

New estimates of early post-settlement mortality for intertidal mussels show no relationship with meso-scale coastline topographic features

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ABSTRACT: For organisms with planktonic larvae, the early post-settlement stage is a particularly vulnerable one, likely to influence distribution patterns in subsequent life stages. Although substantial post-settlement mortality is well known in several marine invertebrate taxa (e.g. barnacles), few estimates exist for benthic invertebrates, such as mussels, that have mobile settlers. Furthermore, estimates of early post-settlement mortality (within 2 d of settlement) are sparse for most groups. In addressing the difficulties involved with quantifying early mortality of mobile settlers, the present study builds on a sequential-deployment method to estimate the post-settlement mortality of the intertidal mussel *Perna perna*. Trials were run at 16 sites, split evenly between bay and open coast locations, and were repeated over 2 sampling cycles. By comparing post-settlement mortality estimates from bays and open coast sites, we investigated whether such topographic features produce differential mortality. Post-settlement mortality estimates showed substantial levels of mortality but did not differ significantly between bay and open coast sites. Early post-settlement mortality (i.e. mortality of primary settlers up to 2 d old) ranged from 31 to 94% but averaged 54 and 64% in the 2 sampling cycles. Estimates of total post-settlement mortality (i.e. inclusive of primary and secondary settlers) had a similar range and averaged 66% in Cycle 1 and 67% in Cycle 2. Apart from confirming significant mortality rates of early mussel settlers, these findings suggest that intertidal abundance patterns of recruits and adults, often associated with topographic features of coastlines, are more likely a result of initial settlement patterns than of differences in post-settlement mortality.

KEY WORDS: Post-settlement mortality · Coastline topography · Bays · *Perna perna* · Intertidal mussels · Pelagic-benthic coupling

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INTRODUCTION

Substantial mortality during early post-settlement life has long been suggested and demonstrated across several marine taxa (Connell 1961, Thorson 1966, Gosselin & Qian 1997, Hunt & Scheibling 1997), including reef fish (Searcy & Sponaugle 2001, Doherty et al. 2004), mobile invertebrates such as crabs, abalone and other gastropod species (Ray & Stoner 1995, Moksnes et al. 1998, Naylor & McShane

2001) and a variety of sessile invertebrates (Hurlbut 1991, Gosselin & Qian 1996, Marshall & Keough 2003). Certainly, juvenile mortality in benthic marine invertebrates is greater than 90% in the majority of studies (Gosselin & Qian 1997), identifying this period as especially important in the life history progression of benthic organisms.

While the relevance and severity of post-settlement mortality is recognized (see Gosselin & Qian 1997, Hunt & Scheibling 1997, Shanks 2009), it is a difficult

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parameter to quantify, particularly for invertebrate organisms with mobile settlers. In general, monitoring of early settlers must be frequent enough to allow separation of the initial settlement pattern from the mortality-altered post-settlement pattern (Hunt & Scheibling 1997). This requirement of high frequency sampling (Minchinton & Scheibling 1993), together with the small size of early settlers makes studies of mortality within the first 24 to 48 h exceedingly difficult (Hunt & Scheibling 1997). Much of our knowledge of post-settlement processes and their importance to benthic invertebrate populations comes from the study of barnacles (Foster 1971, Connell 1985, Wethey 1985, Jarrett 2000, Chan & Williams 2003). Some direct estimates of mortality within 2 to 4 d of settlement are available for several other taxa, including ascidians, bryozoans, oysters and gastropods (Davis 1987, Stoner 1990, Ray & Stoner 1995, Gosselin & Qian 1996, Walters & Wethey 1996, Naylor & McShane 2001). For many groups, including bivalves, post-settlement mortality estimates pertain largely to older settlers and recruits (e.g. Luckenbach 1984, Bartol et al. 1999, Bishop et al. 2005). As a result, little is known about typical levels of early (ca. 2 d) post-settlement mortality, particularly for mussels (but see Bownes & McQuaid 2009).

In this regard, the lack of studies on bivalves (and mussels in particular) suggests that establishing early mortality of mussels is especially difficult. Relative to barnacles, the mobility of mussel settlers makes distinguishing between real mortality and emigration (post-settlement movement, *sensu* Bayne 1964) virtually impossible. Consequently, standard techniques of mapping or photographing experimental plates, such as are used for barnacles (Gosselin & Qian 1997), cannot be employed. Traditional cohort monitoring, or variants of this technique, have been used with some success, finding high rates of mussel mortality within various sampling intervals (Moreno 1995, Cole et al. 2000, McQuaid & Phillips 2007, Peteiro et al. 2007).

Although previous methods of marking or tagging mussel settlers in the numbers necessary for meaningful experimentation have proved unsuccessful (Seed 1969), recent advances, such as the use of calcein staining, have made this method one of the most reliable (Phillips 2002, 2004). Staining methods, however, retain the problem of separating emigration derived losses from actual mortality. Other methods such as the use of caged plots have successfully obtained estimates of monthly juvenile mussel mortality (75–100%) but are only able to explore certain sources of mortality (e.g. Shields et al. 2008).

