

The relative importance of parental nutrition and population versus larval diet on development and phenotypic plasticity of *Sclerasterias mollis* larvae

HADI POORBAGHER¹, MILES D. LAMARE AND MIKE F. BARKER

Department of Marine Science, University of Otago, PO Box 56, Dunedin, New Zealand, ¹Present address: Department of Fishery and Environmental Sciences, University College of Agriculture and Natural Resources, University of Tehran, Karaj, Iran

The relative importance of parental diet/population and larval diet were examined on egg, growth, morphology and biochemistry of Sclerasterias mollis larvae. Adult S. mollis were fed one cockle (Austrovenus stutchburyi) per two animals each week, as a low diet, or two cockles per animal each week, as a high diet. The experiment was run for one year. In addition, two field populations (Otago inshore and offshore) with dissimilar nutritional status (based on the gonad index) were selected. Otago inshore starfish had higher gonad indices and assumed to have better nutritional status. The low and high diet laboratory starfish produced eggs with similar characteristics. Eggs from the low diet laboratory parents had the highest carbohydrate concentration. The eggs from the field parents had higher fertilization rate and lower carbohydrate concentration than eggs from the laboratory parents. The Otago inshore starfish had smaller eggs with a lower carbohydrate concentration than the starfish from Otago offshore. Parents from the laboratory or the field had significant effects on larval growth, morphological phenotypic plasticity (measured by the body length relative to the body width) and development rate. Larvae from Otago offshore parents had highest growth and morphological phenotypic plasticity. Larvae from the low diet laboratory parents and those from Otago inshore had the highest development rate. Larvae from low diet laboratory parents had the highest carbohydrate concentration. Neither the parents nor the larval diet had a significant effect on larval mortality. A higher concentration planktonic diet resulted in higher growth, morphological phenotypic plasticity and development rate. Parents were however more important than larval diet on growth and phenotypic plasticity of the larvae. This study showed that parental nutrition has an important effect on growth, morphological phenotypic plasticity and body composition of S. mollis larvae. The nutritional status of the parents does not influence the larvae through a change in the egg size, protein, lipid, carbohydrate and energy content.

Keywords: egg, larvae, parental nutrition, parental population, starfish

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INTRODUCTION

Two energy sources exist for planktotrophic larvae: an endogenous and an exogenous one, with the latter coming from particulate food and dissolved organic matter. Because planktotrophs tend to produce small eggs with relatively low reserves, they should depend more on exogenous than endogenous energy. Indeed, the strong effect of external energy on the growth and development of planktotrophic echinoderms larvae has been extensively documented (Strathmann *et al.*, 1992; Hart & Strathmann, 1994; Basch, 1996; Meidel *et al.*, 1999).

Endogenous energy stems from the maternal allocation of nutrients to eggs of planktotrophic larvae. Maternal nutrition, maternal habitat and body size have an influence on the egg

size and quality (Jong-Westman *et al.*, 1995; George, 1999). Mothers with a higher nutrition may produce eggs of a larger size and/or with a greater organic content, resulting in higher larval growth, development, survival and metamorphosis rates (George, 1990; George *et al.*, 1990; Jong-Westman *et al.*, 1995). However, others believe that the parental nutritional condition and the endogenous energy source have little or no effect on the subsequent characteristics of planktotrophic larvae (McEdward, 1986a; Bertram & Strathmann, 1998; Meidel *et al.*, 1999). Therefore, the importance of parental food rations on larval growth and development remains unclear for planktotrophic echinoderms.

Although a number of studies have examined the effects of parental diet or population on sea urchin larvae (George, 1990; George *et al.*, 1997; Bertram & Strathmann, 1998; Meidel *et al.*, 1999), little is known about these effects on larval features in starfish (see George, 1999).

To this end, we examined the relative contributions of the parental nutritional status and larval nutrition to the growth, development, shape, mortality rate and biochemical

Corresponding author:

H. Poorbagher

Email: hpoorbagher@yahoo.com

composition of *Sclerasterias mollis* larvae. This species is widely distributed throughout the south-east inshore waters of New Zealand and can be found at depths ranging from 40 to 140 m (Barker & Xu, 1991). *Sclerasterias mollis* has bipinnaria developing brachiolaria larvae with planktotrophic nutrition. Furthermore, to determine whether variation in larval features can be explained by egg characteristics, the egg diameter, fecundity, fertilization percentage, biochemical and energy content of eggs were examined. Eggs and larvae from both laboratory held and field collected adults were studied because it was not known whether the laboratory provided optimal food and environmental conditions for the adults.

MATERIALS AND METHODS

Collection and conditioning of adult *Sclerasterias mollis*

Sclerasterias mollis were collected from inshore waters on the Otago continental shelf (New Zealand; 45°47'218"S 170°51'717"E) using an Agassiz bottom trawl and transferred to the laboratory. Three hundred starfish were randomly allocated to six rectangular plastic tanks (82 × 22 × 22 cm) supplied with sand-filtered (50 µm) running seawater (≈2 l min⁻¹) was provided for each tank. Animals were fed a low diet (three tanks, half a cockle *Austrovenus stutchburyi* with 2–3 cm diameter per starfish every two weeks) or a high diet (three tanks, two cockles per starfish every week), respectively. Tanks were cleaned and scrubbed every two weeks. The experiment was run for one year.

We further sampled two populations with a dissimilar nutritional status from either Otago inshore (45°47'218"S 170°51'717"E; 60 m depth) or offshore (45°48'235"S 170°55'928"E; 100 m depth) waters. The nutritional status of the two populations was inferred through the gonad index, where a higher gonad index indicates better nutrition (Xu & Barker, 1990). The gonad index was measured as the per cent ratio of the gonad wet weight to body wet weight. The Otago inshore *S. mollis* had significantly greater gonad indices (Otago inshore: N = 96, mean ± SE = 5.54 ± 0.57%, Otago offshore: N = 83, 1.71 ± 0.34%; *t*-test: df = 177, *t* = 5.525, *P* < 0.001). When the adults were seasonally ripe (see the section 'Egg studies'), they were sampled by an Agassiz bottom trawl.

