank was fed the standard concentration diet, and the other was fed the high concentration diet. Water changes were completed every two days by adding 30 litres of 1 µm filtered seawater at the same temperature to each tank. Tanks were discharged, scrubbed, and washed with fresh water every two weeks. Every 16 days, three samples of 3000 larvae were taken, concentrated in an eppendorf tube, and rinsed with distilled water. Samples were kept at -80ºC for later biochemical analyses using the methods described earlier for eggs.

Data analysis

Egg studies: Differences in the egg characteristics from Otago Shelf and Doubtful Sound were examined for significance using a t-test.

Larval studies: the growth (size-at-age) of larval body components and shape (the relative length of the arms to the midline length) were studied using principal component analysis (PCA). For each morphometric variable, the mean of the five larvae per replicate jar was calculated prior to analysis to avoid pseudoreplication (Bertram and Strathmann, 1998). The lack of an arm at a sampling time was considered a zero (Hart and Scheibling, 1988). Postoral, anterolateral, posterodorsal, and preoral arm lengths, midline length, body width, stomach length, and rudiment length were used in the analysis (Fig. 1). Total length was not used in the analysis because it was highly correlated with postoral arm length ($r = 0.950$, $N = 1365$). The first two principal components (PC) were chosen to analyse based on a scree plot (Everitt and Dunn, 1991). The correlation matrix was used for PCA, as variables had very different variances (Quinn and Keough, 2002). The coefficient of PC1 was positive for all body components and was considered as the size component describing the growth (size-at-age). The larval morphological phenotypic plasticity was investigated using PC2 based on the method described by Hart and Scheibling (1988) and Meidel et al. (1999). In brief, coefficients of PC2 for larval arms contrasted with those for larval midline length, and thus the greater scores for PC2 (with negative coefficients) would show shorter arms relative to the midline length. To test the difference between the growth and morphology of the larvae from different parental and larval dietary treatments, the principal components scores were examined using a three-way ANOVA, with parental population, larval diet, and age as fixed factors. The normality of data and homogeneity of variance were examined by the Shapiro-Wilk and the Levene tests, respectively (Quinn and Keough, 2002).

The morphometric measurements of later-stage larvae were selected for the analysis because previous studies on larval growth and phenotypic plasticity were carried out on the late-stage larvae (Sewell et al.,

Table 1. Egg characteristics and t-test of egg characteristics of parental sea urchin (*Pseudechinus huttoni*) populations from Otago Shelf and Doubtful Sound. Sample statistics are mean ± SE with sample size in parenthesis.

Egg characteristics	Otago Shelf	Doubtful Sound	df	t	P
Egg diameter (µm)	99.3±1.3 (10)	106.4±1.2 (10)	18	-3.923	0.001
Fecundity (× 10^5)	11.3±2.2 (10)	14.5±3.3 (10)	18	-0.805	0.431
Fertilization rate (%)	82.9±7.9 (10)	81.2±6.2 (10)	18	0.167	0.870
Protein content (ng egg^{-1})	60.5±13.3 (10)	48±11.0 (7)	15	0.677	0.509
Lipid content (ng egg^{-1})	19±3.8 (10)	16±5.5 (7)	15	0.473	0.643
Carbohydrate content (ng egg^{-1})	3.1±0.6 (10)	3.5±1.5 (7)	15	-0.243	0.811
Energy (× 10^{-3} J egg^{-1})	4.3±0.06 (10)	2.5±0.06 (7)	15	2.089	0.054

2004) (see SEWELL et al., 2004). In addition, an initial PCA for the larvae from all sampling times was performed. However, the Levene test for PC1 and PC2 scores showed heterogeneity among all data, which was not improved through transformation. Box plots showed that larval age was the most important source of heterogeneity of variance; however, PC scores had similar variances from day 100 to 132. Larvae older than 132 days started to settle, and culture was terminated in some jars. Therefore, there were not an equal number of replicates (jars) after that time. ANOVA does not need an equal number of replicates; however, a higher number of samples increases the power of the test (Quinn and Keough, 2002). Therefore, to have a greater number of replicates, the period between 100 to 132 days were re-analysed using a PCA followed by an ANOVA for PC scores. Due to a lack of normality of the data and heterogeneity of variance, which were detected by the Shapiro-Wilk and the Levene tests, respectively, PC scores were transformed using the Box-Cox method. The difference in the duration of the larval period was tested between larvae from the two parental populations using a t-test.

