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ABSTRACT

Nutritional provisioning that passes from a mother to her offspring can produce maternal carryover effects. Though the importance of maternal carryover effects on embryos and early juveniles is well established, it is less clear how long the effects persist and whether they can be detected in subsequent life stages of the offspring. Manipulating the amount of food available to a maternal organism is one way to manipulate maternal investment ability and thereby test maternal carryover. We collected adult brooding *Leptasterias aequalis* from three beaches with varying prey communities in the northern Puget Sound. When broods were released, we measured size and survival of the juveniles under starvation conditions. The maternal sea stars were then assigned to different feeding treatments and their diets were controlled for a full year until they spawned again. We measured size and growth of juveniles released from these second broods. Juvenile *L. aequalis* from the initial broods showed surprising resistance to starvation with 80% survival after 6 months and some juveniles living a full year with no food. Juvenile survival over time varied significantly among the mothers from the three study beaches, but we were unable to demonstrate a significant difference in the mean month of juvenile death. Juvenile size also varied significantly among mothers from the three study beaches, even when differences in female size by beach were accounted for. Adult female feeding treatments had no effect on the size of juveniles in the second broods. The patterns of juvenile performance mirrored those seen in the first year regardless of feeding treatment. The beach that a female came from seemed to have a stronger effect than a year of diet treatment. When looking at multiple generations of carryover in *L. aequalis*, it seems that genetic legacies, and possibly full female feeding history, have a greater effect on juvenile quality than a single year of maternal feeding.

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1. Introduction

The nutritional provisioning that passes from a mother to her offspring can produce maternal carryover effects (Podolsky and Moran, 2006). Such carryover effects occur in a variety of organisms including plants (Sakai and Harada, 2001), insects (Fox et al., 1997), fish (Kerrigan, 1997), reptiles (Abell, 1999), birds (Gil et al., 2004) and marine invertebrates (Marshall and Keough, 2008a). Increases in maternal nutrition from either natural variability or through experimental manipulation generally increase some measure of offspring fitness. Podolsky and Moran (2006) developed a model suggesting that carryover effects between life stages in invertebrates have three hypothetical long-term consequences: they disappear through compensation in the next life stage, simply persist or amplify over time.

If offspring are able to compensate for small initial size and poor nutrition carried over from their mothers, maternal carryover will disappear and have little net effect on the population as a whole. For example, larvae of the crab *Chasmagnathus granulata* that develop from smaller eggs follow a longer developmental pathway and actually

metamorphose into larger juveniles despite their initial disadvantage (Giminez et al., 2004).

Egg, larval size or quality differences can also simply persist after metamorphosis. A study of the intertidal snail *Nucella ostrina* showed that initial differences in offspring size following metamorphosis do not result in different post-metamorphic growth rates. Offspring from a single brood with differing initial sizes grow at similar rates, so differences in size simply persist in subsequent life stages (Moran, 1999; Podolsky and Moran, 2006).

The greatest impact of maternal carryover effect occurs when initial differences amplify over time. This can have profound effects on populations and communities. Such amplification was found in the bryozoan *Bugula neritina*, where larger larvae, after metamorphosis, grew faster and went on to produce larger colonies (Marshall and Keough, 2006). This carryover was further amplified in the next generation when the larger colonies subsequently produced larger larvae. Similar amplification has been seen in the colonial tunicate *Botrylloides violaceus* (Marshall et al., 2006).

To clearly demonstrate carryover effects, it is necessary to carefully control the investment potential of individual adults. Changing maternal size is one way to do this, but because maternal size affects reproductive output, size differences can confound effects of provisioning. Manipulating the amount of food available to the adult is an

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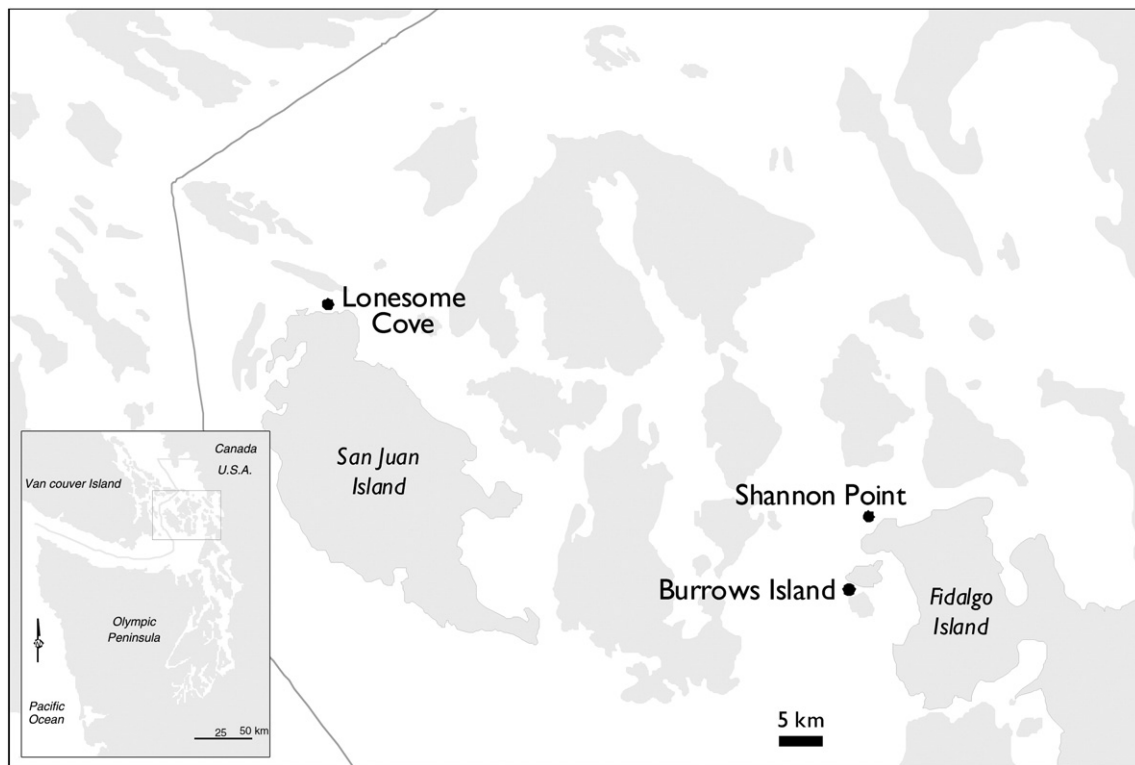