Egg studies

Three months before the beginning of the breeding season (August–September), five *S. mollis* were selected each month randomly from the tanks or sampled from the Otago inshore and offshore populations. A small piece of the gonad (size 1 cm) was biopsied and inspected under a compound microscope to assess egg maturity. When the oocyte diameters were approaching 100 µm, and both the germinal vesicle and the nucleolus were invisible, nine to ten females were taken from the low and the high diet laboratory groups or sampled from the Otago inshore and offshore populations. The sex of the animals was determined on a fresh slide smear under a compound microscope. Ovaries were excised and put in a 250 ml beaker containing 50 ml of 10⁻⁵ M 1-methyladenine. Two hours after the onset of gamete release, ovaries were removed from the solution to avoid a decrease in the fertilization

percentage due to eggs sticking to each other. Most ovaries were spent after two hours. A 500 µl sample was taken to measure egg diameter and fecundity. A 5 or 10 ml sample was taken, concentrated in an Eppendorf tube, rinsed with distilled water, and kept at -80°C for later analyses.

The following egg characteristics were measured: (1) egg diameter—the average egg diameter was determined from 30 eggs per female using an ocular micrometer. If eggs were not spherical, the average of the major and minor axes was used as the egg diameter; (2) egg dry weight—a defined number of eggs were put in a dry pre-weighed eppendorf tube, freeze-dried for 24 hours and weighed; (3) fecundity—the average number of eggs in ten 2 µl subsamples per female; (4) fertilization percentage—for each female, the fertilization percentage of 100 randomly-selected eggs was determined. Inflation of the egg membrane and formation of a perivitelline space was indicative of fertilization.

Protein concentration as well as lipid and carbohydrate content of freeze-dried eggs were measured by the Bradford protein assay (Bradford, 1976) and by colorimetric methods (Mann & Gallager, 1985), respectively. The egg energy content was estimated using the energy equivalent for protein, lipid and carbohydrate: 5.65, 9.45, and 4.0 kcal g⁻¹, respectively (Brody, 1945).

Larval studies

Larvae from each parental treatment were reared at 12°C in six 3 l glass jars at a density of two larvae ml⁻¹ and were kept suspended in water column using a standard paddle system at 10 strokes min⁻¹ (Strathmann, 1987). Larvae were fed *Dunaliella primolecta* at densities of 2000 and 8000 cells ml⁻¹ for low and high concentration planktonic diets, respectively. The experimental design was full-factorial (4 parents: low and high diet laboratory parents, Otago inshore and offshore field parents × planktonic diet concentrations × 3 jars). Algae cultures were replaced every two weeks. Water levels were lowered every four days by 90% and replenished with fresh 1 µm filtered seawater. At the same time, the culture jars were cleaned. Larviculture lasted for 300 days.

Every 15 days, five intact larvae were sampled randomly from each jar. The developmental stage was determined according to Basch (1996) and classified as: (1) early-bipinnaria 1—coelom lower to mouth; (2) early-bipinnaria 2—coelom parallel to mouth; (3) early-bipinnaria 3—coelom upper to mouth and unfused; (4) early mid-bipinnaria—coelom upper to mouth and fused; (5) mid-bipinnaria—coelom extended to anterior process; (6) early-brachiolaria—brachiolar arm buds appear; and (7) brachiolaria—presence of adhesive disc and brachiolarian arms. Body length, body width, mouth width, oesophagus length, coelomic pouch length, stomach length and width (Figure 1) were measured using an ocular micrometer.

Every 15 days, three 5 ml samples were taken from the middle of each jar and the number of larvae was counted using a Bogorov counting tray under a dissecting microscope before being returned to the jars. Larval density in the jars was derived from the average of triplicate measurements at a specific sampling time. Only data for the first 90 days was used for the analysis, because thereafter larval density decreased to levels not measurable by this technique.

Larvae from each parental treatment were reared at a larval density of 2 ml⁻¹ in two 67 l white plastic tanks (0.53 m height × 0.51 m diameter) at 12–14°C, with one tank for

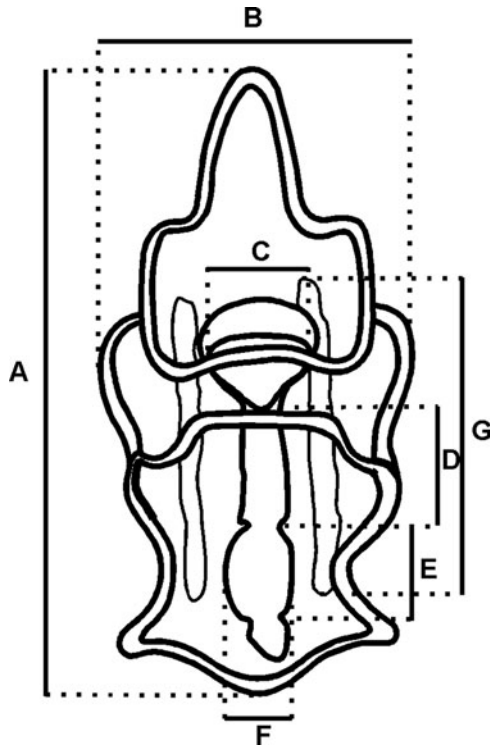


Fig. 1. Morphometric dimensions measured in a *Sclerasterias mollis* bipinnaria larva. (A) Body length; (B) body width; (C) mouth width; (D) oesophagus length; (E) stomach length; (F) stomach width; (G) coelomic pouch length.

the low and another for the high concentration planktonic diet. Tanks were equipped with paddles connected serially to a gearbox motor (1/8 HP) generating 12.5 strokes per minute. A 50 μm mesh round filter (10 cm diameter) protected the outlet on the top of each tank. The diet was as described above for the jars. Water was changed every two days by adding 30 l of 1 μm filtered isothermal seawater to each tank. Tanks were discharged, scrubbed and washed with fresh water every two weeks. Every 16 days, three samples of 3000 larvae each were taken, concentrated in Eppendorf tubes, rinsed with distilled water and kept at -80°C for later biochemical analyses.

Data analysis

EGG STUDIES

Significant differences among the egg characteristics from laboratory or field parents were examined using a one-way ANOVA. Normality and homogeneity of variance were tested using the Shapiro–Wilk and the Levene tests, respectively.