The effects of the parental population, larval diet, and larval age on the protein, lipid, and carbohydrate contents of larvae were examined using a two-way ANOVA on each larval age separately, with the parent and larval diet as fixed factors. A three-way ANOVA could not be used because, in spite of transformation, the variance remained heterogeneous over the data. ANOVA assumptions were tested using the methods described earlier for the previous analyses. Where both parents and larval diet had a significant effect on the response variable, partial eta squared (η_p^2) was used to determine which factor accounted for a greater amount of the variation in the model to a maximum of one (Maxwell and Delaney, 2004). All statistical tests were carried out with SPSS 15.0.

Table 2. Principal component coefficients and eigen values generated by PCA for larvae from the parent sea urchins (*Pseudechinus huttoni*) collected from the Otago Shelf and Doubtful Sound populations.

Body component	PC1	PC2
Postoral arm	0.137	-0.660
Anterolateral arm	0.137	-0.450
Posterodorsal arm	0.138	-0.500
Preoral arm	0.134	-0.281
Midline length	0.142	0.416
Body width	0.135	0.263
Stomach length	0.140	0.538
Rudiment length	0.131	0.674
Eigen value	6.6940	0.512
% of variance	83.678	6.400

Results

Egg studies: Eggs produced by the urchins from the Doubtful Sound population had a significantly greater mean diameter than those from the Otago Shelf (Table 1). None of the other egg characteristics were significantly different between the two populations.

Larval growth and shape: The larvae from the Otago Shelf parents had larger body components than the larvae from the Doubtful Sound parents at most sampling times (Figs. 2, 3). The concentration of the planktonic diet influenced the growth (size-at-age) of larval body components. The larvae fed a high-concentration planktonic diet had body components