Fig. 1. Location of the study beaches in the San Juan Islands of Washington state.

alternative way to control maternal investment ability and experimentally examine carryover effects. Ideally, such experiments would follow single mothers over more than one year of reproductive output to permit comparison of reproductive output under controlled feeding conditions. To do this, it is necessary to have an organism that can survive and reproduce in laboratory conditions. It is also important that the offspring are easy to track and study (i.e., without the planktonic larval stage typical of many marine species). The six-rayed sea star *Leptasterias aequalis* fits these requirements.

L. aequalis is a brooding sea star that is abundant in the northeastern Pacific. Previous research showed that larger *Leptasterias*¹ tend to produce larger eggs (Menge, 1974; George, 1994; Bingham et al., 2004). In addition, George (1994) compared *L. epichlora* populations from exposed and sheltered coastal beaches and found that larger individuals in exposed sites produced larger eggs. The juveniles that developed from larger eggs were, in turn, larger and had a higher protein content. Thus, it is already known that there are initial size-related maternal carryover effects between *Leptasterias* mothers and their juveniles. It is not known, however, what causes these differences, whether the differences persist as the juveniles grow, or how those differences affect juvenile performance. By looking at the relationships between maternal *L. aequalis* resource availability and offspring quality in several field populations, we tested observationally whether mothers with better foraging opportunities produce juveniles that survive better.

By subsequently manipulating the diets of the same maternal *L. aequalis* in the laboratory and looking at juvenile size and growth in a second reproductive period, we tested whether the relationship

between adult provisioning and juvenile size was adaptive (genetically determined) or plastic (responsive to an environmental change). We also tested the possibility that a change in maternal diet could change existing relationships between adult quality and juvenile size.

2. Methods

2.1. Prey communities

To determine the relationship between maternal *L. aequalis* prey availability and quality of their offspring, we collected individuals from three beaches in the northern Puget Sound (Lonesome Cove, San Juan Island; Shannon Point, Fidalgo Island; Burrows Island; Fig. 1). The beaches, which all sustain populations of *L. aequalis*, were selected specifically to represent different levels of *L. aequalis* prey availability. We predicted that adults from beaches with more prey would have more energy to invest in reproduction and would, therefore, produce larger juveniles with higher survival and faster growth rates.

Representative prey communities on each beach were assessed during one tide series between July 29th and July 31st, 2007. We made measurements during the summer because *L. aequalis* feeding during this period is primarily allocated to reproduction (Chia, 1968). Thus, the number and quality of prey available to *L. aequalis* during the summer should have the greatest potential effect on their egg provisioning.

To estimate prey communities on the beaches, we followed the technique of Menge (1970). Fifty-meter line transects were placed parallel to the water at three tide levels (−0.6 m, −0.3 m and 0.0 m). Five locations were randomly selected along each transect and all *L. aequalis* prey items within a 0.25 m² quadrat were counted. Prey items were tabulated using the list of *L. aequalis* prey provided by Menge (1970). All prey directly on the surface within the quadrat were counted. Subsequently, a single layer of cobble was removed and prey on and under the rocks were quantified. In some quadrats, small barnacles and spirorbid polychaetes were too small and numerous to count, so we estimated percent cover in these cases.

¹ The taxonomy of *Leptasterias* populations of the northeastern Pacific is currently problematic. Historically, small six-rayed *Leptasterias* seastars in the Puget Sound region have been called *L. hexactis* (Menge, 1971; Kozloff and Price, 1996). More recently the group has been subdivided and includes *L. epichlora* (George, 1994), and *L. aequalis* (Bingham et al., 2004). Genetic research suggests that it might be best to consider *Leptasterias* in the Puget Sound region as a species complex (Flowers and Foltz, 2001). For this study, we consider *L. aequalis* to represent a species complex that includes *L. hexactis*, *L. epichlora* and *L. aequalis*.

To compare prey communities among the beaches, data for all quadrats were plotted with Multidimensional Scaling (MDS) with Bray-Curtis similarity as the distance metric. We then used ANOSIM (PRIMER software package) to test for significant differences in prey communities among the beaches. To lower the impact of highly abundant species on the analyses, data were square root transformed prior to analysis. SIMPER analysis was used to determine which prey contributed most to differences between beaches.