LARVAL STUDIES

Principal component analysis (PCA) was used to determine differences among parents and larval planktonic diets in larval growth (size-at-age) and shape (the relative size of the body length-to-width). Body length, body width, mouth width, oesophagus length, stomach length, stomach width and coelomic pouch length were analysed. To avoid pseudoreplication, the average of each morphometric variable was calculated over the jar prior to analysis (Bertram & Strathmann, 1998). The first two principal components (PC) were chosen based on a scree diagram (Everitt & Dunn, 1991). A correlation matrix

was used for PCA as the variables had very different variances (Quinn & Keough, 2002). Because all variables were strongly skewed, a logarithmic transformation was applied to improved normality. PC1 was considered as a growth component. PC2 was considered as a shape component, because the body length coefficient contrasted the body width coefficient. Therefore, larger scores for PC2 would show a smaller body length relative to the body width. To test the difference in growth and shape between larvae from different parents and larval planktonic diets, the means of the PC scores were compared using Wilcox's three-way design (Wilcox, 2005), with parents, larval diet and age as fixed factors. This test is based on 20% trimmed means and the bootstrap method. ANOVA could not be applied, because the assumptions for data normality and variance homogeneity were not given.

The instantaneous mortality rate of the larvae was calculated using the exponential model (George *et al.*, 1990):

$$N_t = N_0 e^{-Kt}$$

where N_t = number of larvae alive at time t (days), N_0 = number of larvae at time $t = 0$ (2 ml^{-1}), e = the base of the natural logarithm (≈ 2.71) and K = instantaneous mortality rate (day^{-1}).

The effects of parents, larval diet, and age on larval mortality rates, protein, lipid, and carbohydrate concentrations were examined by the three-way Wilcox test, because it was not possible to meet the ANOVA assumptions. Parents, larval diet and age were fixed factors.

Main effects in ANOVA and the Wilcox three-way design were addressed if no interaction between factors was observed. If a significant interaction between parents and larval diet was present, levels of one factor were compared with each level of the other factor (Underwood, 1997) using a Tukey or t -test. A significant interaction among parents, larval diet and age was interpreted as parents \times larval diet and age (Underwood, 1997). In other words, levels of parents were compared within each level of larval diet (and vice versa) at each larval age. The family-wise probability of Type-I error was adjusted using the Bonferroni method (0.05 divided by the number of multiple comparisons; Underwood, 1997).

Multinomial logistic regression and likelihood ratio tests were used to determine if parents and larval diet had a significant effect on larval development. Due to singularities in the Hessian matrix, jar and larval age were omitted sequentially. Eliminating jar and larval age did not affect the parents and larval diet effects. Only main effects were tested, because the inclusion of the interaction of the parents and the larval diet in the model would decrease the degrees of freedom of main effects to zero. Ordinal regression was not used, as the test of parallelism revealed that the relationships between the independent variables and the logits were not the same for the all logits (Norusis, 2008). Statistical analyses were performed using SPSS 15.0 (release 15.0.0) and R (version 2.7.1, Rand R. Wilcox's allfun package).

RESULTS

Egg studies

Eggs from the low diet and the Otago offshore parents were larger than those from the high diet and the inshore parents

(Table 1). Eggs from parents collected in the field had a higher fertilization rate but a lower carbohydrate concentration than eggs from parents held in the laboratory (Table 1). Thus, restricted diet is associated with an increased egg size.

Larval growth and shape

EFFECTS OF PARENTS

The PC₁ coefficients were positive for all larval body components and indicated body growth, whilst the PC₂ coefficients for the body length contrasted with those for the body width and thus indicated the shape of the larvae (Table 2).

All larval body components increased significantly over time and reached sizes greater than those at the beginning of the experiment (Figures 2 & 3; Table 3). However, in larvae derived from the laboratory held parents and fed a low concentration planktonic diet, the mouth width, stomach length and stomach width did not grow after day 45.

PC₁ and PC₂ scores of larvae from the laboratory and the field parents are shown in Figure 4. There was a significant interaction among parents, larval diet and age in PC₁ scores (Table 3). Tukey tests between levels of parents (low and high diets, Otago inshore and offshore populations) indicated that, under a low concentration planktonic diet, larvae from Otago offshore parents had greater mean PC₁ scores than those from other parents at days 30, 210, 225, 255, 270 and 285. Under a high concentration planktonic diet, larvae from Otago offshore parents had higher mean PC₁ scores at days 30 and 240, indicating their larger body components than larvae from other parents. There was no significant difference in the mean PC₁ scores between larvae from low and high diet laboratory parents. Hence, larvae from Otago offshore parents had the largest body components.

There were significant interactions among parents, larval diet and age in the mean PC₂ scores (Table 3). Further Tukey tests between levels of parents indicated that, under a low concentration planktonic diet, larvae from Otago offshore parents had larger mean PC₂ scores at days 60, 105, 210, 225, 255 and 285 than larvae from other parents. Under a high

Table 2. Principal component coefficients and eigen values generated by PCA for *Sclerasterias mollis* larvae from the parents held with a low or high diet in the laboratory, and from Otago inshore and offshore parents.

Body component	PC ₁	PC ₂
Body length	0.175	-0.178
Body width	0.169	0.286
Mouth length	0.126	0.021
Oesophagus length	0.169	-0.448
Stomach length	0.167	0.315
Stomach width	0.150	0.827
Coelomic pouch length	0.156	-0.774
% of variance	79.961	8.417
Eigen value	5.597	0.589

concentration planktonic diet, larvae from Otago offshore parents had higher mean PC₂ scores at day 240, indicating their smaller body length relative to the body width than larvae from other parents. There was no significant difference in the mean PC₂ scores between larvae from low and from high diet laboratory parents. Thus, larvae from Otago offshore parents showed the highest rate of morphological phenotypic plasticity.

EFFECTS OF LARVAL DIET

PC₁ scores indicated a significant interaction among parents, larval diet and age in mean PC₁ scores (Table 3). A *t*-test was used to compare the PC₁ scores from larvae fed low or high concentration planktonic diets within each level of parents and at each larval age; no significant difference was observed between low and high concentration planktonic diets in the PC₁ scores from larvae derived from low-diet, laboratory-held parents. Larvae from high-diet, laboratory-held parents and fed a high concentration planktonic diet had greater mean PC₁ scores at days 240 and 285 than when fed a low concentration planktonic diet. Larvae from Otago inshore parents and fed a high concentration planktonic diet had greater mean PC₁ scores than low diet larvae at day 30. Larvae from Otago offshore parents and fed a high concentration planktonic diet had a greater

Table 1. Egg characteristics and one-way ANOVA for the effect of *Sclerasterias mollis* held in the laboratory or collected from Otago inshore and Otago offshore on egg features. Sample statistics are mean \pm SE with sample size in parentheses. Carbo., carbohydrate. Significant *P* values are shown in bold. Lack of at least one similar letter between parents indicates a significant difference ($\alpha = 0.05$) detected by a Tukey test ($B > A$).