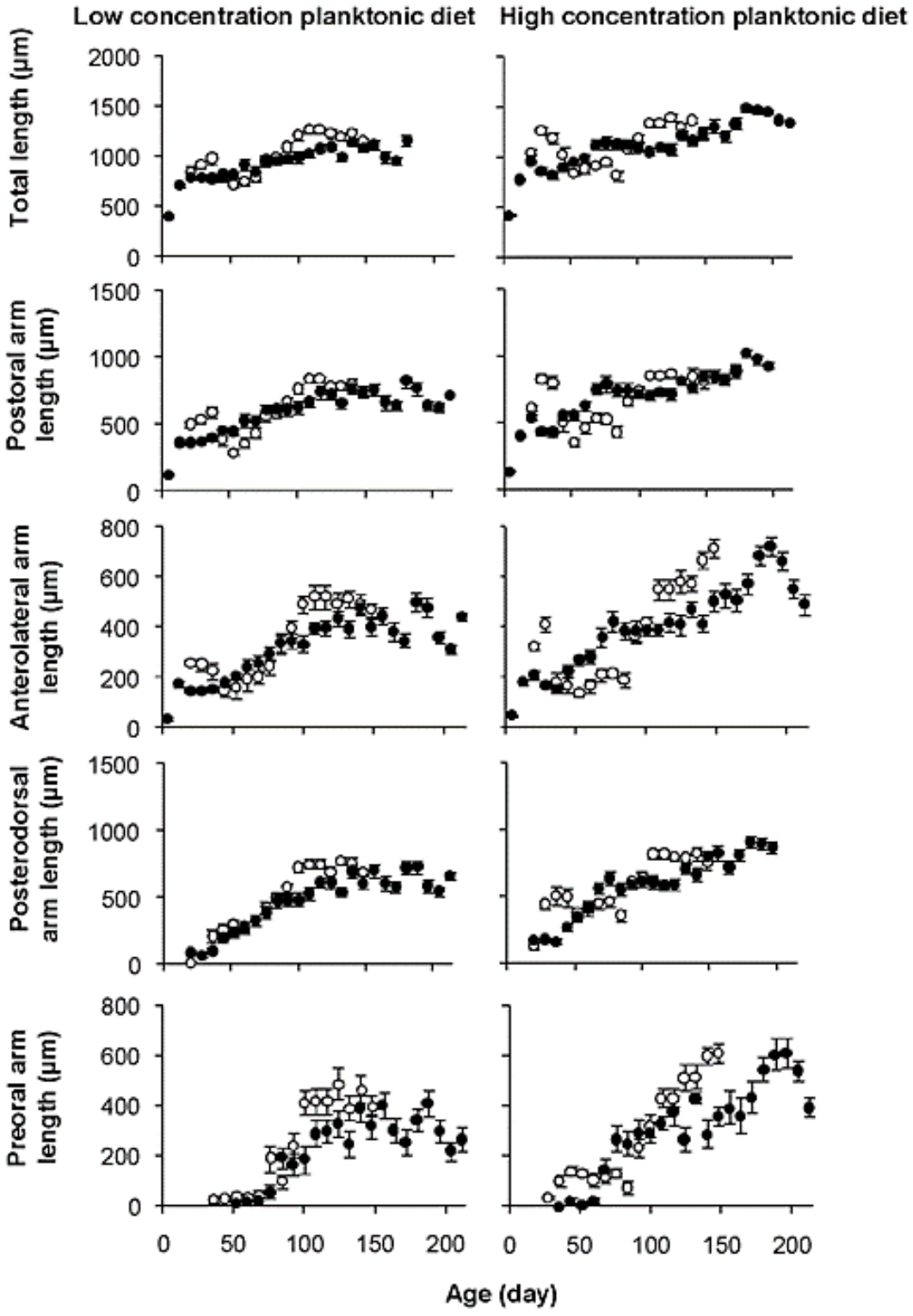

Figure 2. Mean (± SE) of total length and arm lengths of the larvae of the sea urchin (*Pseudechinus huttoni*) from the Otago Shelf (○) or Doubtful Sound (●) parents within standard and high concentrations planktonic diets. Data are pooled over the jar.

larger than those of the larvae fed a low-concentration planktonic diet (Figs. 2, 3).

The first principal component (PC1) explained the major part of the variance (>83%; Table 2). Coefficients of all larval body components were positive for PC1. Hence, higher scores for PC1