2.2. Collection of brooding females

To measure the relationship between maternal size, source beach and the size and survival of offspring, we surveyed the intertidal area of each beach at low tide, collecting female *L. aequalis* with broods. To minimize lab artifacts, we collected the brooding females as late in the brooding cycle as possible. Beginning in January (2007), we monitored adults at Shannon Point and Burrows Island approximately once a month to document when adults spawned and began brooding. Adults at these sites began brooding at the same time (late January), so Shannon Point females (which were readily accessible) were examined weekly and were used as an indicator for both Shannon Point and Burrows Island *L. aequalis* populations. To monitor individuals from Lonesome Cove (a more remote site), a few brooding *L. aequalis* were collected from that site in early January and the developing embryos were monitored daily in the lab.

When we saw evidence of metamorphosis in the broods, we collected fresh brooding individuals from each of the beaches. Lonesome Cove *L. aequalis* ($n=10$) were collected on March 10th, 2007, Shannon Point and Burrows Island *L. aequalis* ($n=11$) were collected on March 23rd, 2007. The difference in collection dates was due to the differences in the timing of brooding at the sites (Lonesome Cove females began brooding approximately two weeks earlier than Shannon Point and Burrows Island females).

Brooding females from Lonesome Cove were held in individual glass containers in a 12:12 hour light/dark incubator for 13 days until the Shannon Point and Burrows Island sea stars were collected. While in the incubator, all the Lonesome Cove *L. aequalis* dropped their broods. Though previous research has suggested that removing the brood from a female would prevent the juveniles from undergoing metamorphosis (Chia, 1966), the Lonesome Cove juveniles continued to develop normally and metamorphosed at the expected time, presumably because they were so far developed at collection. When we had obtained brooding females from all three beaches, they were placed individually in 500- μ m, mesh-lined boxes (12.5 cm \times 7.5 cm \times 9.5 cm) equipped with a single piece of brick and partially submerged in a single flow-through seawater tank.

2.3. Adult and juvenile size measurements

Female size is related to brood size in *L. aequalis*, so we wet-weighed each female immediately after her brood had been released. We then tested for differences in female size among the beaches with a one-way ANOVA. Prior to the analysis, we tested the homogenous variance assumption with a Levene's test.

To test for differences in initial juvenile size and in subsequent growth, we collected twelve juveniles from the broods of each of the maternal seastars. The juveniles were placed individually in 6 cell-well trays and kept at 9 °C in a 12:12 h light/dark incubator. The juveniles were supplied with 5- μ m filtered seawater that was changed once a week. They were not fed. To monitor their growth over time, each was photographed immediately after release, then once a month for 6 months and every other month up to a year (a total of ten measurements).

We later used the photographs and Image-Pro Plus software to determine the size of each juvenile, measured as the distance from the center of the central disk to the tip of the longest arm. We tested for differences in juvenile size at the three beaches immediately upon

release and 6 months later using ANCOVA analyses with adult size as the covariate. Juvenile mortality after 6 months was sufficiently high that it was impractical to statistically compare treatments in subsequent months. To permit use of adult size as a covariate, we had to average sizes of all individuals within a brood to produce one juvenile size per adult. We then compared treatments with beach as the independent variable. This analysis allowed us to test for differences in juvenile size at the beaches, while accounting for differences in maternal size. The analysis for newly released juveniles tested the initial carryover effect (i.e., do females from a beach with more food produce larger offspring), while the six-month measurement showed whether any carryover effect persisted under starvation conditions. The assumptions of equal variance and homogeneity of covariate slopes were tested and met.

2.4. Juvenile survival

Upon release from the brood, *Leptasterias aequalis* juveniles carry yolk stores provided by their mothers. If females from prey-rich beaches better provision their juveniles, those juveniles should survive longer under starvation conditions. To compare survival among starved juveniles from the three beaches, we documented the condition of the juveniles held for size measurements once a month for 6 months, then once a week until all of the juveniles were dead. A juvenile was considered dead if it was not attached to a surface and its unattached tube feet were not moving. We compared survival of juveniles from the three beaches by calculating the percent survival of juveniles from each mother on each date. The data were square root transformed to homogenize variance and analyzed by ANOVA with month as the within factor (fixed) and beach (fixed) and female (random and nested in beach) as the between factors. Mauchley's *W* was used to evaluate the ANOVA circularity assumption.

2.5. Female nutrition manipulation

To determine experimentally whether nutrition of the maternal sea star affected juvenile size and subsequent juvenile performance, or whether those features were simply a consequence of maternal genetics, we maintained in the laboratory the females collected for the 2007 sampling described above. For the following year, we manipulated their food availability and examined their subsequent broods (in 2008). We predicted that females that experienced lower food availability would produce poorly provisioned juveniles that would be smaller regardless of the beach they came from.

To successfully induce reproduction in the laboratory, it was necessary to provide males for fertilization. Brooding season is the only time of year that it is possible to determine the sex of *L. aequalis* without dissection. However, even during brooding season, it is only possible to positively identify females. Non-brooding starfish could either be males or females that are simply not brooding. To collect at least a few males from each beach, 10 non-brooding *L. aequalis* were collected at the same time and location as the brooding females in the first year. These sea stars were held in a flow-through seawater tank and fed *ad libitum* until they were used 10 months later to fertilize the females.

