# Fast versus Slow Larval Growth in an Invasive Marine Mollusc: Does Paternity Matter?

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## Abstract

Reproductive strategies and parental effects play a major role in shaping early life-history traits. Although polyandry is a common reproductive strategy, its role is still poorly documented in relation to paternal effects. Here, we used as a case study the invasive sessile marine gastropod *Crepidula fornicata*, a mollusc with polyandry and extreme larval growth variation among sibling larvae. Based on paternity analyses, the relationships between paternal identity and the variations in a major early life-history trait in marine organisms, that is, larval growth, were investigated. Using microsatellite markers, paternities of 437 fast- and slow-growing larvae from 6 broods were reliably assigned to a set of 20 fathers. No particular fathers were found responsible for the specific growth performances of their offspring. However, the range of larval growth rates within a brood was significantly correlated to 1) an index of sire diversity and 2) the degree of larvae relatedness within broods. Multiple paternity could thus play an important role in determining the extent of pelagic larval duration and consequently the range of dispersal distances achieved during larval life. This study also highlighted the usefulness of using indices based on fathers' relative contribution to the progeny in paternity studies.

**Key words:** larval dispersal, life-history traits, marine invader, paternity analysis, protandry

Early life-history traits, including those determined by conditions experienced early in development, can profoundly influence recruitment success and overall population dynamics (Benton et al. 2006). This has been notably demonstrated in marine species (Roughgarden et al. 1988; Pechenik 2006) for which, in addition, parental effects on early life-history traits have been documented with a special emphasis on maternal effects (i.e., nongenetic effects of the maternal environment and phenotype; reviewed in Marshall et al. 2008). Paternal genetic effects (additive or nonadditive) or paternal environmental effects (Yasui 1998; Jennions and Petrie 2000; Garcia-Gonzalez and Simmons 2007) have been much less studied because they were first believed to have less influence than maternal contributions (e.g., through egg provisioning). Moreover, experimental crosses are difficult to carry out in most marine species, particularly for those with internal fertilization. Despite such theoretical expectations and practical limitations, the paternal influence on early life-history traits has been documented for some taxa, mainly fishes (e.g., Bang et al. 2006; see also Trippel et al. 2005 and references therein) but also in some invertebrates, for example, in tunicates (e.g., Marshall and Evans 2007).

Considering early life-history traits, polyandry (i.e., females mate with more than 1 male) could play a significant role in conferring fitness benefits due to the intrinsic genetic quality of males, beneficial interactions between maternal and paternal genotypes, or through paternal environmental effects (Yasui 1998; Simmons 2005). For instance, polyandry was shown to contribute to an increase of hatchling size (e.g., Sprenger, Faber, et al. 2008), embryonic viability (e.g., Garcia-Gonzalez and Simmons 2005), and offspring size (e.g., Sprenger, Anthes, and Michiels 2008; Sprenger, Faber, et al. 2008). These effects all conferred a potential increase of female mean fitness through a greater average value for the trait under consideration within a set of siblings. Yet, for some characteristics, the range of variation might be more important than the average value: considering larval phase in benthopelagic life cycles, which characterizes 55–85% of marine invertebrates (Thorson 1950), larvae that spend more time in the plankton may be particularly good dispersers as compared with larvae that are able to

metamorphose sooner and recruit in the vicinity of the parental populations (Marshall and Keough 2003). In unpredictable environments like the seawater column, multiple mating can provide genetic benefits to females as a more genetically diverse clutch decreases both the risk of reproductive failure and the sibling competition (Yasui 1998; Zeh AJ and Zeh DW 2003; Neff and Pitcher 2005; Simmons 2005). Although being an important expected outcome of polyandry, increased trait variability within brood due to multiple paternity is still poorly documented. Without using quantitative genetic methods, paternal effects can still be examined based on correlation analyses between the sire identity and/or diversity and larval phenotypes. Using this approach, we investigated the extent to which polyandry could influence variation in larval growth rate of sibling larvae, one early life-history trait that has received considerable attention as it determines the duration of the larval dispersal period (Pechenik 1999).