Egg characteristics	Laboratory parents		Population parents		ANOVA		
	Low diet	High diet	Otago inshore	Otago offshore	df	F	P
Egg diameter (μm)	124.2 \pm 1.2 (10) AB	121.3 \pm 0.9 (10) A	121.2 \pm 1.5 (10) A	126.1 \pm 0.8 (9) B	3	5.052	0.005
Fecundity ($\times 10^5$)	10.6 \pm 5.2 (10)	8.0 \pm 1.7 (10)	21.7 \pm 5.8 (10)	19.4 \pm 4.6 (9)	3	2.081	0.121
Fertilization rate (%)	29.9 \pm 7.7 (10) A	36.3 \pm 5.5 (10) A	82.0 \pm 6.5 (10) B	66.9 \pm 7.4 (9) B	3	13.538	<0.001
Protein (mg g ⁻¹ dry weight)	399.4 \pm 24.7 (9)	355.0 \pm 17.1 (10)	338.6 \pm 28.2 (10)	409.9 \pm 58.6 (9)	3	0.976	0.416
Protein content ($\times 10^{-2}$ $\mu\text{g egg}^{-1}$)	8.9 \pm 1.6 (9)	8.5 \pm 1.0 (10)	11.5 \pm 0.9 (10)	11.1 \pm 1.7 (9)	3	0.951	0.427
Lipid (mg g ⁻¹ dry weight)	126.8 \pm 4.1 (10) A	124.6 \pm 2.5 (10) A	137.2 \pm 5.7 (10) A	139.3 \pm 4.1 (9) A	3	2.958	0.046
Lipid content ($\times 10^{-2}$ $\mu\text{g egg}^{-1}$)	2.8 \pm 0.4 (10)	3.0 \pm 0.3 (10)	4.7 \pm 0.7 (10)	3.8 \pm 0.4 (9)	3	2.700	0.061
Carbo. (mg g ⁻¹ dry weight)	73.2 \pm 4.3 (10) B	67.4 \pm 3.0 (10) B	31.9 \pm 3.3 (10) A	44.0 \pm 3.2 (9) A	3	30.847	<0.001
Carbo. content ($\times 10^{-2}$ $\mu\text{g egg}^{-1}$)	1.5 \pm 0.2 (10)	1.6 \pm 0.2 (10)	1.1 \pm 0.2 (10)	1.2 \pm 0.1 (9)	3	1.681	0.189
Energy ($\times 10^{-7}$ kcal)	9.0 \pm 1.4 (10)	8.2 \pm 0.9 (10)	11.3 \pm 1.8 (10)	10.3 \pm 1.2 (9)	3	0.000	1.000

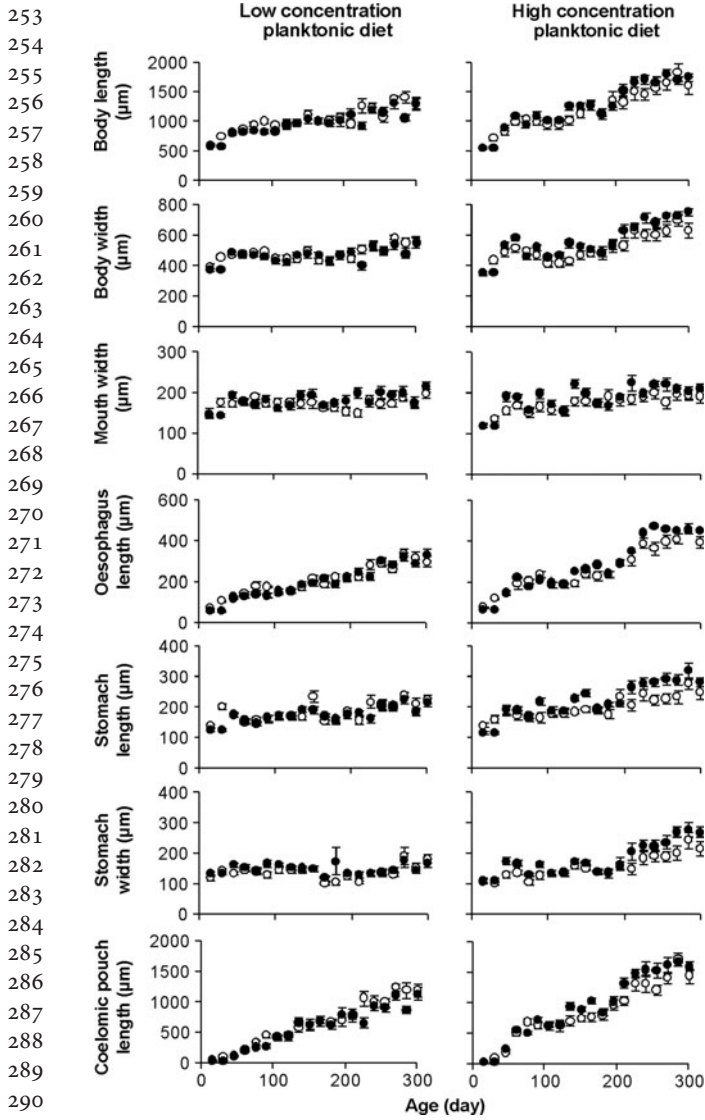


Fig. 2. Mean (\pm SE) body components of *Sclerasterias mollis* larvae from low (\circ) and high (\bullet) diet laboratory parents under low and high concentrations planktonic diets. Means were calculated from 15 larvae from three jars.