To test whether the relationship between adult condition and juvenile size is a plastic trait that can change with food availability or is a genetically determined trait that persists regardless of food resources, we established two adult feeding treatments: a high-food group that was fed twice a week and a low-food group that was fed twice a month. At each feeding, all test starfish were fed one snail (*Littorina sitkana*) collected from the Shannon Point beach. Females were placed on their feeding schedule immediately following the laboratory release of their broods in May and June of 2007. Females from the study beaches were divided among the feeding treatments

so there was even representation from each of the beaches in both of the feeding treatments.

Feeding treatments were applied for 9–10 months until females put on their broods in February and March 2008. During the entire feeding experiment, maternal adults were kept separated in individual boxes (12.5 mm × 7.5 mm × 9.5 mm) lined with 1-mm mesh. In January, during the spawning period, a single presumptive male was added to each box. To increase the chance of brood fertilization, all remaining sea stars (thought to be males) were placed outside the boxes in the same flow through tank. The *L. aequalis* were left to spawn naturally and monitored 3 times a week to detect the first signs of brooding. As soon as the maternal stars started brooding, they were moved from the 1-mm mesh boxes to 500- μ m mesh boxes to prevent juvenile escape and the males were placed in the surrounding tank. The females were monitored for the following weeks to detect metamorphosis of the juveniles.

Immediately upon metamorphosis and release, the juveniles were collected for size analysis. These measurements were made as described for the first year, except that the juveniles were fed to determine whether fed, and therefore growing, juveniles would show persistence, compensation or amplification of maternal carryover effects. All *L. aequalis* juveniles were fed two juvenile *L. sitkana* per week (collected from recently hatched egg masses).

Due to adult mortality over the year of the feeding treatments, there was insufficient replication to run a single ANOVA with both feeding treatment and beach as between subject factors. Instead, we ran separate ANOVAs with the same data set, first testing the effect of feeding treatment then separately testing the effect of maternal beach of origin on juvenile size.

3. Results

3.1. Prey availability

There were distinct differences in the variety of *L. aequalis* prey on the three study beaches. An MDS analysis showed that, while there was much within-site variability in prey communities (related to tidal height and microhabitat), the sites separated from one another (Fig. 2). ANOSIM verified the separation of the study beaches (Global $R = 0.47$, $p = 0.001$).

SIMPER analysis indicated that separation of the sites resulted largely from differences in the abundance of limpets, barnacles, spirorbid polychaetes, and small snails (Fig. 3). We separated these prey organisms into prey quality categories depending on their caloric yield, ease of capture and consumption and the selectivity of *L. aequalis* for that prey (Menge, 1971). Based on these criteria, we considered

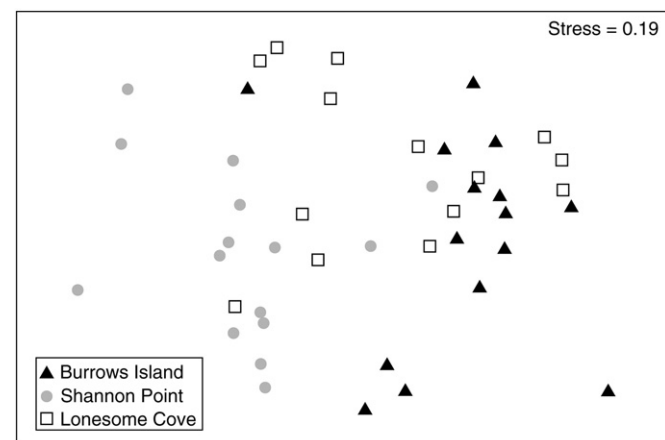


Fig. 2. Multi-dimensional scaling analysis of prey availability at Burrows Island, Shannon Point beach and Lonesome Cove.

limpets (*Lottia* spp.) high quality prey, snails (*Littorina scutulata*, *Lacuna vincta*) medium quality prey and barnacles (*Balanus* spp.) and spirorbid polychaetes low quality prey.

Lonesome Cove had low abundances of all potential prey except spirorbid polychaetes (a relatively low quality prey). Shannon Point had high numbers of barnacles (*Balanus* spp.) and the snail *L. vincta*. Burrows Island had high abundances of the snail *L. scutulata*, limpets in the genus *Lottia* and spirorbid polychaetes. Based on these results, we believe that Burrows Island has the richest prey field for *L. aequalis*, Shannon Point is an intermediate site and Lonesome Cove is a relatively food-poor environment.

3.2. *L. aequalis* female and juvenile size

Female *L. aequalis* size varied significantly among the study beaches ($F_{2,31} = 5.63$, $p = 0.009$). Burrows Island had the largest females, followed by Shannon Point beach and Lonesome Cove (Fig. 4). Tukey's HSD pairwise comparisons showed that females on Burrows Island were significantly larger than those at Lonesome Cove (Fig. 4).

Source beach of the maternal sea star also had a significant effect on the size of juveniles immediately after they were released from the brood, even when female size was removed as a covariate (Fig. 5, Table 1). A Bonferonni pairwise comparison of the covariate-adjusted means showed that Lonesome Cove juveniles were significantly smaller than those from Burrows Island. After six months of starvation, juveniles still differed in size. Lonesome Cove juveniles were significantly smaller than those from both Shannon Point and Burrows Island females.