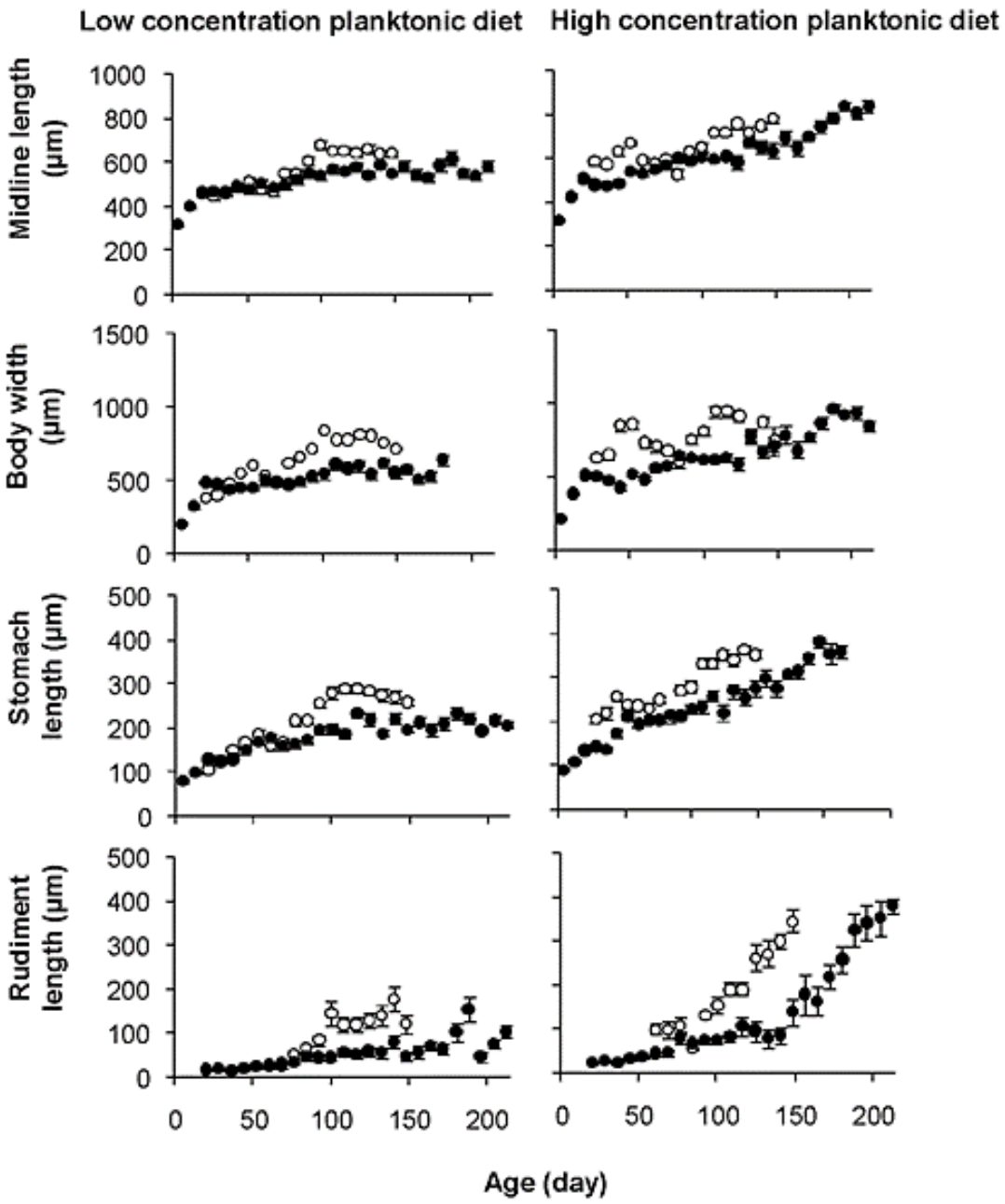

Figure 3. Mean (± SE) of body components of the larvae of the sea urchin (*Pseudechinus huttoni*) from the Otago Shelf (○) or Doubtful Sound (●) parents within standard and high concentrations planktonic diets. Data are pooled over the jar.

indicated larger body components. The coefficients of all arms were negative for the second principal component (PC2), while the coefficient of the midline length was positive for PC2 (Table 2). Thus, higher scores for PC2 showed shorter arms relative to the midline length. There was little overlap between scatter plots of the PC1 and PC2 scores of larvae from the Otago Shelf and Doubtful Sound parents (Fig. 4 a, b).

The larvae from the Otago Shelf parents had significantly greater mean PC1 and PC2 scores than those from the Doubtful Sound parents (PC1: df = 1, F = 76.204, P<0.001, PC2: df = 1, F = 4.417, P = 0.042). That is, larvae from the Otago Shelf had larger body components but had shorter arms relative to the midline length than those from the Doubtful Sound parents. The effect of larval age was not significant (PC1: df = 4, F = 1.617, P = 0.189; PC2: df = 4, F = 1.059, P = 0.389). There was no significant interaction between the parental population and larval