We used the sessile marine gastropod *Crepidula fornicata* as a case study. This mollusc is native to the east coast of North America but has successfully invaded many bays and estuaries along the western and northern coasts of Europe. Dispersal ability is a critical aspect of marine biological invasions, and studying the factors that may influence larval development and recruitment is of central importance in understanding sessile marine invaders (Dunstan and Bax 2007; Dupont, Ellien, and Viard 2007). This marine gastropod has a number of traits that make it well suited to study the role of paternity in larval characteristics. An inverse relationship between larval growth rate and duration of larval life has been previously demonstrated for this species as individual growth rates are linear until competence to metamorphose, which is occurring at sizes between 900 and 1100 µm (Pechenik 1984). Also, the larvae of *C. fornicata* can be reared in the laboratory with negligible mortality (Pechenik and Lima 1984) so that loss in genetic diversity due to genetic drift effect is extremely limited. They grow substantially during development, and larval growth rates show remarkably wide variation in laboratory culture, ranging from about 20 µm/day to more than 100 µm/day, even among siblings reared in the same containers, so that slow-growing and fast-growing larvae can be readily discriminated (Hilbish et al. 1999; Pechenik and Levine 2007). Although narrow-sense heritability studies using experimental crosses and quantitative genetic approaches have not previously been carried out in this species, broad-sense heritability for larval growth rate, based on analyses within and among full-sib families, has been estimated as 0.35 (Hilbish et al. 1999), showing that a proportion of the total phenotypic variance is explained by genetic components. *Crepidula fornicata* exhibits an interesting feature as this sequential hermaphrodite (i.e., sex-changing species) has the specificity of forming long-lived associations (stacks) that typically contain a number of males, females, juveniles, and individuals in transition, on the way from being male to becoming female (Conklin 1898; Coe 1938). Fertilization is internal with male transmitting sperm during mating. Mothers release veliger larvae that swim and feed in the plankton for at least several more weeks before becoming competent to metamorphose (Pechenik 1999). Previous paternity studies in native (Gaffney and McGee 1992; Proestou et al. 2008) as well as in introduced populations (Dupont et al. 2006) showed that the eggs of a given female are commonly fertilized by multiple males mostly found in the mother's "stack." Thus, when collecting a stack of animals from the field, most potential fathers for any brood produced by females from that stack are simultaneously collected (Dupont et al. 2006; Proestou et al. 2008).

In this study, by analyzing offspring from fertilizations that took place in the wild, we could measure natural rates of polyandry and analyze with accuracy reproductive patterns occurring within a natural population to examine the relationships between the sire identity or/and diversity and larval growth rates. Based on a paternity analysis of 437 fast- and slow-growing larvae from 6 broods, we hypothesized 1) that the slow versus fast growth observed among sibling larvae might be determined by specific fathers and 2) that the amount of within-brood variation for growth rate could be influenced by the number of participating fathers.

## Materials and Methods

### Collection of Stacks and Sampling of Slow- and Fast-Growing Larvae

*Crepidula fornicata* individuals ($N = 93$) in 7 stacks were dredged from the Bay of Morlaix (Brittany, France) during the peak of the breeding season in June 2007 and maintained in aquaria for about 4 days, until 1 of the females in each stack released larvae. The sex, position, and shell length of each adult in each stack was then recorded, and the presence of eggs beneath other females was noted; for details, see Dupont et al. (2006). The animals' heads were preserved in 96% ethanol for subsequent genetic analyses. The larvae from 6 of the broods were cultured in 6 separate glass containers at room temperature in 800 ml of 0.45 µm filtered seawater. Seawater was changed and phytoplankton provided ($2 \times 10^5$ cells per milliliters of *Isochrysis aff. galbana*, clone T-ISO) daily.

*Crepidula fornicata* is characterized by an average fecundity of 7500 ovocytes per female (Richard et al. 2006). All larvae were collected within about a week and were sorted according to size. For each brood, 41 among the largest larvae were isolated as "fast-growing larvae" and 41 among the smallest larvae were isolated as "slow-growing larvae" for subsequent genetic analyses. During the precompetent period, larvae grow at constant rates in terms of both shell length and biomass (Pechenik and Levine 2007), so that a given difference in shell length means the same amount of growth regardless of the starting size. Individual larval growth rates (in microns per day) were calculated as the size difference between 410 µm, the average hatchling size in *C. fornicata* (Pechenik J, personal observation), and the size at sampling date divided by the number of days in culture. The mean growth rate and standard deviation for fast- and
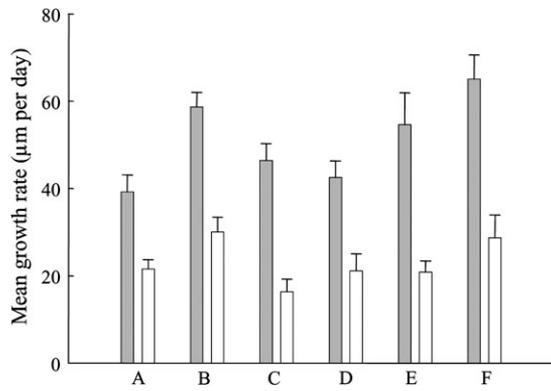
**Figure 1.** Mean growth rates of slow-growing (white) and fast-growing (gray) larvae for each brood. Standard deviations are indicated above bars.