3.3. Juvenile mortality

Juveniles under starvation conditions lasted much longer than we had expected; over 80% survived with no food for a full six months. The longest lasting individuals survived for 13 months. ANOVA showed a significant interaction between month and beach, reflecting differences in the mortality pattern of juveniles from different beaches (Table 2). In general, juveniles from Shannon Point had the highest survival for the first 8 months. After 8 months, there was a large drop in survival of the remaining juveniles from this site (Fig. 6). Lonesome Cove juveniles showed a similar pattern, but all individuals had died at least a month before those from the other 2 sites. Burrows Island had the lowest survival for the first 7 months, but juveniles remaining after that time survived longer than those from either Shannon Point or Lonesome Cove.

The relationship between juvenile size and month of death was also analyzed to test the hypothesis that larger juveniles, in general, are better provisioned and survive longer under starvation conditions. We found no clear relationship between juvenile size and survival ($r^2 = 0.012$, $p = 0.82$, Fig. 7).

3.4. Female nutrition manipulation

There was high female mortality during the one-year nutrition manipulation experiments. Of a total of 10 sea stars, only one Burrows Island female and two Shannon Point beach females survived in the low food treatment. One Burrows Island female, three Shannon Point females and two Lonesome Cove females survived in the high food treatment.

ANOVA testing for effects of feeding showed that only month had a significant effect on juvenile size; juveniles (which were being fed) grew significantly in the 5 weeks after metamorphosis (Table 3, Fig. 8). Food treatment had no statistically significant effect on juvenile size. Due to female mortality, replication was low, data were unbalanced and statistical power was low. However, any effects that may have been present were subtle; the average size of juveniles newly released from well-fed mothers was only 5% higher than that of juveniles from mothers in the low-food treatment.

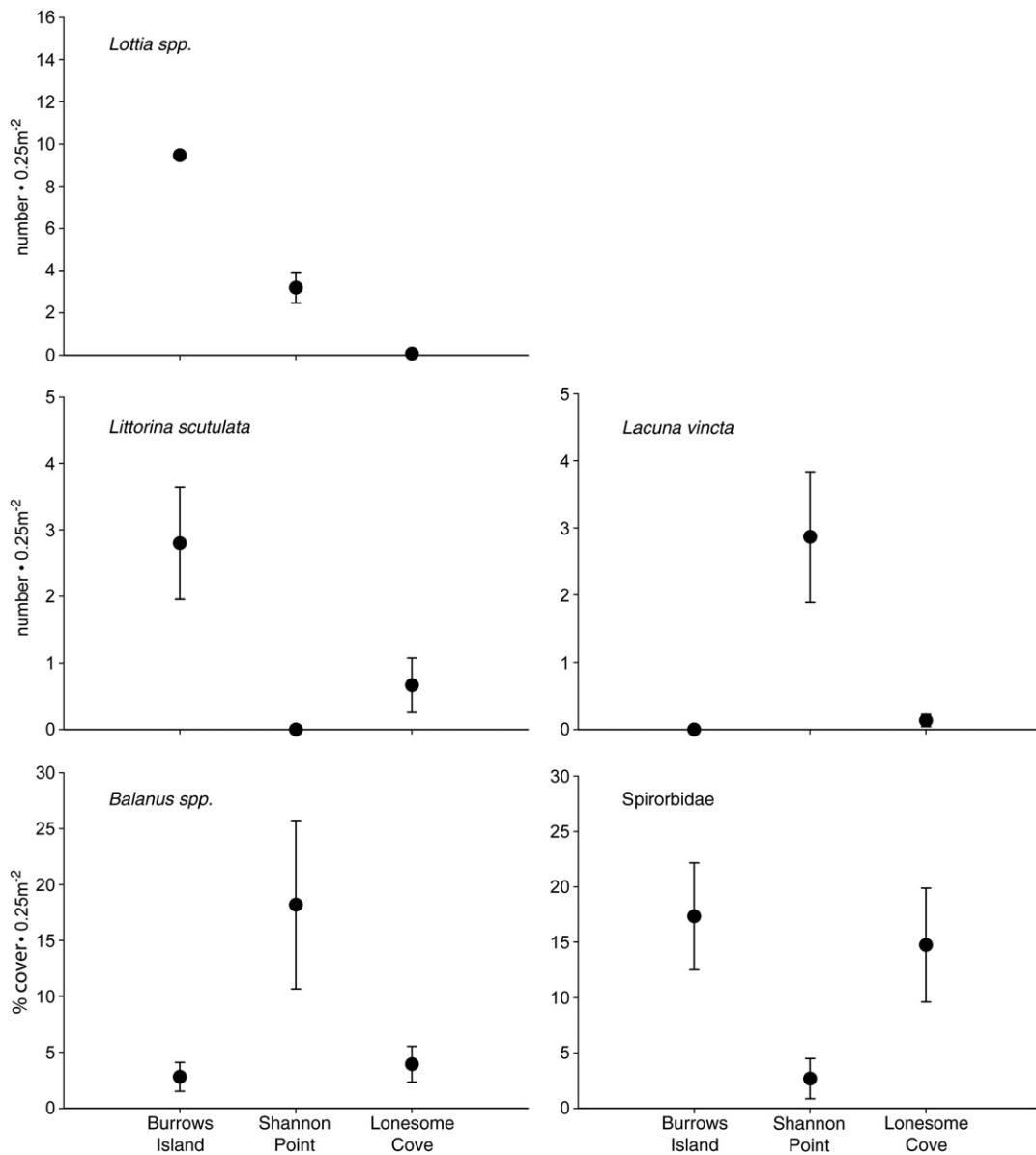


Fig. 3. Prey groups identified by SIMPER as contributing most to differences among study beaches. Standard errors are shown.