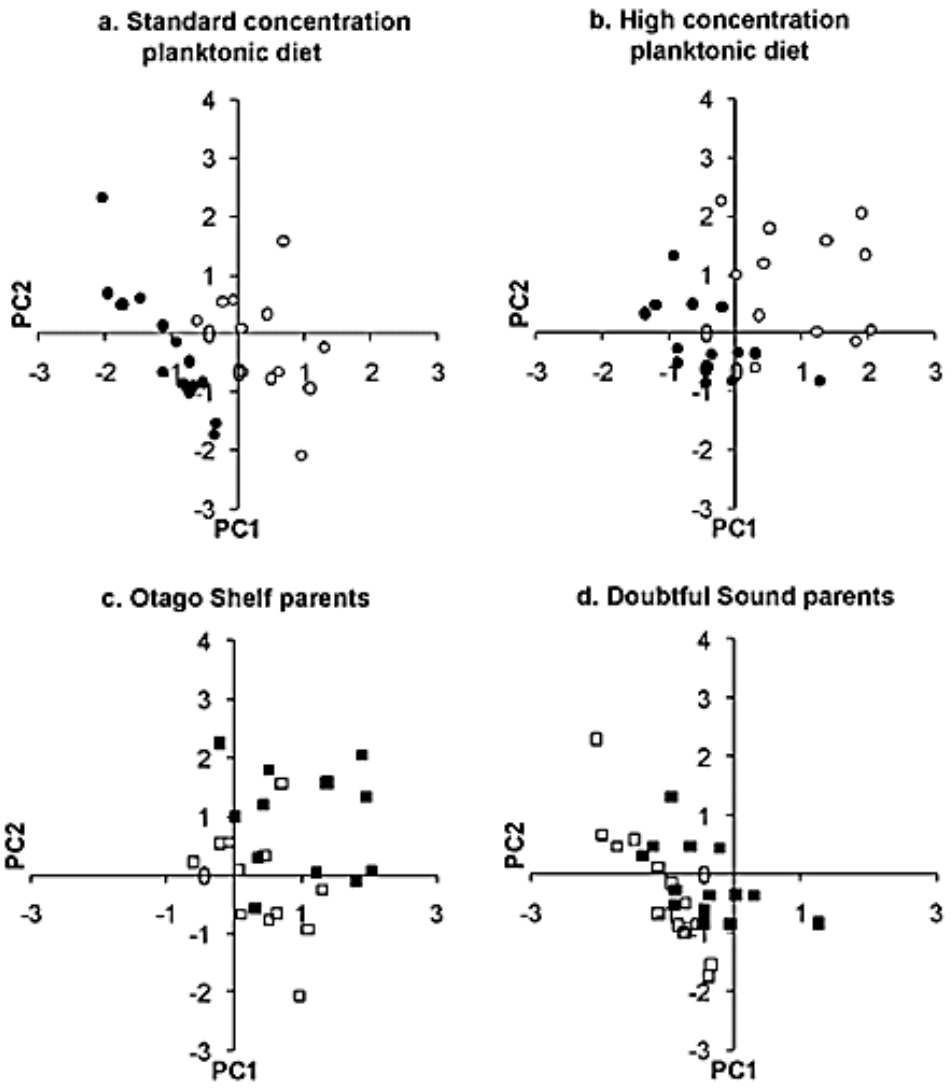

Figure 4. The first and second principal components scores of the larvae of the sea urchin (*Pseudechinus huttoni*) from Otago Shelf (○) or Doubtful Sound parents (●) fed a standard (a) or high (b) concentration planktonic diet; and larvae fed a standard (□) or high (■)concentration planktonic diet from Otago Shelf (c) or Doubtful Sound (d). Data are pooled over jar and sampling time.

diet for PC1 and PC2 scores (PC1: df = 1, F = 0.003, P = 0.959; PC2: df = 1, F = 3.461, P = 0.070).

Across the two parental populations, the larvae fed a high-concentration planktonic diet had significantly greater mean PC1 and PC2 scores than the larvae fed a standard-concentration planktonic diet (Fig. 4c and d; PC1: df = 1, F = 13.310, P = 0.001, PC2: df = 1, F = 6.393, P = 0.016). Hence, a high concentration of planktonic diet resulted in larvae having larger body components but shorter arms relative to the midline length. In summary, the importance of parental population was higher than that of larval diet on growth of the larvae (η_p^2 : 0.656 vs. 0.250) but lower than that of larval diet on morphological phenotypic plasticity of the larvae (η_p^2 : 0.099 vs. 0.138).

Larval development: Larvae from Otago Shelf parents had faster development than those from Doubtful Sound parents, e.g., the time to 8-arm stages was shorter in larvae from the Otago Shelf parents (compare Figure 5 a with c and b with d). Under a standard concentration planktonic diet, only the larvae from the Otago Shelf parents reached competency. Under a high-concentration planktonic diet, the larvae from the Otago Shelf parents became competent earlier (day 124 vs. day 140, Figure 5 b and d) and had a shorter larval period than the larvae from the