slow-growing larvae of each brood are shown in Figure 1. Tests for the difference of growth rates 1) between broods and 2) between the 2 groups of larvae referred as "slow growing" and "fast growing" for each brood were carried out. Using a nested 2-way analysis of variance for ranked data (Sokal and Rohlf 1995) on the distribution of individual growth rates, we found significant differences among broods ($P < 0.001$) and between slow- and fast-growing larvae of a given brood. The brood effect explained 13.3% of the variance, and the slow or fast character of the larvae explained 82.8% of the variance. The mean growth rate of slow- and fast-growing larvae was not statistically different between broods ($P = 0.416$, Kruskal–Wallis test). Based on these results, the 6 broods were used for subsequent genetic analyses.

## DNA Extraction and Microsatellite Genotyping

DNA extraction of the 93 sampled adults was performed using a NucleospinRMulti-96 Tissue Kit (Macherey-Nagel, Düren, Germany) following the manufacturer's protocol and then checked on a 1% agarose gel. DNA extraction of the larvae of the 6 study broods was performed following the protocol of Higuchi (1989): in brief, each larva was washed in phosphate-buffered saline 1×, pH 7.4, to remove residual ethanol and incubated for 6 h at 56 °C in 100 µl of Higuchi buffer and 5 µl of proteinase K (10 mg/ml), followed by 15 min at 95 °C.

Adults and larvae were genotyped at 4 microsatellite loci previously characterized for *C. fornicata* and previously shown to be polymorphic and accurate for paternity analyses: CfCA2 and CfCA4 (Dupont and Viard 2003), CfH7 (Dupont et al. 2006), and Cf8 (Proestou 2006). Amplifications and genotyping on a Li-Cor 4200 automatic sequencer were carried out following protocols given in Dupont and Viard (2003) and Dupont et al. (2006).

## Paternity Assignment

For each brood, paternity assignment was based on the mother and larvae multilocus genotypes with reference to the allelic composition of the adult population (93 individuals from the 7 sampled stacks).

The minimum method (Clapham and Palsboll 1997) was first used as a preliminary assessment of the minimum number of fathers for each brood considering the genotypes of the larvae and mother. For brood A, 10 larvae presented a genotype inconsistent with the other larvae and the mother's genotype for all 4 loci but could be associated with another female of the same stack (female A2). This indicates that 2 females released larvae simultaneously, and these 10 larvae were therefore excluded from subsequent analyses. A paternity analysis was then performed using maximum likelihood computations as implemented in the software CERVUS version 3.0 (Marshall et al. 1998).

## Fatherhood of Slow- and Fast-Growing Larvae and Clutch Relatedness Coefficient

For each brood, a Fisher's Exact test was made to determine whether all assigned fathers contributed equally to the offspring. Additionally, the number of fast-growing and slow-growing larvae that could be attributed to each father was determined, and a binomial test was then used for each fatherhood to test for a contribution bias between larvae of the 2 growth rate categories.

Larval growth rate variability was compared between broods by calculating the difference in mean growth rates between fast- and slow-growing larvae. The relationship between this growth rate variability and the male reproductive success was investigated for each brood, through correlation analyses using 3 indices: 1) the number of fathers contributing to the offspring of each brood, 2) the sire diversity index calculated as a Shannon index of diversity on fathers' contributions in each brood (Schmoll et al. 2007):

$$I = -\sum_i \frac{n_i}{N} \ln\left(\frac{n_i}{N}\right),$$

where $i = 1$ to total number of father in the brood, $n_i =$ number of larvae assigned to the father $i$, and $N =$ total number of assigned larvae in the brood; the higher this index the higher is the number of father and the evenness of the sire's contribution.

3) a clutch relatedness (CR) coefficient giving the average relatedness coefficient between 2 individuals sampled randomly from a brood and obtained from the following formula:

$$CR = \frac{1}{2}\sum_i \frac{n_i}{N}\frac{n_i-1}{N-1} + \frac{1}{4}\sum_i\sum_{j\neq i} \frac{ni}{N}\frac{nj}{N-1},$$

as full sibs have a relatedness of ½, whereas half sibs have relatedness of ¼. As compared with single paternity, the average relatedness among offspring (i.e., the CR value) is decreased if multiple fathers make a genetic contribution to a clutch because the offspring will be related as half sibs. The CR value is an important parameter to further interpret genetic data.