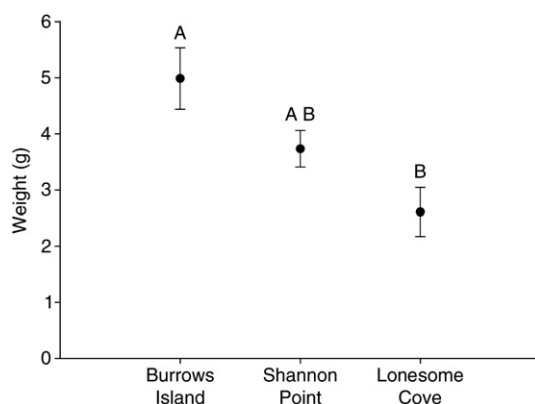


Fig. 4. Wet weight of female *L. aequalis* from the collection beach ($n = 10$ to 11 for each site). Wet weights were taken after all juveniles had been released from their brooding mothers. Error bars represent standard errors. Letters show results of Tukey's HSD pairwise comparisons.

To determine whether original habitat had a persistent effect on juvenile size despite a year on a feeding treatment, we re-analyzed these same data but with beach replacing feeding treatment as the main effect. Beach was not statistically significant (Table 4), though power was again low. Despite the lack of a significant effect, the trend in the data mimicked the previous year; Burrows Island had the largest juveniles, followed by Shannon Point and Lonesome Cove (Fig. 8). Newly released juveniles from Burrows Island females were, on average, 14% larger than those from Lonesome Cove (Fig. 9).

4. Discussion

The original intent of this work was to determine whether maternal nutrition affects juvenile quality in an abundant intertidal sea star. In the course of the study, we discovered that juvenile *L. aequalis*, in general, are surprisingly resilient. We initially expected starved juveniles to last one or two months at most instead, most survived at least six full months with no food.

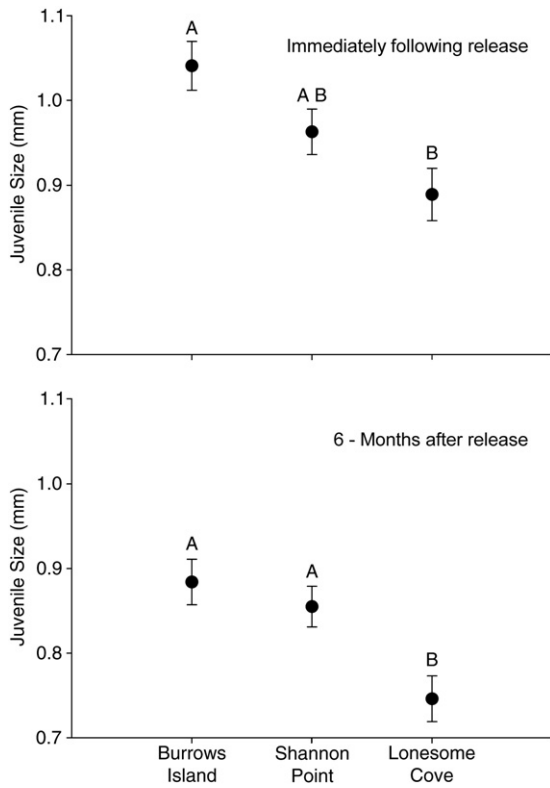


Fig. 5. Covariate-adjusted mean size of juveniles from each of the beaches with female size as a covariate. Standard errors are shown. Letters show results of Bonferroni pairwise comparisons.

It has generally been presumed that, among marine invertebrate species, larger offspring are more resistant to starvation and will survive better under adverse conditions (Marshall and Keough, 2008a). We found no such relationship between *L. aequalis* juvenile size and starvation resistance. Instead we found that juveniles from the beach we considered to have the best prey resource survived longer than juveniles from the other beaches independent of juvenile size. Juveniles from Lonesome Cove, which had the smallest prey field, were all dead a full two months before the juveniles from the other beaches.

Because juveniles survived so long, we have to consider the possibility that they were receiving some form of nourishment during the starvation experiment. The juveniles were held in 5- μ L filtered seawater in an incubator that received 12 h of light each day. There may have been some growth of a bacterial/algal microfilm in their containers. A preliminary feeding experiment showed that juvenile *L. aequalis* are, in fact, capable of feeding on biofilm. Juveniles fed natural biofilm scraped from the underside of intertidal rocks showed growth

Table 1 ANCOVA testing for differences in juvenile size among the beaches with adult size removed as a covariate.

	SS	df	MS	F	P
Immediately following release					
Covariate (female weight)	0.04	1	0.043	5.3	0.03
Beach	0.08	2	0.044	5.4	0.01
Error	0.23	28	0.008		
Total	0.35	31			
Six months after release					
Covariate (female weight)	0.008	1	0.008	1.23	0.278
Beach	0.085	2	0.042	6.67	0.004
Error	0.172	27	0.006		
Total	0.265	30			

Table 2 Survival of starved juvenile *L. aequalis*, analyzed by ANOVA. Because data did not meet the sphericity assumption, results are reported with the Huynh-Feldt adjusted degrees of freedom.