Most notably, Bownes & McQuaid (2009) used a novel method of sequential deployment of paired settler collectors to estimate juvenile mussel mortality over 6 d. Mortality was calculated by subtracting the total settlement on the settlement pads left in place for the duration of the experiment ('6-d pads') from the cumulative settlement on successive daily pads and expressing this as a percentage of the cumulative daily settlement. As in other studies such as Shanks (2009), discrete daily settlement was essentially considered mortality free. In Bownes & McQuaid's (2009) calculations, 'settlement' included all sizes of settlers, and it was assumed that there were no differences in attraction of settlers to the 'clean' daily collectors and the established weekly collectors. In reality, weekly collectors would have rapidly developed bacterial communities and biofilms, probably within the first 24 h of being deployed (Zobell & Allen 1935), and would have carried increasing numbers of conspecific settlers. These factors have been found to increase primary and secondary mussel settlement, respectively (von der Meden et al. 2010).

Primarily, the present study further develops the sequential-deployment method of Bownes & McQuaid (2009), taking into account differences in settler attraction to biofilms and conspecifics, to estimate early post-settlement mortality of the intertidal mussel *Perna perna* on the south coast of South Africa (Fig. 1). Using these estimates, this study examines the influence of coastal topographic features (bays/headlands) on post-settlement mortality. In the study area, settlement and the abundance of adult intertidal mussels are greater in bays than on the open coast (C.E.O. von der Meden unpubl., von der Meden et al. 2008). How this pattern comes about is not known, although a supply-side influence has been suggested here and in several other regions (e.g. Helson & Gardner 2004, Lagos et al. 2005, Pfaff et al. 2011). An additional explanation, however, may lie in patterns of post-settlement mortality. Since juvenile survival can be affected by physiological stresses related to desiccation or emersion (Tan 1975, Iwasaki 1995) and latent effects of larval food availability (Phillips 2002, 2004), differences in exogenous 'causal' factors between bay and open coast habitats (such as wave exposure) may well drive patterns of post-settlement mortality (e.g. Gosselin & Chia 1995, Naylor & McShane 2001). By investigating post-settlement mortality across multiple bays and open coast sites, we test the hypothesis that post-settlement mortality is lower in bays than on the open coast.

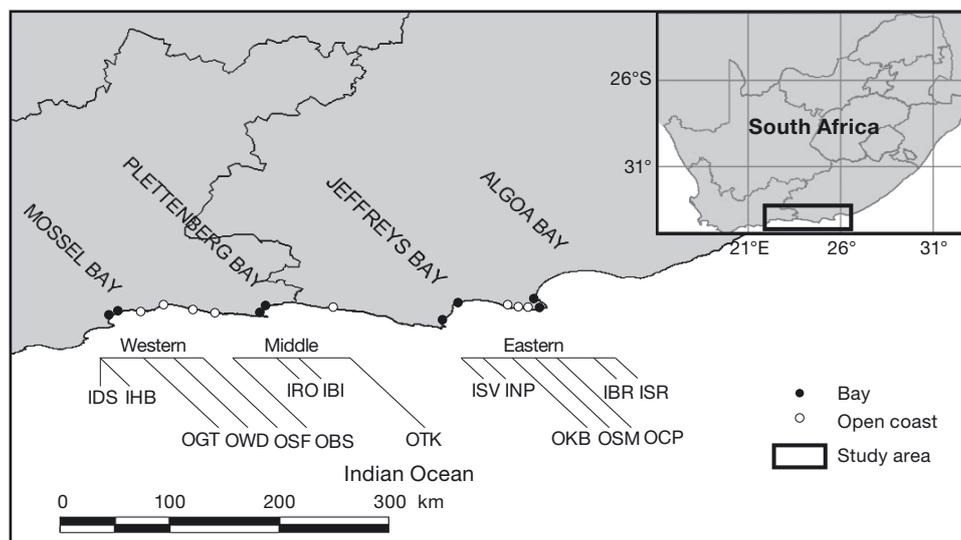


Fig. 1. Study sites along the south coast of South Africa. Bay sites have the prefix 'T'; open coast sites have the prefix 'O'

MATERIALS AND METHODS

Laboratory procedures

Experimental setup

Following Bownes & McQuaid (2009), paired larval collectors (plastic mesh pot-scourers) were deployed on low-shore rocks (within the lower balanoid zone) in 3 replicate pairs at each of 16 sites. Collectors were attached in pairs to individual eye bolts, which were separated by >50 cm. Sites were split evenly between bay and open coast habitats (Fig. 1). One collector from each pair was replaced daily while the other remained in place for the 7 d duration of the experiment.

Because sites were spread out over a considerable distance (ca. 500 km, Fig. 1), the study area was subdivided into 3 sections. The most easterly of these sections included 7 sites (ISV, INP, OKB, OSM, OCP, IBR, ISR) the middle section 4 (OBS, IRO, IBI, OTK) and the westerly section 5 (IDS, IHB, OGT, OWD, OSF) (Fig. 1). Each section was sampled over a week, collecting and replacing the daily collectors every morning during low spring tides. Sampling of the first section began on 18 January 2007, with the middle and western sections being sampled on the 2 subsequent spring tides (starting 31 January and 15 February, respectively). This procedure was repeated directly after the completion of this first 3-section 'cycle', starting with the eastern section on 1 March, and sampling the middle and western sections starting on 15 March and 1 April, respectively. This produced 2 sampling cycles of 3 sections each: Cycle 1 and 2. Collectors were stored in jars of 70% ethanol immediately after collection.