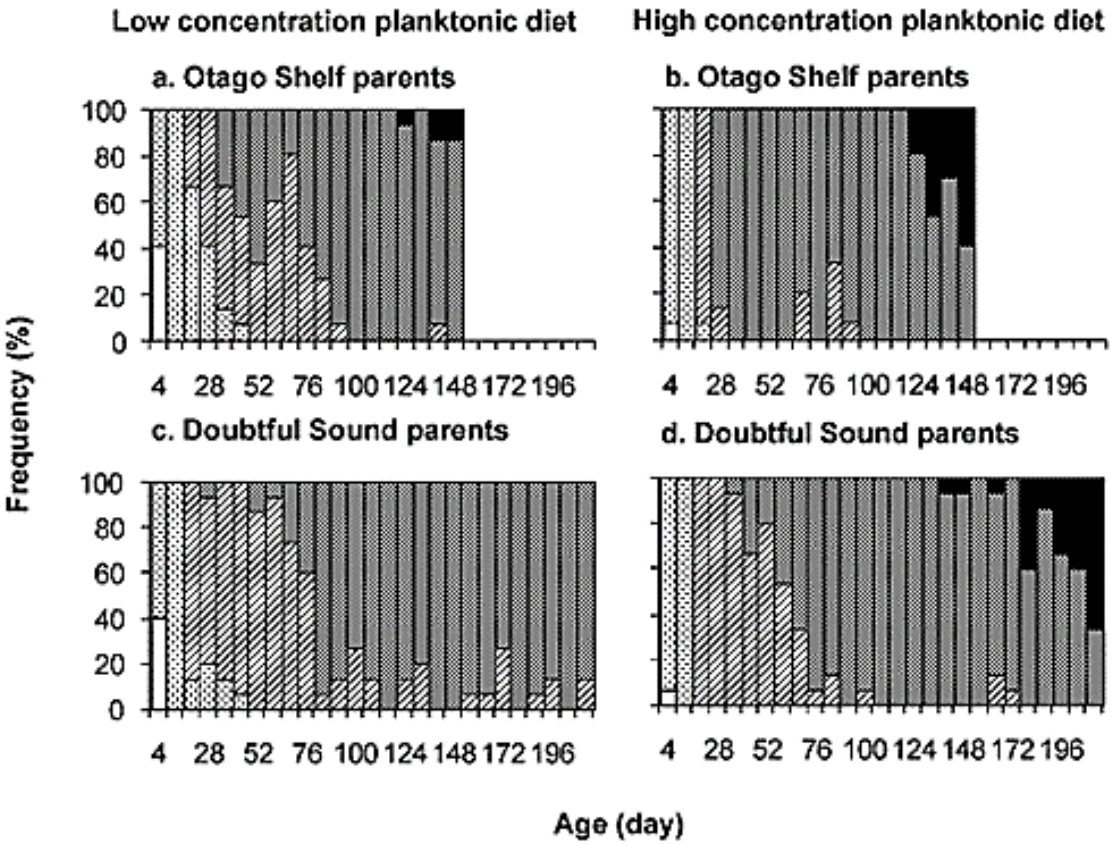

Figure 5. The developmental stage (2-arm: □, 4-arm: ⬚, 6-arm: ▨, 8-arm: ▩, and competent: ■) percentage of the larvae of the sea urchin (*Pseudechinus huttoni*) from the Otago Shelf or Doubtful Sound parents under a standard or high concentrations planktonic diets.

Doubtful Sound parents (mean ± SE: 142±4.6 vs. 193±10.6 days, df = 4, t = -4.359, P = 0.012).

Biochemical content of the larvae: The biochemical and energy content of the larvae increased over time (Fig. 6). There was a significant difference between the larvae from the Otago Shelf and Doubtful Sound parents in protein at day 1, in lipid at days 1 and 17, in carbohydrate content at day 17 and energy at day 1 (Table 3, Fig. 6). At days 1 and 17, larval diet had a significant effect on the lipid content of the larvae. On day 1, the larval diet had a significant effect on the carbohydrates of the larvae. In all detected significant differences, larvae from the Doubtful Sound parental population had greater biochemical and energy content.

On days 1 and 17, the parental population was more important than the larval diet in the lipid content of the larvae (η_p^2 : day 1, 0.844 vs. 0.605; day 17, 0.698 vs. 0.672). On day 17, the parental population was more important than the larval diet in the carbohydrate content of the larvae (η_p^2 : day 1, 0.405 vs. 0.401).

Discussions

Eggs from Doubtful Sound parents were larger than those from Otago Shelf, but there was no significant difference in biochemical and energy content. Also, our results indicate that egg size is not a good estimator of egg content, which had already been demonstrated by Mcedward and Carson (1987) and Mcedward and Chia (1991) (1991), and smaller eggs may even have denser materials that are consistent with Strathmann and Vedder (1977). As suggested by George (1996), an insignificant difference between eggs' biochemical and energy content between the two populations of the present study may just indicate a difference in water content.

Sea urchin populations may show a change in egg size that may be related to food availability (George, 1999). Our finding on egg size is seemingly consistent with the assumption of higher food availability for the

Table 3. The F values ($F_{1,8}$) and significance levels of the two-way ANOVA for the effects of parental population and larval diet on biochemical content of components of the body of the larvae (of the sea urchin *Pseudechinus huttoni*) at each sampling time.

Variable	Age	Parental population	Larval diet	Parental population × larval diet
Protein	1	7.057*	1.694 NS	1.694 NS
	17	1.771NS	1.744 NS	1.744 NS
	33	0.176 NS	0.385 NS	0.313 NS
	49	1.601 NS	0.002 NS	0.115 NS
Lipid	1	43.344***	12.265**	12.265**
	17	18.516**	16.410**	16.410**
	33	0.332 NS	1.816 NS	0.171 NS
	49	1.302 NS	0.707 NS	0.658 NS
Carbohydrate	1	4.980 NS	3.663 NS	3.663 NS
	17	5.449*	5.355*	5.355*
	33	0.901 NS	0.324 NS	0.657 NS
	49	0.397 NS	0.015 NS	0.012 NS
Energy	1	14.363**	3.945 NS	3.945 NS
	17	2.181 NS	2.130 NS	2.130 NS
	33	0.248 NS	0.993 NS	0.266 NS
	49	1.348 NS	0.575 NS	0.481 NS

Significance ***P<0.001, **P<0.01, *P<0.05, NS = non-significant

Doubtful Sound population. However, egg size may be influenced by confounding geographic/genetic factors, including fertilization success (Levitan, 1993; Podolsky and Strathmann, 1996), temperature, and salinity (Moran, 2004; Moran and McAlister, 2009), which were not measured in the present study. Hence, the reason for the difference in egg size between Otago Shelf and Doubtful Sound populations remains unknown.