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## Results

### Paternity Analysis

Three of the loci were highly polymorphic, with 15–37 alleles observed over the 93 adults analyzed, whereas the fourth locus (CfCA2) had only 3 alleles. The exclusion probability of the combined set of loci (i.e., power to exclude a randomly selected unrelated candidate parent of an arbitrary offspring, given the genotype of the offspring and of the mother) was remarkably high (>0.99). Each adult was characterized by 1 unique multilocus genotype. Altogether, 437 larvae (221 slow-growing larvae and 216 fast-growing larvae, i.e., 91% of the 492 larvae genotyped) could be convincingly assigned to known fathers (Table 1). All the analyzed broods exhibited multiple paternity, and 20 fathers were identified. Most of the assignments (89.5% or 491 larvae) were made to adults found on the maternal stacks: in 4 broods (A, C, D, and F), all the larvae were assigned to a father in the maternal stacks and in broods B and E 43 out of 82 (52%) and 3 out of 74 (4%) larvae, respectively, could not be assigned to an individual from the maternal stack but showed a genotype compatible with a single father. This father was identified from the comparison between the larval genotype and the maternal alleles. For each locus, 2 paternal alleles could be determined leading to a genotype heterozygous over all loci and characterized by a mendelian segregation of the alleles in the progeny. These genotypes were added to the list of candidate parents to run the analysis again. Note that in agreement with the high exclusion power of the microsatellites used, these genotypes were not observed in the adult population.

The number of fathers for each brood varied from 2 to 5 (3.3 on average, Table 1). In brood B, the paternity was balanced between 2 fathers (Fisher's test, $P < 0.001$), namely, b4 and x2 assigned to 48% and 52% of the larvae, respectively (Figure 2). In all the other broods, fathers did not contribute equally to the paternity of the larvae (Fisher's test, $P < 0.001$ in broods A, D, E, and F, $P = 0.03$ in brood C). A single father contributed to the majority of the larvae in these broods except in brood C with 2 dominant fathers observed, namely, c2 and c4 assigned to 39% and 47.5% of the larvae, respectively (Figure 2).

All 17 fathers, whose position in the stack was known, were indeed found to be located above the mother with most of them being situated directly attached to (5; i.e., 29% of the 17 fathers), 1 individual away (4; 24%), or 2 individuals away (4; 24%) from the mother (Table 1). Four fathers were located further from the mother in the maternal stack (a12 of brood A and f5, f6, and f9 from brood F). Only 3 out of 20 assigned fathers (15%) had an unknown location: 1 moved away from the stack when transferred back to the laboratory (a15 of brood A) and 2 were unsampled (x1 and x2 of broods E and B). At the time of sampling, the fathers were either males (8; i.e., 40% of the sampled fathers), in sexual transition (i.e., an individual which was changing sex at the time of sampling; 2; 20%) females with eggs (7; 35%), or even in one case an individual that had been incorrectly thought to be immature at the time of sampling (1; 5%).

### Growth of the Larvae and Fatherhood

Only 3 fathers out of 20 (15%) contributed exclusively to either fast-growing larvae or slow-growing larvae, and each was responsible for fathering only a small proportion of those larvae (see Table 1). The remaining fathers (17 fathers) were assigned both to fast- and slow-growing larvae with only 30% (6 fathers) preferably assigned to 1 category or the other (Figure 2, Table 1). Overall, the dominant fathers generally produced about equal numbers of slow- and fast-growing larvae.

Brood mean growth rates were not correlated to the number of sires identified ($r^2 = 0.10$, $P = 0.525$). In the same way, the difference of mean growth rate between fast- and slow-growing larvae was not correlated to the number of fathers represented in the brood ($r^2 = 0.03$, $P = 0.337$; Figure 3a). For example, with the same number of fathers observed, brood A presented a much smaller growth rate variation than brood B. However, growth rate variability increased significantly with the sire diversity index ($r^2 = 0.80$, $P = 0.010$; Figure 3b). Similarly, the difference in the growth rate of slow- and fast-growing larvae was significantly and inversely correlated with the degree of relatedness ($r^2 = 0.74$, $P = 0.017$; Figure 3c): the more closely related within a clutch the lower the variation in growth rate.