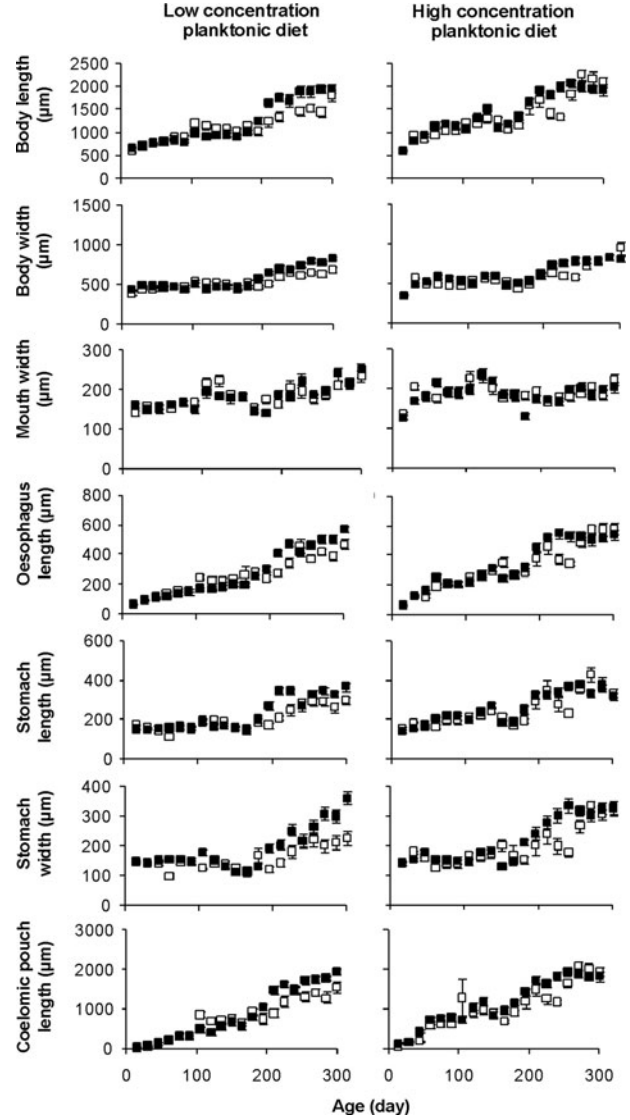


Fig. 3. Mean (\pm SE) body components of *Sclerasterias mollis* larvae from Otago inshore (\square) and Otago offshore (\blacksquare) field parents under low and high concentrations planktonic diets. Means were calculated from 15 larvae from three jars.

mean PC1 scores at days 45 and 60, indicating larger body components than those of larvae fed a low concentration planktonic diet at these days. Therefore, concentration of planktonic diet had a short-term effect on the size of larval body components.

PC2 scores indicated a significant interaction among parents, larval diet and age in the mean PC2 scores (Table 3). Using a *t*-test, PC2 scores from larvae fed low or high concentration planktonic diets were compared within each level of parents and at each larval age. Larvae from low-diet laboratory parents and fed a high concentration planktonic diet had greater mean PC2 scores at day 165 than when fed the 'low' diet. Larvae from high-diet laboratory parents and fed a high concentration planktonic diet had greater mean PC2 scores at days 240, 255 and 300. Larvae from Otago offshore parents and fed a high concentration planktonic diet had significantly greater mean PC2 scores at days 15 and 285, indicating a smaller body length-to-body width-ratio than that of the 'low' diet group. Larval diet had no significant effect on mean PC2 scores of larvae from

Otago inshore parents. Hence, concentration of planktonic diet had a short-term effect on shape of the larvae.

Based on the Wilcoxon test Q statistics, parents had a stronger impact on larval growth (based on PC1 scores) and shape (based on PC2 scores; Table 3) than the planktonic diet.

Table 3. Three-way Wilcoxon test for the effects of parents, larval diet and age on the first and second principal component scores of *Sclerasterias mollis* larvae. Significant *P* values are shown in bold.

Source of variation	PC1		PC2	
	Q	P	Q	P
Parents	194.611	<0.001	155.769	<0.001
Larval diet	181.280	<0.001	2.755	0.100
Larval age	3417.335	<0.001	1417.533	<0.001
Parents \times larval diet	14.567	0.005	13.064	0.008
Parents \times larval age	426.456	<0.001	300.293	<0.001
Larval diet \times larval age	100.514	<0.001	181.809	<0.001
Parents \times larval diet \times larval age	186.173	<0.001	168.561	0.002

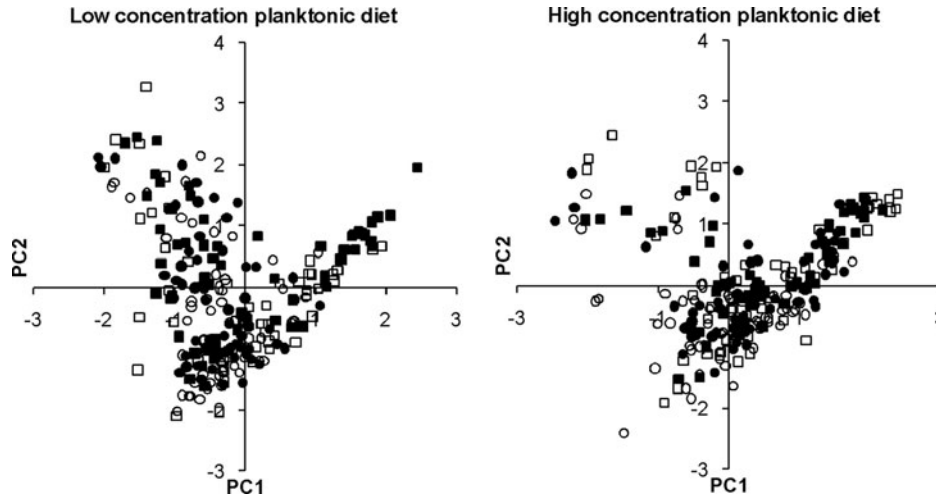


Fig. 4. Means of the first and second principal components scores of *Sclerasterias mollis* larvae from low (○) and high (●) diet laboratory parents and from Otago inshore (□) and Otago offshore (■) field parents under a low or high concentration planktonic diet. Data are pooled over jar and larval age.

Larval development

Parents had a significant effect on the larval development rate ($-2 \log$ likelihood of reduced model: 430.600; likelihood ratio tests: $df = 21, \chi^2 = 156.072, P < 0.001$). Larvae from parents held in the laboratory on a low diet and those from Otago inshore parents had a faster development than the larvae from high diet laboratory and Otago offshore parents, respectively (compare Figure 5A with C and B with D).