The concentration of the planktonic diet significantly affected the growth, development rate, and morphological phenotypic plasticity of the larvae. Larvae fed a higher concentration of algal cells had larger body components, faster development, and shorter arms relative to the midline length. This result is not new; *P. huttoni* larvae have a planktotrophic nutritional habit, and former studies have already demonstrated the importance of exogenous food for the growth and development of planktotrophic larvae (Boidron-Metairon, 1988; Lamare and Barker, 1999; Meidel et al., 1999). The present study also showed the occurrence of morphological phenotypic plasticity in *P. huttoni* larvae to improve the feeding capability, which had been found previously in other sea urchins (Bertram and Strathmann, 1998; Hart and Scheibling, 1988; Hart and Strathmann, 1994; Sewell et al., 2004).

The larvae from the Otago Shelf population, which were assumed to have a lower nutritional status, produced larvae with faster growth and development rates and shorter arms relative to the midline length. Parental population was found to be more than two times as important as larval diet on larval growth. However, the parental population was less important than the larval planktonic diet in the morphological, phenotypic plasticity of the larvae. The present study agrees with Byrne et al. (2008), indicating that egg quality is more important than egg size because our larvae from smaller eggs (with higher quality) had a faster development rate, although there was no significant difference between eggs from the two parental populations. There is no universal pattern on the effects of egg size/reserve and exogenous food on larval characteristics in sea urchins. For instance, some studies show that the effects of egg reserves on larval features are similar to that of the planktonic diet, with larger eggs producing larvae with faster development (Emlet and Hoegh-Guldberg, 1997) and/or a lower capacity for phenotypic plasticity (Strathmann et al., 1992). Contrastingly, some studies believe that smaller eggs have a greater capacity for phenotypic plasticity and development of larvae, which is dissimilar to that of exogenous food (Reitzel