Collectors were soaked for 3 min in a weak (20%) sodium hypochlorite solution to dissolve byssal threads, allowing easy removal of settlers (Davies 1974). The collectors were then vigorously washed and all material collected in a 75 μm sieve. All *Perna perna* settlers were identified following Bownes et al. (2008) and enumerated. Larval collectors often contain a range of sizes of post-larval mussels, even if recovered from the shore after only 24 h. These include primary settlers and those that have immigrated as secondary settlers. We separated mussels into 3 size classes: primary settlers (<360 μm); primary settlers plus 1 wk of growth (360–660 μm , referred to as size class B); and secondary settlers/recruits (>660 μm , referred to as size class C). The range for the middle size class (B) was based on potential weekly growth from a minimum initial settlement size of 260 μm or a maximum initial settlement size of 360 μm , calculated using growth rates estimated by Bownes (2005) for the study species (12 ± 8.4 to 32.5 ± 5.0 $\mu\text{m d}^{-1}$, mean \pm SD).

Calculation of post-settlement mortality

In refining the methods of Bownes & McQuaid (2009), the present study separated primary post-settlement mortality (i.e. mortality of primary settlers) and total post-settlement mortality (i.e. mortality of all individuals arriving on collectors). Both biofilm and the presence of conspecifics have been shown to

affect settlement rates (von der Meden et al. 2010), so we incorporated field-derived correction factors to compensate for these effects on weekly collectors. Correction factors were determined for the study species by analogous week-long manipulative experiments at 2 sites, which estimated the effects of both biofilm and the presence of conspecifics on settlement rates (see von der Meden et al. 2010 for detailed methods). In the present study, correction factors were applied to daily settlement rates from Day 2 onwards, allowing 24 h for the establishment of biofilms and arrival of conspecifics on the weekly collectors. The equations were developed as follows:

Primary post-settlement mortality

The corrected cumulative (total) primary settlement (D_{psT}) on daily collectors was calculated using Eq. (1.1), where D_{1ps} is the primary settlement (ps) on the Day 1 daily collector (uncorrected for biofilm and conspecifics), and $\sum D_{2-7ps}$ is the sum of primary settlers from Days 2–7, which was multiplied by the primary settler correction factor $C_1 = 3.8$, derived from von der Meden et al. (2010).

$$D_{psT} = D_{1ps} + D_{2-7ps}C_1 \quad \text{Eq. (1.1)}$$

The total number of primary settlers on each weekly collector (W_{psT}) was calculated according to Eq. (1.2), where W_{ps} is the number of primary settlers on the weekly collector; W_B is the number of settlers of size class B (360–660 μm) on the weekly collector (indicating the number of settlers that either grew out of the primary settler size class during the week or immigrated to the weekly collector at a size larger than 360 μm); and $\sum D_{1-7B}$ is the sum of settlers on the daily collectors that were of size class B. Since settlers of size class B on daily collectors must have immigrated rather than settled and grown, this sum ($\sum D_{1-7B}$) provides an estimate of immigration at a given site. Subtracting this estimate of immigration from W_B leaves the number of primary settlers that had arrived on a weekly collector but grew out of the primary size class into class B during the sampling week. W_{psT} is therefore simply the total number of primary settlers on the weekly collector, including those that were no longer in the primary settler class at the end of the experiment.

$$W_{psT} = W_{ps} + (W_B - \sum D_{1-7B}) \quad \text{Eq. (1.2)}$$

Note that no correction factor was applied to the daily immigrants ($\sum D_{1-7B}$) in Eq. (1.2) since no effect

of biofilm or conspecific presence was found for this size class (von der Meden et al. 2010). Putting Eqs. (1.1) & (1.2) together, mortality of primary settlers, or primary post-settlement mortality (M_{primary}) over the 7 d duration of the experiment, was calculated as follows:

$$M_{\text{primary}} = \frac{D_{psT} - W_{psT}}{D_{psT}} \times 100 \quad \text{Eq. (1.3)}$$

The calculation of M_{primary} as described by Eqs. (1.1) to (1.3) has several difficulties and comes with 5 assumptions:

(1) There is negligible mortality on daily collectors, within each 24 h deployment time, relative to the corresponding weekly collectors.

(2) We are forced to assume that mortality of W_B settlers (Eq. 1.2) is negligible.

(3) Rates of immigration and emigration of settlers to and from collectors are assumed to be similar if not equal and therefore balance each other. Based on this assumption, calculated mortality rates describe only those settlers that die.

(4) Differential settler attraction to weekly collectors remains relatively consistent across sites and in time, meaning the calculated correction factor adequately accounts for these differences at all sites.

(5) Given no increased attraction of size class B to biofilm (sensu von der Meden et al. 2010), it is assumed that the $\sum D_{1-7B}$ (Eq. 1.2) approximates immigration onto weekly collectors.