## Discussion

With multiple paternity observed in every brood and a mean number of 3.3 fathers per brood, our data provide a confirmation of the ubiquity of polyandry in the slipper limpet *C. fornicata*. Previous paternity studies carried out on the same species have similar output both from the native range, with 1 population showing 66% of the broods with multiple paternity and on average 1.8 fathers per brood (Proestou et al. 2008) and 3 populations from the introduced area with 78% of multiple paternity and 3.1 fathers per brood (Dupont et al. 2006). A lower value was observed in the native population of Saunderstown, RI (Proestou et al. 2008), possibly due to differences in the number of reproductive individuals (excluding the mother) per stack. Only 2–5 individuals per stack (3.7 per stack on average) were reported in the study by Proestou et al. (2008), instead of 3–11 (6.1 on average over 18 stacks from 3 populations) in Dupont et al. (2006) and 9–13 (11.3 on average) in our study. The introduced populations appeared as more gregarious, possibly based on evolutionary and ecological changes in the new habitat as documented in other taxa (Whitney and Gabler 2008). Because gregarious behavior may favor rapid population growth and efficient reproduction (Dupont et al. 2006), perhaps an increased number of individuals per stack has been selected for in the French introduced populations. Raw data from a previous study (Dupont and Viard 2003) give some support to this hypothesis: the mean stack size over 12 French populations is 5.4 compared with 4.6 in Saunderstown, RI (Proestou

**Table 1.** Stack description and paternity assignments

| Stack | Nb$_{adults}$ | Mother | Larval growth rate (μm per day) | | | Number of larvae assigned | | % Assigned | Father | | | | | |
| | | | Mean | Min | Max | Fast growing | Slow growing | | Name | % Larvae | | Total | Position | Sex |
| | | | | | | | | | | Fast growing | Slow growing | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | 12 | 4 | 29.84 | 17.27 | 47.27 | 30 | 37 | 82 | a5 | 42 | 58 | 93 | 5 | Fb |
| | | | | | | | | | a12 | 80 | 20 | 7 | Secondary stack | M |
| B | 9 | 3 | 44.35 | 18.33 | 65 | 41 | 41 | 100 | b4 | 64 | 36 | 48 | 4 | M |
| | | | | | | | | | x2 | 37 | 63 | 52 | Unknown | Unknown |
| C | 11 | 1 | 31.6 | 9.17 | 52.5 | 30 | 29 | 72 | c2 | 48 | 52 | 39 | 2 | Fb |
| | | | | | | | | | c3 | 87 | 13 | 14 | 3 | Fb |
| | | | | | | | | | c4 | 43 | 17 | 47 | 4 | Fb |
| D | 13 | 3 | 32 | 6 | 42 | 38 | 37 | 91 | d4 | 33 | 67 | 4 | 4 | Fb |
| | | | | | | | | | d5 | 42 | 58 | 78 | 5 | M |
| | | | | | | | | | d6 | 100 | 0 | 15 | 6 | M |
| | | | | | | | | | d15 | 50 | 50 | 3 | Unknown | M |
| E | 13 | 2 | 36.42 | 15 | 65 | 35 | 39 | 90 | e3 | 29 | 71 | 55 | 3 | T |
| | | | | | | | | | e4 | 95 | 5 | 30 | 4 | M |
| | | | | | | | | | e5 | 25 | 75 | 11 | 5 | M |
| | | | | | | | | | x1 | 0 | 100 | 4 | Unknown | Unknown |
| F | 10 | 1 | 46.86 | 18.75 | 81.25 | 41 | 40 | 99 | f3 | 55 | 45 | 69 | 3 | Fb |
| | | | | | | | | | f4 | 40 | 60 | 6 | 4 | Fb |
| | | | | | | | | | f5 | 54 | 46 | 16 | 5 | M |
| | | | | | | | | | f6 | 0 | 100 | 1 | 6 | T |
| | | | | | | | | | f9 | 17 | 83 | 8 | 9 | M |