	SS	df	MS	F	P
Between					
Beach	0.46	2	0.23	1.79	0.185
Female	3.69	29	0.13		
Within					
Month	89.47	4.3	20.52	405.97	>0.001
Month \cdot Beach	1.69	8.7	0.24	3.85	>0.001
Female \cdot Month	6.39	126.4	0.05		
Total	101.7	170			

rates similar to those of juveniles fed *Littorina sitkana* (C. Altaras, pers. com.). As dishes in our experiment were cleaned only once a week, there was some opportunity for biofilm to develop. We cannot reject this possibility. However, if laboratory biofilm was present, it should have been consistent among treatments and produced no treatment bias.

It is also possible that juveniles absorbed dissolved organic matter (DOM) through their epithelium or tube feet. Such transfer of nutrients has been reported in other invertebrates (Fontaine and Chia, 1968; Jaekle and Manahan, 1989). To assess DOM absorption as a possible source of nutrition, we placed juvenile *L. aequalis* in nutrient-free artificial seawater and monitored their survival over three months. Juveniles in this treatment had mortality rates similar to those seen in our 5- μ L filtered seawater treatments. It seems likely, therefore, that juveniles were surviving solely on yolk stores provided by their mothers.

Menge (1970, 1975) studied survival of *L. hexactis* from metamorphosis to reproduction and predicted Type III survival; high mortality prior to reproductive maturity and low mortality after. Hypothesized causes of early mortality included predation, desiccation and starvation. In light of the high survival of starved juveniles in our study, and the observation that juveniles can survive and grow while feeding only on natural intertidal biofilms, starvation may be less important than previously assumed. It should be noted, however, that our survival experiments were run in a laboratory; low food availability could contribute to mortality from other sources (e.g., predation, desiccation, washing away). The absolute importance of juvenile starvation probably varies in space and time. Creative field studies will be necessary to demonstrate its importance.

It is well established that embryo and juvenile size in *L. aequalis* correlate with maternal size (Menge, 1974; George, 1994; Bingham

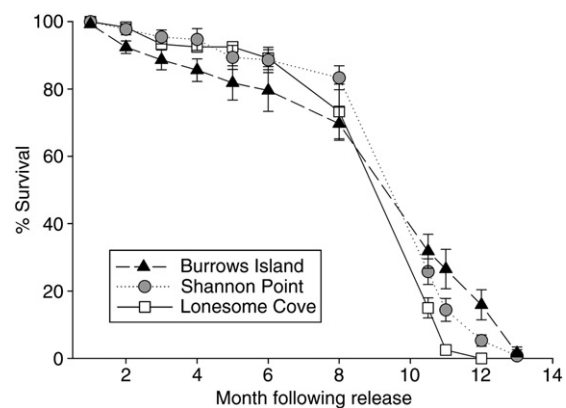


Fig. 6. Survival of starved juvenile *L. aequalis*. Each data point represents the average % survival of the offspring from 10–11 females (with 12 juveniles from each female). Standard errors are shown.

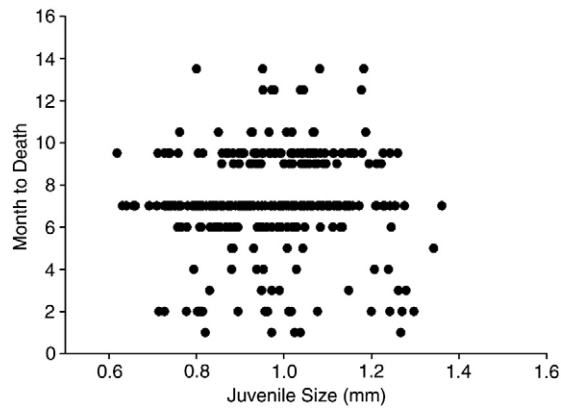


Fig. 7. Mean month to death of starved juvenile *L. aequalis* as a function of juvenile size.

et al., 2004). This was also clear in our study; the largest females (from Burrows Island) produced the largest offspring (followed, as predicted, by Shannon Point and Lonesome Cove). However, our data suggest that something about the beach that the females came from has an even greater effect on juvenile size than maternal size alone can account for. Juvenile size differences were significant even when female size was removed as a covariate. Similar variation in offspring size by region was found in the bryozoan *Watersipora subtorquata* (Marshall and Keough, 2008b). It remains unclear, however, whether these differences were genetically derived or a result of phenotypic plasticity.

The diet of maternal *Arbacia lixula* (Echinoidea, Echinoidea) affects the nutritional composition of their eggs (George, 1990). Larvae from better provisioned eggs, in turn, grow faster and have greater survival. The different prey communities on the beaches we studied may produce similar effects with mothers from beaches with high quality prey producing juveniles with, for example, greater lipid stores. Burrows Island had high numbers of high-calorie, high-preference prey. In contrast, the only abundant prey at Lonesome Cove were small spiroboid polychaetes. While the relative caloric value of these worms has not been measured, their small, tightly coiled calcareous tubes should make them particularly difficult to consume, decreasing their value as a prey item. Differences in caloric yields of prey may have contributed to differences in maternal provisioning and to the increased survival we found in juveniles from Burrows Island beach females.