Total post-settlement mortality

Total post-settlement mortality (M_{total}) was calculated by subtracting the total number of settlers of all 3 size classes on the weekly collector (W_{TS}) from the cumulative settlement of all 3 size classes on the 7 daily collectors (total daily settlement, D_{TS}). C_1 was applied to the daily primary settlers as in Eq. (1.1). A second correction factor ($C_2 = 6.6$) was applied to size class C. This corrected for the combined effect of biofilm and conspecifics on these larger recruits, as calculated by von der Meden et al. (2010). D_{TS} was calculated as:

$$D_{TS} = (D_{1ps} + \sum D_{2-7ps}C_1) + \sum D_{1-7B} + (D_{1C} + \sum D_{2-7C}C_2) \quad \text{Eq. (2.1)}$$

and W_{TS} as:

$$W_{TS} = W_{ps} + W_B + W_C \quad \text{Eq. (2.2)}$$

M_{total} for the 1 wk period was therefore calculated from D_{TS} and W_{TS} as follows:

$$M_{\text{Total}} = \frac{D_{\text{Ts}} - W_{\text{Ts}}}{D_{\text{Ts}}} \times 100 \quad \text{Eq. (2.3)}$$

The terms for Eqs. (2.1) to (2.3) are the same as in Eqs. (1.1) to (1.3), with the addition of D_{1C} , the number of settlers of size class C on the Day 1 daily collector; $\sum D_{2-7C} C_2$, the corrected sum of settlers of size class C on daily collectors from Day 2–7, and finally W_C , the number of weekly settlers in size class C. Assumptions (1) and (3) above also apply to the calculation of total post-settlement mortality.

Data handling and statistical analyses

Mortality was calculated for all sites; however, the stochastic nature of settlement means that weekly and daily collectors on the same eye bolt inevitably differ in the number of settlers arriving on them, with the difference ranging between positive and negative so that subtracting weekly settlement from cumulative daily settlement should result in a normally distributed spread of values. Because of this and the possibility of non-conformance to the assumptions of the equations (above), some post-settlement mortality percentages were biologically unrealistic (i.e. outside the 0–100% range). These were therefore not included in statistical analyses.

Two-way ANOVAs were used to examine primary and total post-settlement mortality in each of the 2 sampling cycles. These analyses tested for effects of topography (fixed, 2 levels: bay and open coast) and site (random, nested in topography) using all data within the 0–100% range in each case.

Settlement rates and assumptions

To try to understand why so many mortality percentages were not realistic, we examined the potential influence of our assumptions on primary post-settlement mortality estimates. This was done using hypothetical cases, each correcting for the situation arising from non-conformance to a particular assumption. For example, increasing the number of primary settlers on daily collectors (D_{1ps} and D_{2-7ps} , Eq. 1.1) corrects for the possibility of non-conformance with Assumption 1 (negligible mortality of daily settlers). This provided a way of modelling the influence of different terms in the post-settlement mortality equation on our mortality estimates, as each term or group of terms can be related to a specific assumption. Increases or decreases in equation terms were arbitrarily chosen, and involved doubling, tripling and quadrupling or reducing by half, a third and a quarter (see Table 1). In the case of changes to C_1 (3.8) a lower value of 1.5 and 2 higher values 5 and 8 were selected for modelling.

RESULTS

Settlement patterns

Cumulative daily settlement of all 3 size classes displayed order-of-magnitude differences among several of the 15 study sites (reduced from 16 sites due to loss of Site OCP in this cycle) as seen in Cycle 1 (Fig. 2). Of the 3 size classes, primary settle-

Table 1. Summary results from modelling of the primary post-settlement mortality equation. Equation terms were altered to simulate non-conformance of a particular assumption. The range of alterations is shown as the factor by which a given term was multiplied. The direction of change produced in the mortality estimates is described and the number of additional realistic estimates rendered is listed in the final column. D_{psT} : cumulative primary settlement on daily collectors; D_{ps} : primary settlers on Day 1 daily collector; D_{2-7ps} : primary settlers from daily collectors over Days 2–7; W_B : number of settlers of size class B on weekly collectors; C_1 : primary settler correction factor; $\sum D_{1-7B}$: sum of settlers on daily collectors that were size class B; +ve: positive; -ve: negative

Relevant assumption	Terms altered	Range of alterations	Direction of result	No. additional realistic percentages
(1) Negligible daily mortality (higher D_{psT} to compensate for mortality)	D_{1ps} & D_{2-7ps} , Eq. (1.1)	× 2, 3, 4	Lowers +ve but increases -ve percentages	2–7
(2) Negligible mortality W_B (higher W_B to compensate for mortality)	W_B , Eq. (1.2)	× 2, 3, 4	All percentages get smaller as W_B increases	4–5
(4) Consistent correction factor (higher or lower correction factor)	C_1 , Eq. (1.1)	$C_1 = 1.5, 5, 8$	Higher $C_1 =$ lowers +ve but increases -ve percentages	2–6
(5) & (3) Weekly immigration = cumulative daily (higher or lower immigration)	$\sum D_{1-7B}$, Eq. (1.2)	× 2, 3, 4 × 0.5, 0.33, 0.25	Percent changes in direction of alteration	2–6