For each stack (brood), the position in the stack of the mother analyzed (see below for the numbering) and the number of other reproductive adults (Nb$_{adults}$), are indicated. For each mother, the mean, maximum, and minimum larval growth rates observed in the samples. The number of fast- and slow-growing larvae as well as the total percentage of larvae assigned is given for each brood. Then, the percentage of larvae assigned to each identified father is indicated together with the proportion of fast- and slow-growing larvae, father's name, position, and sex at the time of the sampling. Adult individuals within the stacks were numbered according to their position in the stacks: from the bottom (number 1) to the top of the main stack (e.g., I5, individual number 5 in the main stack) or in secondary stack (e.g., I12, individual number 12 in a secondary stack). The position of a father identified but either not attached to the stack or unsampled is indicated with "unknown." Fathers' sex is specified as follows: M, male, T, sexual transition, Fb, brooding female.
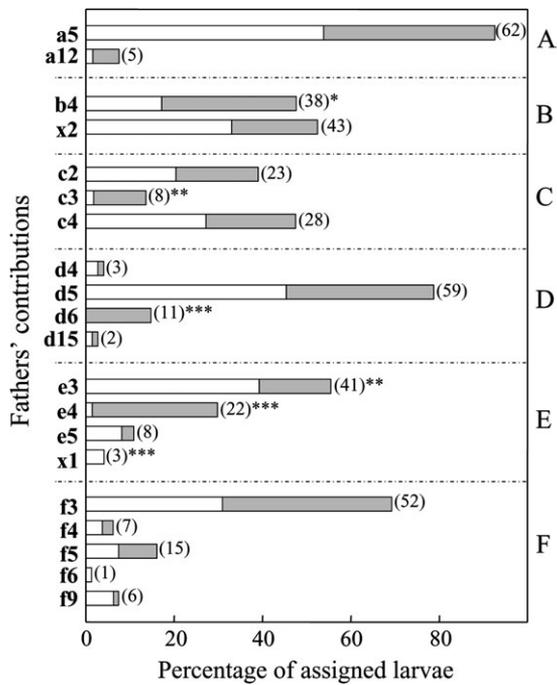
**Figure 2.** Relative contributions of fathers to the fast- and slow-growing larvae. For each brood, the percentage and number of larvae assigned to each father are indicated by the bar and a number in brackets, respectively. For each father, the relative proportion of slow-growing larvae (white) and fast-growing larvae (gray) are then indicated. The proportion of slow-growing larvae and fast-growing larvae assigned to a given father were compared with a binomial test (statistical confidence $*P < 0.05$, $**P < 0.01$, $***P < 0.001$).

et al. 2008), and 2.5 in Fairhaven, MA (Dupont and Viard 2003). Other environmental drivers (e.g., chemical cues inducing larval settlement) could also explain the variation in the number of individuals per stack. Precise comparison of stack structure between populations within and among the native and introduced range deserves to be carried out to validate this observation and investigate what can explain this apparent shift in the stack composition.

The specific behavior of the slipper limpet of aggregating in perennial stacks presents a great advantage: the individuals within a stack behave as an almost closed mating group (89.5% of the larvae analyzed had a father within the maternal stack). Patterns of polyandry form copulations that had taken place in the wild can thus be observed with an accuracy similar to controlled crossing. Together with such stacking behavior, protandry (sex-changing trait) is a primary characteristic that may favor polyandry in the slipper limpet. A nonnegligible proportion of the sires were identified as brooding females at sampling time (35%). The brooding female A5 in stack A was assigned as a father to 92.5% of the larvae of brood A (Figure 2), showing that a brooding female can even be the "dominant" father of a brood. It is noteworthy that bisexual individuals have never been documented in the literature
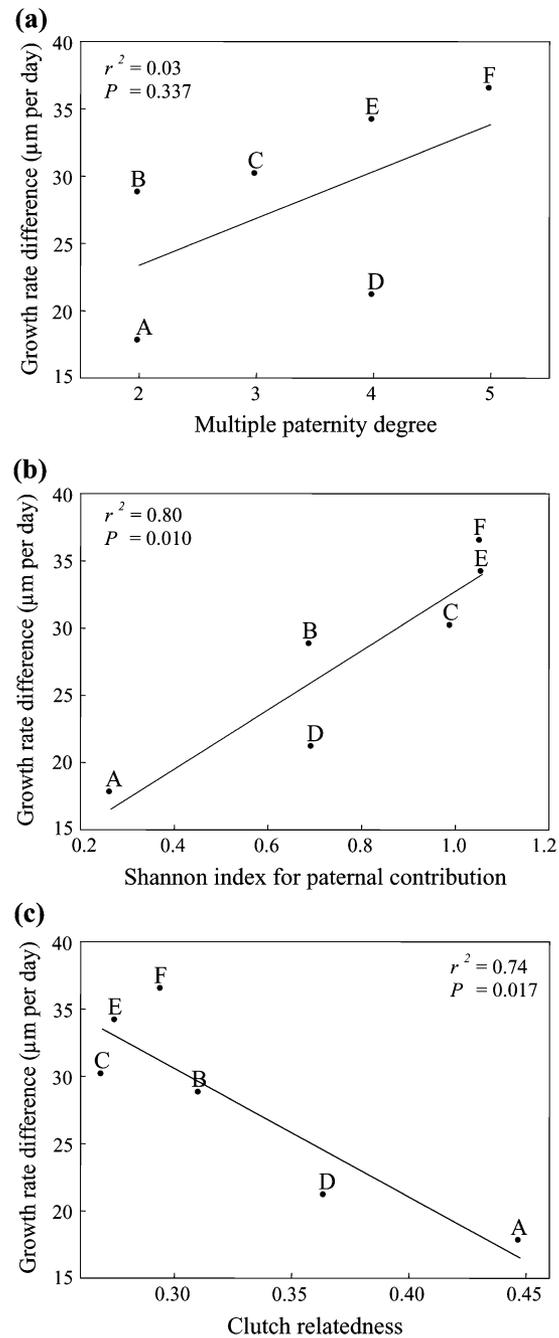