Larval diet had a significant effect on the larval development rate, with larvae on a high planktonic diet having a faster development rate ($-2 \log$ likelihood of reduced model = 446.466, likelihood ratio tests: $df = 7, \chi^2 = 171.939, P < 0.001$; compare Figure 5A with B, C with D, E with F, and G with H).

Larval mortality

Neither parental nor nutritional conditions had a significant effect on larval mortality (Table 4; Figure 6). The only parameter significantly affecting the larval mortality rate was time.

Biochemical composition of the larvae

EFFECTS OF PARENTS

Parents had no significant effect on protein and lipid concentration of the larvae (Table 5). A three-way Wilcoxon test detected a significant interaction between parents and larval diet with regards to the larval lipid concentration (Table 5). However, further Tukey and t -tests at each larval age indicated that parents and larval diet had no significant effect on the lipid concentration (Figure 7).

There was a significant interaction among parents, larval diet and age regarding the larval carbohydrate concentration (Table 5). Thus, further Tukey and t -tests comparing parental levels within each level of the larval diet and at each larval age were performed. Larvae from low-diet, laboratory-held parents had a greater mean carbohydrate concentration than the larvae from other parents at days 8 and 86 (Figure 7). Hence, parent only had a significant effect on carbohydrate concentration of the larvae.

EFFECTS OF LARVAL DIET

Larval diet had no significant effect on the protein and lipid concentration of larvae (Table 5). There was a significant interaction among parents, larval diet and age regarding the

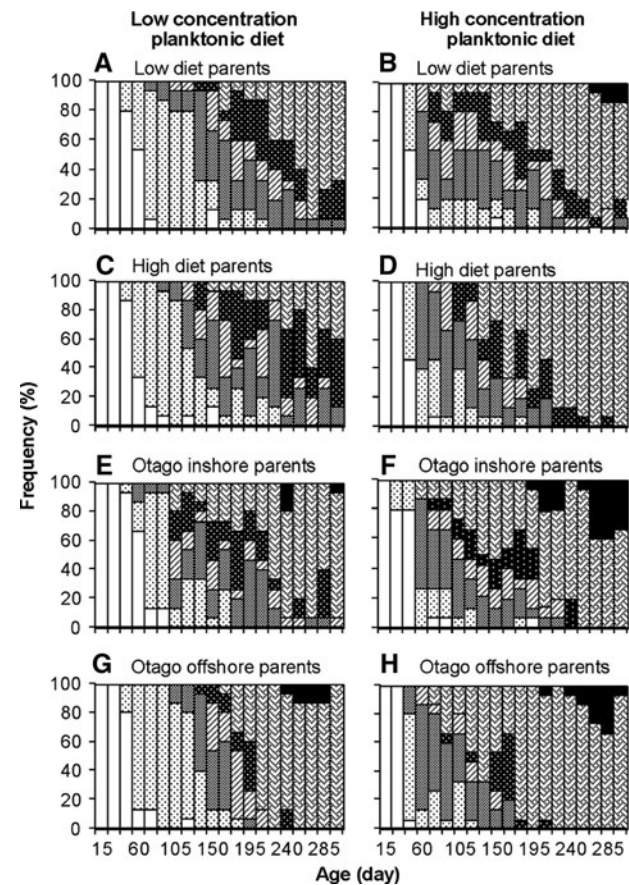
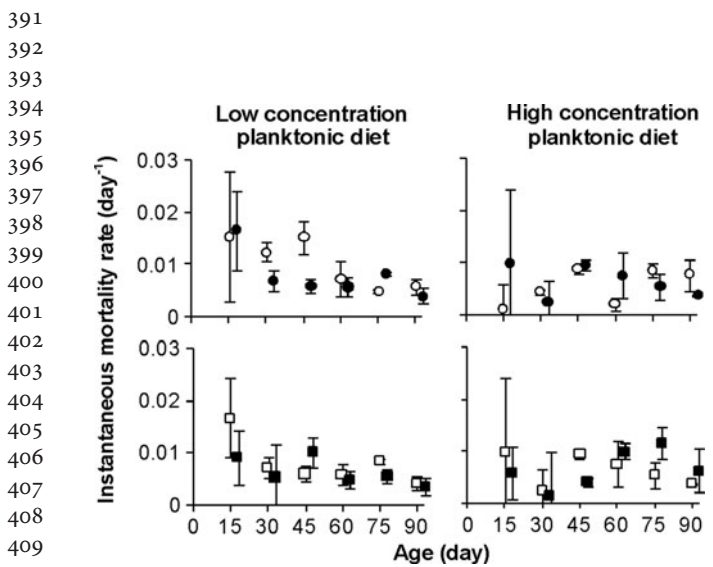


Fig. 5. Percentage of *Sclerasterias mollis* larvae in developmental stages from low and high diet laboratory parents and from Otago inshore and offshore field parents under a low or high concentration planktonic diet. Each bar is calculated from measurement of 15 larvae. The developmental stages are early-bipinnaria 1: □, early-bipinnaria 2: ▨, early-bipinnaria 3: ▩, early mid-bipinnaria: ▤, mid-bipinnaria: ▥, early-brachiolaria: ▦ and brachiolaria: ■.

379 **Table 4.** Three-way Wilcoxon test for the effects of parents, larval diet and
380 age on mortality rate of *Sclerasterias mollis* larvae. Significant *P* value is
381 shown in bold.