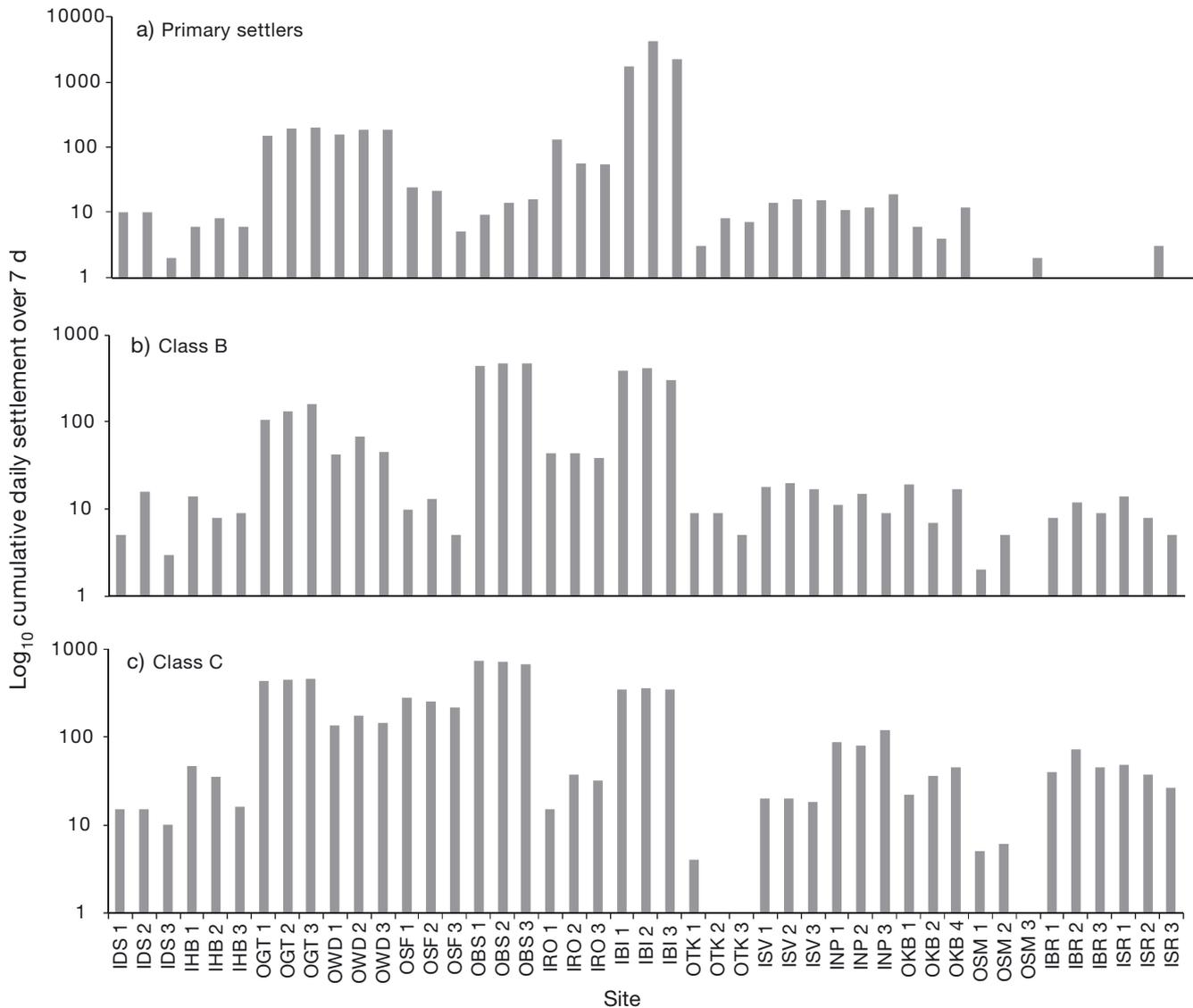


Fig. 2. Log-scaled cumulative daily settlement in each size class (a) primary settlers (<360 μm), (b) size class B (360–660 μm) and (c) size class C (>660 μm). Cumulative settlement values are summed over 7 daily collections. Sites are in geographical order from west to east. As in Fig. 1, site codes for bays have the prefix 'I'; open coast sites have the prefix 'O', with numbers 1 to 3 indicating replicates

ment was particularly variable, having the greatest number of replicates with zero settlement as well as the maximum cumulative settlement rate of 4216 settlers (over 7 d). For settlers between 360 and 660 μm (size class B) a maximum cumulative settlement rate of 471 settlers was recorded, while for those larger than 660 μm (size class C) the maximum cumulative rate was 756 settlers. Spatially, settlement patterns were similar across all 3 size classes. The general pattern was an increase in settlement from the westerly sites in Mossel Bay (IHB, IDS) to peak values in Brenton-on-Sea (OBS) and Plettenberg Bay (IBI), followed by decreasing rates across the easterly sites of

the Algoa Bay area (ISR, IBR, OSM, OKB, see Fig. 2). The same pattern was evident in Cycle 2.