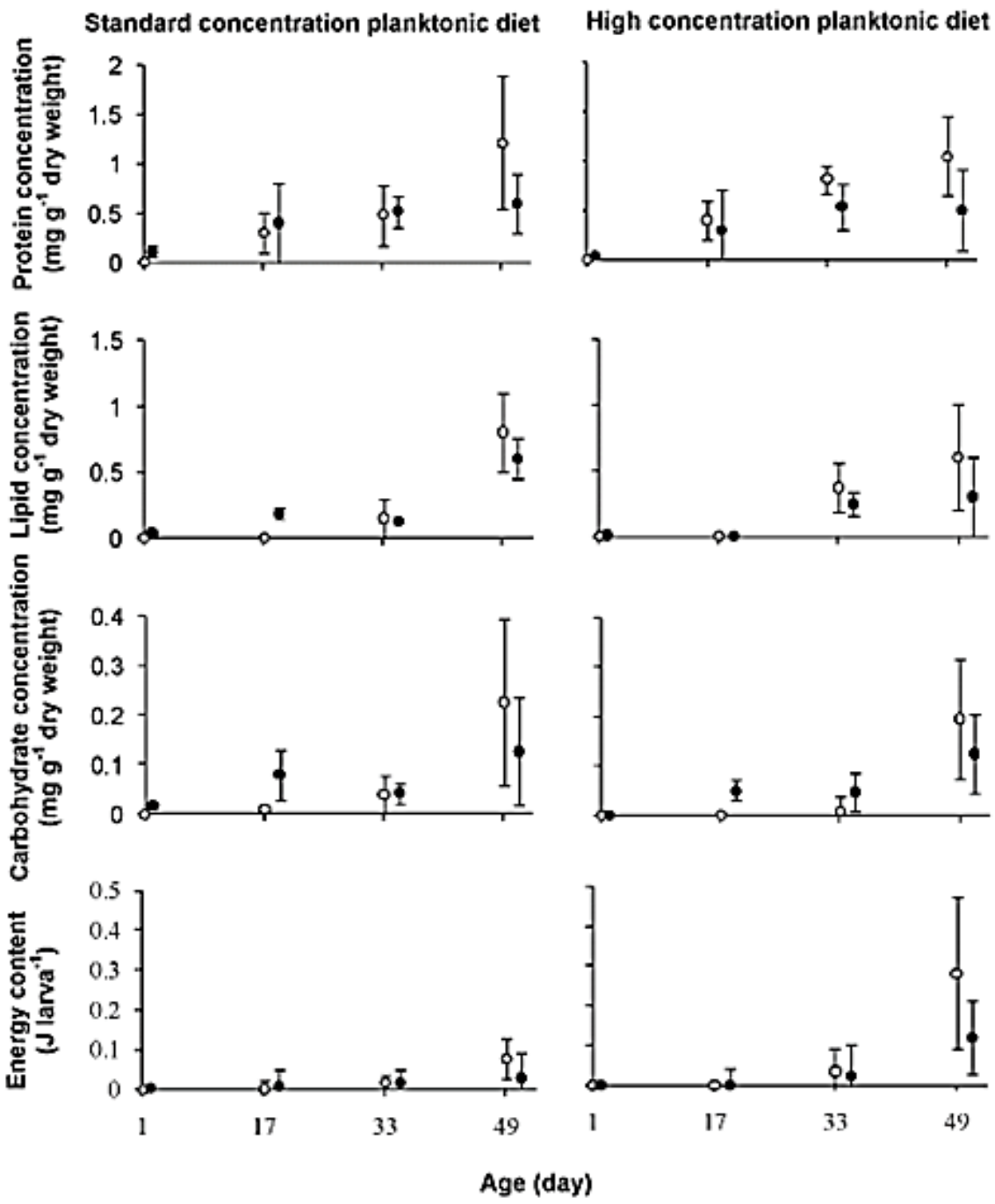

Figure 6. Mean (± SE) of protein, lipid, carbohydrate, and energy content of the larvae of the sea urchin (*Pseudechinus huttoni*) from the Otago Shelf (○) or Doubtful Sound (●) parents fed a standard or high concentration planktonic diet.

et al., 2005; McAlister, 2007). Furthermore, Soars et al. (2009) found contrasting phenotypic plasticity patterns in *Heliocidaris tuberculata* and *Centrostephanus rodgersii,* suggesting that non-nutritional may be involved in phenotypic plasticity of echinoplutei. The present study is apparently in agreement with McAlister (2007). However, a study on *P. huttoni* (Poorbagher et al., 2010) proposed that phenotypic plasticity may be consistent with Reitzel and Heyland's (2007) contention. Such a conflict between the current study and Poorbagher et al. (2010) may hint at an egg parameter other than biochemical and energy content egg as a responsible factor for the capacity of phenotypic plasticity in larvae from the

two populations. In particular, larvae from Doubtful Sound parents that produced eggs with lower biochemical and energy contents had significantly greater protein, lipid, carbohydrate, and energy in some sampling times. Our results may thus further suggest that egg content cannot fully justify parental effects on sea urchin larvae. In this respect, Jaeckle (1995) warned that measuring protein, lipid, and carbohydrate contents and converting these values to energy, *per se*, is useful for comparing different eggs because their forms may have greater importance. Also, the two parental populations of our study were exposed to different geographic/genetic factors that may have an effect on their life-history traits, which has been suggested to be taken into account in addition to egg size and reserve (McAlister, 2007; McAlister, 2008).

Faster development of larvae from Otago Shelf parents may be related to phenotypic plasticity. The PCA indicated that there was an inverse relationship between arm lengths and stomach and rudiment lengths, i.e., between the allocation of materials to structures used for acquiring food and settling. Developing shorter arms may suggest that a greater amount of resources are allocated to the growth of the stomach and rudiment. A larger stomach may let a greater amount of food to be digested (Miner, 2005) and a larger rudiment may reduce the time to metamorphosis (Bertram and Strathmann, 1998).

Conclusion

Although it has been shown that planktonic diet is more important than the effect of parents on the characteristics of the planktotrophic larvae (Bertram and Strathmann, 1998; Meidel et al., 1999), our study indicated that parents were more important than the concentration of the planktonic diet on the growth and morphological phenotypic plasticity of *P. huttoni* larvae. We also found that parental population has a substantial effect on larval period and development rate. While the parental population can affect larval features, the egg quality, measured by analysis of protein, lipid, carbohydrate, and energy content, may not be the only reason. It has been shown that differences in the development of larvae may be related to differences in the amount of growth hormone (thyroxin) in the egg (Saito et al., 1998; Heyland et al., 2004) or egg carotenoids (Tsushima et al., 1995). Further study is required to find a factor in the egg that changes in response to the parental population and subsequently influences larval life.

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