**Figure 3.** Relationships between polyandry patterns and range of variation of larval growth rate. For each brood (featured by its name along the $x$ axis), the difference of mean growth rate between fast- and slow-growing larvae is regressed against (**a**) the number of father per brood, (**b**) the mean relative fertilization success of the fathers per brood, and (**c**) the CR coefficient.

based on histology, genetics, or morphological studies (see Le Gall 1974; Martin 1985 and references therein). Individuals in transition or even brooding females can be fathers (this study; Dupont et al. 2006; Gaffney and McGee

1992; Proestou et al. 2008). Together with the results by Hoagland (1978) who showed that isolated females were able to store sperm for 1 year, these findings evidence a capacity for sperm storage either between reproductive seasons or possibly during the same season. Sperm storage is often linked to multiple paternity because females can receive sperm from consecutive males (Adams et al. 2005).

The number of successful fathers in our study ranged from 2 to 5 per brood. As compared with the number of potential fathers in each stack (9–13; see Table 1) and given the rather large amount of larvae studied, the number of fathers was low. This result could first be due to the sampling design as we assigned fathers to 2 extreme categories of larvae (i.e., the largest and smallest ones after 1 week of culture): perhaps additional fathers would be identified with the analysis of intermediate size larvae. It is nevertheless noteworthy that 17 out of 20 fathers contributed to both fast- and slow-growing categories. Paternal identity thus appears to behave neutrally regarding the larval growth (see below) with consequently little bias on overall paternity assignment. In a previous paternity analysis (Dupont et al. 2006), based on 11–16 larvae randomly sorted, the number of fathers identified was similar. Together with a limited number of fathers, the second main outcome of the present paternity analysis is the observation of a skewed reproductive pattern: more than 50% of the larvae were fathered by 1 or 2 individuals in 5 out of the 6 broods analyzed. Such variation in the degree of paternity among sires has been observed in wild populations of numerous polyandrous species (e.g., Neff et al. 2008) and has been attributed to various causes like sexual selection (Birkhead and Møller 1998) or sperm amount (Stockley 1997) and embryo viability (Simmons 2005). In the slipper limpet, this variation could be explained by the position of the study mothers within the stacks: all fathers with a known position were found close to and above the mother. Among the 17 fathers belonging to the maternal stack, 13 (76.5%) were at most 2 individuals away from the mother. There is, however, no significant correlation between the fatherhood and the number of individuals separating the mother and assigned father. Differences in sperm amount due to repeated matings by the same male may also account for these effects. Precise data about the reproductive behavior of the slipper limpet, like the frequency of repeated mating with the same male, are needed to test this hypothesis.

In this study, we have shown that the paternity diversity, rather than the paternal identity, is linked to the range of variation in growth rate. We indeed found that the range of growth rates within a brood is not caused by any particular father, as most of the fathers (75%) were reliably assigned both to the fastest and slowest growing larvae within a brood and only 25% had a fatherhood biased toward larvae in only 1 of those 2 categories. It was also not correlated with the number of fathers although this number varied across broods, ranging from 2 to 5. Interestingly, the range of larval growth rates was significantly related to the relative contribution of the fathers: the magnitude in the difference of mean growth rates, between fast- and slow-

growing larvae, increased significantly with the sire diversity index and decreased with the degree of CR (Figure 3). Thus, a brood can exhibit a large number of fathers without showing a high degree of variability among offspring if most of those fathers fertilize only a small number of eggs. For example, in brood D, 3 out of 4 fathers sired less than 15% of the larvae and growth rate variation was relatively low. On the other hand, brood B exhibited only 2 sires, but because they fathered a similar proportion of larvae, CR is lower than that of brood D; consequently, the range of growth rate variability was higher in brood B than in brood D. Therefore, it is not only the number of fathers involved but also the degree to which each of those fathers contribute to offspring, which enhances growth rate variability in the offspring. Our results indicate that paternity analyses should consider the number of fathers involved as well as sire diversity (Schmoll et al. 2007) or the CR (this study).