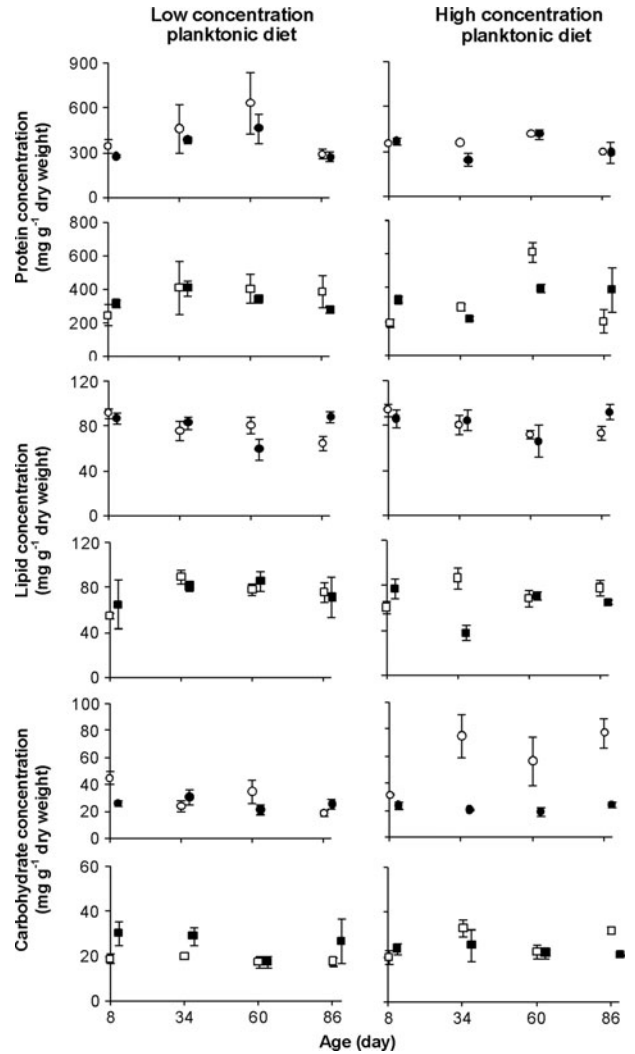
Source of variation	Q	P
Parents	0.634	0.910
Larval diet	2.267	0.150
Larval age	17.613	0.024
Parents × larval diet	2.179	0.600
Parents × larval age	13.627	0.820
Larval diet × larval age	6.544	0.360
Parents × larval diet × larval age	33.185	0.150



392 **Fig. 6.** Mean (\pm SE) instantaneous mortality rate of the larvae from low (\circ)
393 and high (\bullet) diet laboratory parents and from Otago inshore (\square) and
394 offshore (\diamond) parents under a low or high concentration planktonic diet.
395 Means are calculated from three measurements.

402 **Table 5.** Three-way Wilcoxon test for the effect of parents, larval diet and
403 age on protein, lipid and carbohydrate concentrations of *Sclerasterias*
404 *mollis* larvae. Significant *P* values are shown in bold.

Source of variation		Q	P
Protein	Parents	2.361	0.570
	Larval diet	1.760	0.210
	Larval age	21.590	0.005
	Parents × larval diet	0.998	0.830
	Parents × larval age	17.354	0.270
	Larval diet × larval age	5.589	0.230
	Parents × larval diet × larval age	30.060	0.570
Lipid	Parents	9.441	0.057
	Larval diet	0.777	0.390
	Larval age	1.792	0.660
	Parents × larval diet	4.076	0.320
	Parents × larval age	67.016	<0.001
	Larval diet × larval age	4.312	0.300
	Parents × larval diet × larval age	10.172	0.560
Carbohydrate	Parents	37.005	<0.001
	Larval diet	13.040	0.003
	Larval age	4.809	0.300
	Parents × larval diet	34.516	<0.001
	Parents × larval age	8.122	0.740
	Larval diet × larval age	28.809	0.002
	Parents × larval diet × larval age	45.558	0.006



405 **Fig. 7.** Mean (\pm SE) of biochemical composition of the larvae from low (\circ)
406 and high (\bullet) diet laboratory parents and from Otago inshore (\square) and
407 offshore (\diamond) parents under a low or high concentration planktonic diet.
408 Means are calculated from three measurements.

409 larval carbohydrate concentration (Table 5). Further Tukey
410 and *t*-tests comparing the various larval groups revealed that
411 larvae from low-diet, laboratory-held parents and fed a high
412 concentration planktonic diet had a greater mean carbo-
413 hydrate concentration than the larvae on a low planktonic
414 diet at day 86. Hence, concentration of planktonic diet had
415 a short-term significant effect on carbohydrate concentration
416 of larvae.

417 **DISCUSSION**

418 **Egg studies**

419 Parents with a lower nutritional status (i.e. low diet in the lab-
420 oratory or from Otago offshore) produced larger eggs than
421 those with a high nutritional status. Compared to laboratory-
422 held parents, the field starfish produced eggs with a higher
423 fertilization rate and a lower carbohydrate concentration.

424 Most life-history models have interpreted changes of the
425 egg size in relation to the egg number (Vance, 1973a, b;

442 McEdward, 1997). These models predict that in favourable
443 conditions females produce a large number of small eggs
444 but few large eggs in unfavourable conditions. However,
445 these models cannot explain the larger eggs from parents
446 with a lower nutritional status as observed in this study,
447 because egg number was not inversely related to the egg
448 size. Furthermore, it has frequently been demonstrated that
449 the relationship between the egg size and number deviates
450 from what models on reproductive strategy have predicted.
451 For example, George (1994) found that the starfish
452 *Leptasterias epichlora* from a site with more resources pro-
453 duced a higher number of larger eggs than those from a
454 resource-poor site. Additionally, in spite of many existing
455 studies, it is difficult to draw a general inference on the
456 topic. For instance, Thompson (1982), George *et al.* (1991)
457 and George (1999) found larger eggs in food-limiting echino-
458 derms, while George *et al.* (1990) and George (1990) found
459 larger eggs in populations that seemed to have better nutri-
460 tional status. Thus, changes of the egg size corresponding to
461 nutritional status may be a species-specific trait and further
462 studies are suggested.

463 Field and laboratory parents had different effects on the egg
464 diameter and carbohydrate concentration. Two potential
465 scenarios may explain this difference: firstly, when the mean
466 egg diameter of starfish fed a high diet was compared to
467 that of starfish fed a low diet, the latter tended to be larger,
468 albeit at non-significant levels. Meidel & Scheibling (1999)
469 have shown that the duration of feeding experiments influ-
470 ences oocyte size in *Strongylocentrotus droebachiensis*. They
471 found that females fed low and high diets had a similar
472 oocytes size at first reproduction but a significantly different
473 oocyte size at second reproduction. Hence, in the present
474 study, the food-dependent changes in *Sclerasterias mollis* egg
475 dimensions may take a longer time to develop and may
476 become significant in the following years. Secondly, the bio-
477 chemical egg composition is influenced by parental diet
478 (Thompson, 1982; Jong-Westman *et al.*, 1995). Therefore,
479 the higher egg carbohydrate concentration in laboratory
480 held parents may suggest that laboratory and field diets
481 were not similar in the current study.