Post-settlement mortality

Estimates of primary post-settlement mortality varied greatly in both sampling cycles; an example of this can be seen in Cycle 1, for which 17 of the 45 replicate collector pairs produced biologically realistic primary mortality percentages within the range of 0 to 100% (Fig. 3a). A similarly wide range of estimates resulted from calculations of total post-settle-

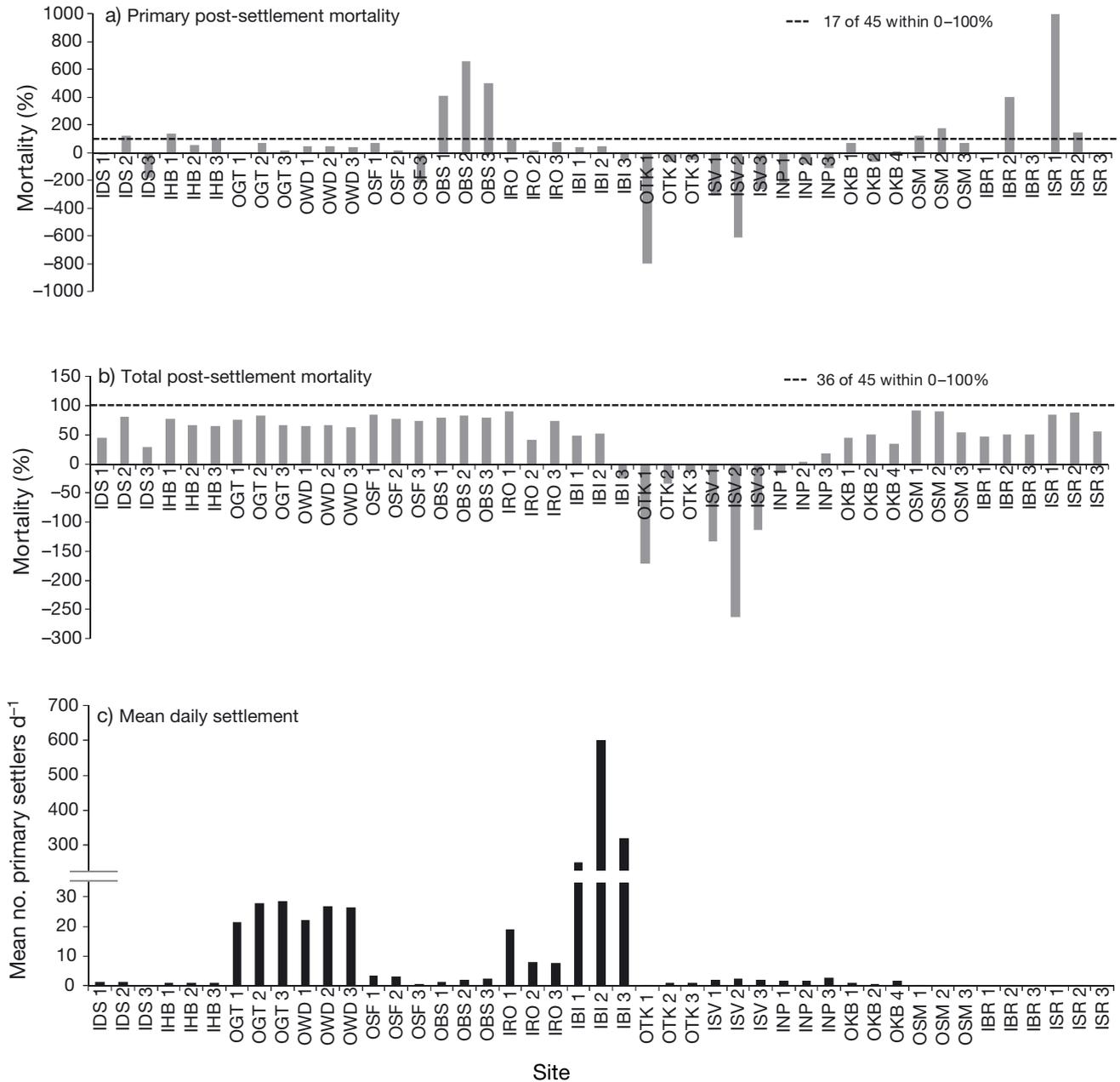


Fig. 3. Calculated (a) primary and (b) total post-settlement mortality percentages for all 3 replicates at each of the 15 study sites from the first sampling cycle, Cycle 1. Bars with values between 0 and the dashed line (100%) are the biologically realistic values calculated. (c) Mean daily primary settlement rate for corresponding replicates during Cycle 1. Sites are in geographical order from west to east. As in Fig. 1, site codes for bays have the prefix 'I'; open coast sites have the prefix 'O', with numbers 1 to 3 indicating replicates

ment mortality, although many more fell within the biologically realistic range (36 of 45 collector pairs, Fig. 3b). Although not presented, numbers of biologically realistic percentages were very similar in Cycle 2 (19/48 for primary mortality; 38/48 for total mortality). No obvious pattern or relationship was noticed between mean daily settlement rates and the calculated mortality percentages (e.g. Fig. 3c).

Modelling of assumptions

Increasing D_{psT} (see Assumption 1) either directly through settler numbers or indirectly by changing C_1 (see Assumption 4) resulted in lower positive mortality percentages and smaller negative percentages, and improved the number of replicates with realistic mortality percentages by between 2 and 7, depend-