We predicted that the differences in offspring quality found among the study beaches could be removed by a year of maternal nutritional manipulation. Instead, we found that the beach that the mother came from (its original habitat) had a greater effect on juvenile size than a year of controlled feeding.

Maintenance of large juvenile size may have come at the expense of the number of juveniles produced. There could have been a tradeoff

Table 3

ANOVAR analysis testing the effect of food treatment on juvenile size immediately following release and after 5 weeks of growth. Juvenile size was averaged from twelve juveniles for each female.

	SS	df	MS	F	P
Between					
Treatment	0.0020	1	0.0020	0.27	0.622
Individual	0.0500	7	0.0070		
Within					
Month	0.0660	1	0.0660	35.19	0.001
Month • Treatment	0.0004	1	0.0004	0.19	0.678
Month • Individual	0.0130	7	0.0020		
Total	0.1314	17			

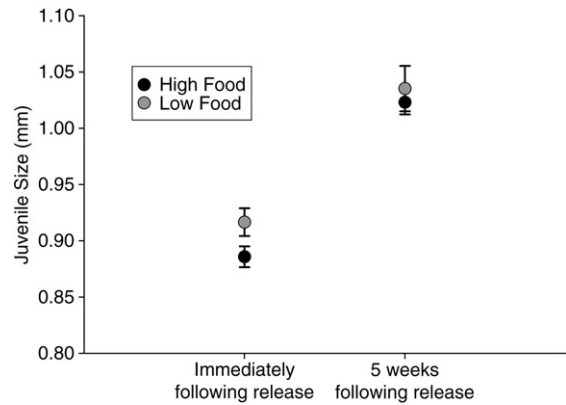


Fig. 8. Juvenile *L. aequalis* size immediately after release and after 5 weeks of growth (juveniles were fed two *Littorina sitkana* once a week). Juveniles are the offspring from the high and low-fed female treatments. Error bars represent standard errors.

between the size of the embryos and the size of the broods. This hypothesis remains to be tested as we did not count embryos in the second year female broods. Alternatively, the production of large juveniles in the second year may have resulted from year-to-year maternal nutritional carryover. The *L. aequalis* maternal sea stars may have been able to store enough nutritional provisions to reproduce normally and maintain juvenile size despite a year of low food. Finally, there could simply have been a genetic predisposition for Burrows Island females to produce larger eggs.

Padilla and Miner (2004) introduced the idea of a genetic legacy, a type of carryover effect passed on genetically in invertebrate species. Under this scenario, size of juveniles is controlled genetically and not by environmental condition (e.g., food). Our results suggest that, in *L. aequalis*, juvenile size is at least partly a genetic legacy, resulting in different size juveniles at different sites. Because there is no planktonic larval stage in *L. aequalis*, dispersal is probably quite limited; beaches separated by relatively short distances could, therefore, be reproductively isolated, leading to population-level size differences. Burrows Island females produce larger offspring with long starvation resistance; those offspring may grow into larger adults. Conversely the Lonesome Cove females are producing smaller offspring with shorter survival. The Lonesome Cove offspring are likely to continue to demonstrate the costs of their small juvenile size throughout their life cycle. Although persistence can't be ruled out, it seems likely that this pattern represents an amplification of maternal carryover effects in these organisms.

Our study showed that a year of maternal feeding did not change site-based differences in juvenile size. This does not eliminate the possibility that several years of maternal feeding would have carryover effects. Female feeding history and genetic legacies are likely linked in a way that makes it impossible to separate out the

Table 4

ANOVAR testing of the size of juveniles by beach immediately following release and after five weeks of growth. Juvenile size was averaged from twelve juveniles for each female.

	SS	df	MS	F	P
Between					
Beach	0.02	2	0.011	2.38	0.174
Individual	0.03	6	0.005		
Within					
Month	0.08	1	0.079	53.09	>0.001
Month • Beach	0.01	2	0.002	1.53	0.290
Month • Individual	0.01	6	0.001		
Total	0.15	17			

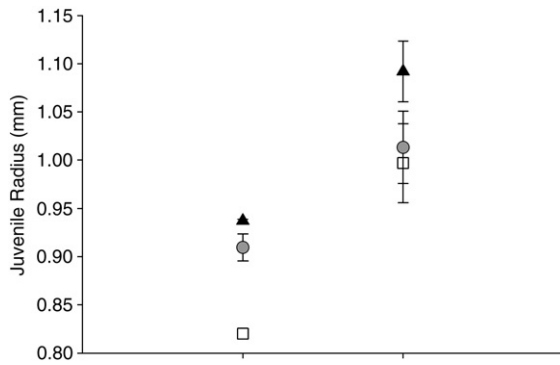


Fig. 9. Juvenile *L. aequalis* size by beach immediately following release from the brood and after five weeks of growth (juveniles were fed two *Littorina sitkana* once a week). Juveniles are the offspring from the high and low-fed female treatments. Error bars represent standard errors.

effects of each. Furthermore, attempting to separate the two effects may provide little useful insight into the natural history of an organism. It may be more productive to focus on the specific outcomes of maternal carryover with an eye to the geographic and environmental conditions of the maternal animals.

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