482 483 484 **Larval growth, development and shape** 485

486 Larval nutrition had a significant influence on the larval devel-
487 opment rate, growth and shape. Larvae fed a high concen-
488 tration planktonic diet had larger body components, a faster
489 development rate and smaller body length relative to the
490 body width at some sampling times. The influence of plank-
491 tonic diet on growth and shape was however limited to a
492 few sampling times. These results were expected as
493 *Sclerasterias mollis* larvae have a planktotrophic development,
494 and an exogenous diet is their main energy source. These find-
495 ings are consistent with previous studies on other starfish
496 larvae such as *Luidia clathrata* (George *et al.*, 1991), *Pisaster*
497 *ochraceus* (George, 1999) and *Asterina miniata* (Basch,
498 1996). In this study, the limited effect of planktonic diet on
499 larval growth and development suggests that *Dunaliella pri-*
500 *molecta* was not the preferred diet for *S. mollis* larvae. This
501 suggestion is further supported by the fact that the larvae
502 had a slow development rate under both low and high concen-
503 tration planktonic diets, resulting in a long period to reach the
504 brachiolaria stage.

The larval growth, shape and development rate were differ-
ent between laboratory and field parents. Field parents but not
laboratory held parents had a significant influence on larval
growth and shape, with larvae from Otago offshore parents
having a stronger growth and a smaller body length relative
to the body width than larvae from other parents. In addition,
both larvae from low diet laboratory parents and from Otago
inshore parents (assumed to have higher nutritional status)
had a fast development rate. The inconsistencies in larval
growth, shape and development rates between laboratory
and field parents suggest that the difference in the diet of
the low and high fed laboratory parents was not severe
enough to affect larval characteristics. In addition, other par-
ameters (e.g. depth and temperature) which could not be
assessed with the present study design may have contributed
to these differences. However, most echinoderm studies on
this topic have investigated mainly the parental nutritional
status as a factor affecting larval features (George, 1990,
1994, 1996, 1999; Bertram & Strathmann, 1998). In this
respect, the importance of non-nutritional factors (e.g. depth
and temperature) has been widely neglected in echinoderm
research and needs to be addressed in future studies.

Parents and larval diet had significant effects on the larval
shape. Phenotypic plasticity in response to varying concen-
trations of planktonic diet has been reported previously
(George, 1999). Nonetheless, the present study is the first to
report a significant effect of parents on morphological pheno-
typic plasticity of starfish larvae.

Echinoderms with a better nutritional status may produce
larger eggs and/or eggs with a greater amount of biochemical
reserves (protein, lipid and carbohydrate) and energy, which
may subsequently result in stronger larval growth, develop-
ment, survival and metamorphosis rates (George, 1990;
George *et al.*, 1990, 1991; Jong-Westman *et al.*, 1995). In the
current study, Otago offshore parents produced the largest
eggs and their larvae had the best growth and greatest mor-
phological phenotypic plasticity. Thus, our results appear to
agree with previous studies suggesting that parents affect
larvae through egg features. However, it is unlikely that egg
size is the only factor affecting larval growth and phenotypic
plasticity because Otago inshore parents had smaller eggs
than Otago offshore parents but their larvae had a faster devel-
opment rate. Furthermore, the faster development rate of the
larvae from both low diet laboratory and Otago inshore
parents cannot be explained by egg reserves and energy,
because their egg sizes were similar. Therefore, effects of star-
fish parents on their larvae are not exerted solely through the
size and the biochemical content/energy of the eggs.

505 506 507 **Larval mortality and biochemical 508 composition**

Neither parents nor planktonic diet concentrations had a sig-
nificant effect on the larval mortality rate. The effect of parents
on larval mortality rates has been related to egg resources
(Jong-Westman *et al.*, 1995; George, 1999) and feeding effi-
ciency (George *et al.*, 1990) in echinoderms. In the present
study, the resources allocated to eggs (the biochemical and
energy content of the egg) were not influenced by the parental
nutritional status. However, there was a difference in pheno-
typic plasticity of the larvae from laboratory and field
parents (see above, section 'larval growth, development and

shape'), which might be related to shape changes that ultimately affect feeding efficiency (McEdward, 1986a, b; George, 1999). However, the similar mortality rates of the larvae from laboratory and field parents in this study are unlikely to be related to the feeding efficiency, because the concentration of planktonic diet had no influence on larval mortality.

It is believed that food availability is not a serious problem for the survival of marine invertebrate larvae (Manahan, 1989; Moran & Manahan, 2004), even though a low planktonic diet may prolong the larval period and thus increase the risk of mortality by non-nutritional factors (Morgan, 1995). The results of the present study are consistent with this view, given that 'low diet' larvae had a slower development rate and consequently a longer larval period than 'high diet' larvae. However, the planktonic diet concentration had no effect on the mortality rate of the larvae.

Parents and larval diet had a significant effect on the larval carbohydrate concentration. These results were expected, as low-diet, laboratory-held parents that produced eggs with the highest carbohydrate concentration also had larvae with the greatest carbohydrate content. This result is consistent with studies by George (1990, 1994) who assigned the biochemical composition of the larvae to the egg reserves, and with studies by Schiopu *et al.* (2006) and George *et al.* (2008) who demonstrated that larval diet influences larval composition.

In summary, this study indicates that: (i) parents have a significant effect on the characteristics of *Sclerasterias mollis* larvae; and (ii) parents can be more important than larval diet with respect to some larval features (e.g. growth, shape and biochemical composition). The influence of the parents on *S. mollis* larvae cannot be simply explained by egg characteristics such as size, organic content and energy. Indeed, biochemical egg components other than protein, lipid and carbohydrate can significantly affect the development rate of echinoderm larvae. For example, Saito *et al.* (1998) have observed that the levels of thyroid hormones in the egg influence the larval development rate in the sea urchin *Peronella japonica*. Further, egg carotenoids have also been associated with the larval development in the sea urchins *Heliocidaris tuberculata* and *H. erythrogramma* (Tsushima *et al.*, 1995). Therefore, our study is consistent with the presence of uncharacterized egg compounds that mediate the parental effects on the characteristics of *S. mollis* larvae, and suggests searching for these factors in future studies.

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- Correspondence should be addressed to:**
H. Poorbagher
Department of Fishery and Environmental Sciences
University College of Agriculture and Natural Resources
University of Tehran
Karaj
Iran
email: hpoorbagher@yahoo.com