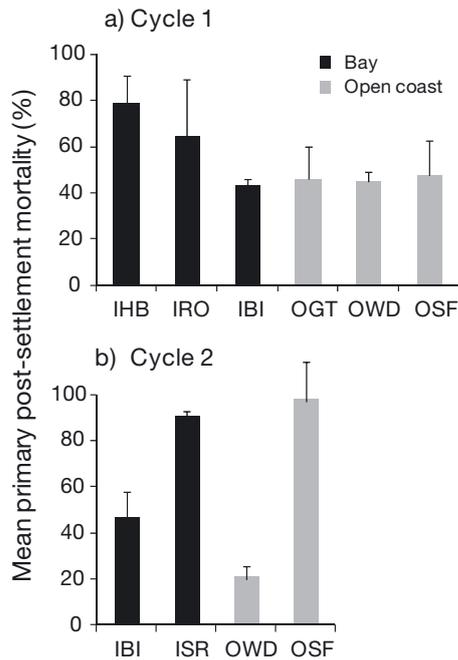


Fig. 4. Mean primary post-settlement mortality percentages (+SE) calculated for sites in (a) Cycle 1 ($n = 6$) and (b) Cycle 2 ($n = 4$). Within each topographic class, sites are arranged in geographic order from west to east (see Fig. 1)

ing on the amount increased (Table 1). By increasing the number of W_B (Assumption 2), mortality percentages were lowered and the number of biologically realistic percentages was increased by 4 and 5 replicates (Table 1). Similarly, altering $\sum D_{1-7B}$ (Assumption 5) caused the mortality percentages to shift in the direction of the alteration. Both increases and decreases in immigrating settlers produced between 2 and 6 additional realistic mortality percentages (Table 1).

Topography-related patterns

Using only biologically realistic sites in each case, the mean percent primary post-settlement mortality was 54.3% in Cycle 1 and 64.3% in Cycle 2. On a by-site basis, mean primary post-settlement mortality ranged across sites from 43.2 to 78.7% in Cycle 1 and from 31.1 to 94.7% in Cycle 2 (Fig. 4). While the average mortality for bay sites was greater than that for open coast sites in both cycles, these differences between bay and open coast sites were not statistically significant (Table 2, Fig. 4).

The biologically realistic total post-settlement mortality percentages were similar to

those of primary post-settlement mortality, with an overall mean of 65.9% in Cycle 1 and 67.2% in Cycle 2. By site, total post-settlement mortality percentages ranged from 43.9 to 81.3% in Cycle 1 and from 49.2 to 81% in Cycle 2 (Fig. 5). As with primary post-settlement mortality, no effect of topography was found (Table 3, Fig. 5). Significant site-level differences were found in both cycles, but in each case, differences were among sites of one or the other topographic group. Student-Newman-Keuls post-hoc tests could not fully resolve these differences (Table 3).

DISCUSSION

Principally, our work adds methodologically and quantitatively to the small number of studies providing estimates of early post-settlement mortality (within the first 2 d of settlement) in benthic marine invertebrates, and to the even smaller number of works on bivalves (e.g. Roegner & Mann 1995, Bownes & McQuaid 2009). Clearly, in raw form our mortality equations and related assumptions did not give realistic estimates of mortality for all sites and replicates. However, modelling changes to the primary post-settlement mortality estimates showed that by altering daily settlement, immigration rates and the correction factor to simulate non-conformance with our original assumptions, the output of the primary post-settlement mortality equation could be improved in many instances (see Table 1). These improvements demonstrate that the poor performance of the equation was probably due to one or more of the assumptions not being met. Using the equation under our original assumptions, we obtained 17 of 45 realistic values. As our modelling

Table 2. ANOVA results for primary post-settlement mortality among sites in (a) Cycle 1 ($n = 6$) and (b) Cycle 2 ($n = 4$). *Italics* denote significant p-values ($\alpha < 0.05$). SNK: Student-Newman Keuls test

Source	df	MS	F	p	F versus
(a) Cycle 1 (n = 6)					
Topography	1	1144.76	2.37	0.1986	Site (Topography)
Site (Topography)	4	483.256	0.81	0.5434	Residual
Residual	12	598.015			
Total	17				
(b) Cycle 2 (n = 4)					
Topography	1	2657.52	1.53	0.3411	Site (Topography)
Site (Topography)	2	1731.45	4.81	<i>0.0426</i>	RES
Residual	8	360.311			
Total	11				
SNK could not resolve among-site differences					

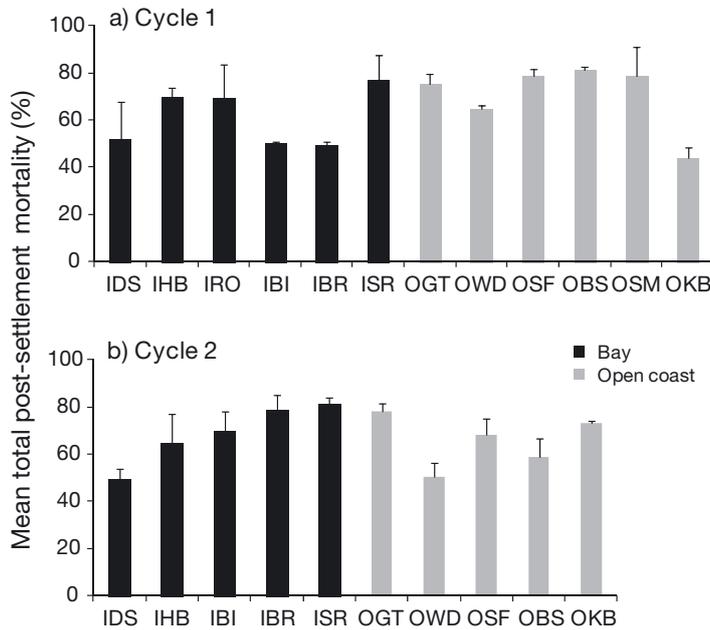


Fig. 5. Mean total post-settlement mortality percentages (+SE) calculated for sites in (a) Cycle 1 (n = 12) and (b) Cycle 2 (n = 10). Within each topographic class, sites are arranged in geographic order from west to east (see Fig. 1)

showed, however, this number can be increased (e.g. to 24 of 45, Table 1). With more accurate estimation of daily mortality parameters and immigration rates it will be possible to get many more realistic estimates from our equations.