Polyandry, a common feature of marine invertebrates (e.g., Toonen 2004; Marshall and Evans 2007; Sprenger, Anthes, and Michiels 2008; Sprenger, Faber, et al. 2008; for a review, see also Baur 1998), is expected to indirectly increase the female fitness either through paternal genetic effects (additive or nonadditive) or through paternal environmental effects (Yasui 1998; Jennions and Petrie 2000; Garcia-Gonzalez and Simmons 2007). Our results show that the number of fathers and their relative contribution to the brood influences the magnitude of variation in larval growth rates. This finding has ecological and evolutionary implications. On the one hand, the fact that growth rate variability within a brood decreased with higher CR gives some support to the "genetic diversity hypothesis" where there is no single best sire for any given female but diversity is per se advantageous (Yasui 1998; Zeh AJ and Zeh DW 2003; Marshall and Uller 2007). As most marine invertebrates, adults of *C. fornicata* typically have limited or no ability to move so that the persistence of the metapopulation relies on recruitment of free-living, independent larval phase. Fast-growing larvae will reach competence more rapidly and thus may metamorphose and settle earlier than slow-growing ones. As most of the fathers identified within a brood contribute to both fast- and slow-growing larvae, the 2 sets of sibling larvae that may ultimately recruit at different time will be genetically similar and diversified. This result is congruent with the large genetic diversity in juveniles coupled with a genetic stability between juveniles and adults in 2 French introduced populations (Dupont, Bernas, and Viard 2007). Slow-growing larvae might appear to be disadvantaged as they are more susceptible to planktonic predation. However, Pechenik et al. (1996) showed that larval growth rate is a poor predictor of juvenile performance; in particular, slow-growing larvae can metamorphose into unusually fast-growing juveniles. Slow-growing larvae may also have an advantage under different circumstances, for example, if the early settlers (i.e., resulting from metamorphosis of fast-growing larvae) failed to survive because of unfavorable environmental conditions. Altogether, as environmental conditions encountered by the larvae are highly unpredictable,

decreasing CR through multiple matings reduces the risk of reproductive failure of the mother and full-sibling competition (Yasui 1998). On the other hand, selection may favor female preference for males through multiple mating but not necessarily based on differences in the trait that we here studied, namely, growth rate (good genes or compatible genes hypothesis; Neff and Pitcher 2005). Skewed reproductive pattern observed among fathers might be an outcome of male–male competition or cryptic mechanism of female choice. Finally, larval growth rate could not only be due to the paternity diversity but also be affected by the maternal phenotype. For instance, Simmons (2001) and Sprenger, Anthes, and Michiels (2008) observed females modifying their reproductive output in response to mating possibilities that they encounter. In our case study, part of the differences in growth rate could result from differences in egg size so that differential allocation by mothers, and not male identity, would be the source of variations in growth rates. A logical next step would be to determine more precisely the underlying mechanisms of variability in larval growth, in particular disentangling the phenotypic plasticity and genetic bases responsible for this variability. Deciphering between those alternative hypotheses would mostly require controlled laboratory crosses, which are unfortunately technically difficult to carry out in a long-lived species with internal fertilization like *C. fornicata*.

We here showed that a reproductive behavior, that is, polyandry and more specifically the sire diversity, was significantly correlated to variations in growth rate of a benthopelagic species. Such variations in the growth rate may have implications for dispersal and recruitment success, important facets of the invasion success of the slipper limpet. Previous results based on larval modeling approaches and population genetics studies suggested that larval dispersal was an important contributor to the invasion success of *C. fornicata* in Europe (Viard et al. 2006; Dupont, Ellien, and Viard 2007). Similar to the variability in settlement behavior observed in other marine invertebrates (Marshall and Keough 2003; Toonen and Tyre 2007), variable growth rates might be associated with variable dispersal potential. Slow-growing larvae, by exhibiting a longer pelagic larval duration, could be particularly good dispersers and provide selective advantages for sessile or sedentary organisms by reducing competition between siblings and reducing mating between close relatives (review in Ronce 2007). Nevertheless, if dispersal distance scales directly with the time larvae spend in the plankton for many animals (Shanks et al. 2003; Siegel et al. 2003), many additional factors contribute to the effective dispersal range (for a review, see Cowen and Sponaugle 2008). Pineda et al. (2007) suggested that if pelagic larval duration is a good proxy of larval transport, it could be a poor predictor of the dispersal distance. Numerous environmental drivers (e.g., temperature) or specific traits (e.g., cues inducing metamorphosis; Pechenik 1990; Hadfield 1998) are likely to play a significant role in determining the length of the pelagic larval duration in *C. fornicata*. Further studies are needed to establish if in an invasive species like *C. fornicata*, larvae with long larval duration effectively contribute to range expansion. Insights about larval dispersal variation might also come from indirect approaches like genetic assignment of larvae and juveniles sampled in the wild to determine the relative proportion of local versus immigrant larvae contributing to the local populations.

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