The set of biologically realistic mortality estimates put forward in the present study are in agreement with several previous findings, including estimates for the study species (e.g. Gosselin & Qian 1997, Bownes 2005), and put post-settlement mortality within the first 24 h of settlement at between ca. 54 and 64%. These rates are, however, higher than findings for conch (20–40% within ca. 2 d, Ray & Stoner 1995) and abalone species (ca. 25–36% within 3 d, Naylor & McShane 2001). Estimates of total post-settlement mortality in the present study were also substantial (ca. 65–67%) but these agree less consistently with results from other studies. For example, while Phillips (2002, 2004) found mortality over 2 wk to be between ca. 70 and 97% for *Mytilus galloprovincialis*, Bownes & McQuaid (2009) showed a far lower range of between 0 and 30% mortality on the low shore over a 6 d period. The latter result is especially relevant to the present study as it describes mortality on the low shore, for the

same species and from the same coast examined here. It could be that these low percentages were a result of increased attraction to established weekly collectors, which was unaccounted for in their study.

While post-settlement mortality varied among sites, there was no significant effect of topography on either primary or total post-settlement mortality; meaning that post-settlement mortality was similarly substantial across bay and open coast sites. This result suggests that wave exposure and other physical factors that differ between these habitats did not have a strong effect on early mussel mortality. Nicastro et al. (2008), however, showed a topographic (bay/open coast) effect on adult mussel mortality, with greater mortality on the open coast, suggesting that differences in wave exposure and other factors can act directly on adult mortality. A possible explanation for the lack of a topographic effect on post-settlement mortality could be that the minimum ‘fatal’ level of wave exposure for settlers lies below the level of wave action commonly occurring in bays on the study coast. In other words, mussel settlers may have been equally disturbed by wave action in both habitats. Nonetheless, the lack of differences in post-settlement mortality between bay and open coast shores suggests that patterns of greater recruitment and adult abundance in bays as seen in the study area and in several other regions (Gaines & Bertness 1992, Helson & Gardner 2004, McQuaid & Phillips 2007,

Table 3. ANOVA results for total post-settlement mortality among sites in (a) Cycle 1 (n = 12) and (b) Cycle 2 (n = 10). SNK: Student-Newman-Keuls test. As in Fig. 1, site codes for bays have the prefix ‘I’; open coast sites have the prefix ‘O’. *Italics* denote significant p-values ($\alpha < 0.05$)

Source	df	MS	F	p	F versus
(a) Cycle 1					
Topography	1	755.186	1.44	0.2577	Site (Topography)
Site (Topography)	10	524.3	2.66	<i>0.0243</i>	Residual
Residual	24	197.445			
Total	35				
SNK: OBS, OSM, OSF, OGT = OWD > OKB = OWD, remainder not resolved					
(b) Cycle 2					
Topography	1	57.9118	0.14	0.7228	Site (Topography)
Site (Topography)	8	428.964	3.14	<i>0.018</i>	Residual
Residual	20	136.613			
Total	29				
SNK: ISR = IBR = IHB > IDS = IHB, remainder not resolved					

von der Meden et al. 2008, Pfaff et al. 2011) are shaped by supply-side factors, rather than early mortality. This predominance of supply-side influence is supported by findings of larval accumulation and retention within bays and upwelling shadows (Graham & Largier 1997, Wing et al. 1998, Roughan et al. 2005, Mace & Morgan 2006), as well as by the spatial consistency with which recruitment may occur across taxa (e.g. Pfaff et al. 2011). It seems then that the topography-associated pattern in the study region is set up largely during delivery and initial settlement and, being unchanged by post-settlement losses, propagates through to recruit and adult populations. Although our results support previous work identifying the importance of initial colonization patterns over post-colonization mortality in establishing mussel distributions (Hunt & Scheibling 1998a), post-settlement mortality and density effects are known to be capable of de-coupling recruitment from initial settlement in other taxa (e.g. Shanks 2009) and are still likely to be important factors, particularly over smaller spatial scales.

Effects of predation on settler mortality can be significant for epibenthic invertebrates (Osman et al. 1992, Osman & Whitlatch 1995); however, little is known about specific sources of mortality in mussel settlers under natural conditions. In terms of predation, whelks and a variety of benthic and pelagic species are known to prey on mussels as small as 2 mm, but no substantial effects of predation on smaller settlers have been documented (Hunt & Scheibling 1998b, Plass-Johnson et al. 2010). Rather, previous work on post-settlement mortality of *Perna perna* indicates that mortality increases with tidal height, suggesting abiotic sources of mortality related to increasing physiological stress (Bownes & McQuaid 2009). In agreement, while dead mussels (empty valves) were present in samples from the present study, very few had been drilled by whelks (C. E. O. von der Meden pers. obs.). Given the likelihood of abiotic causes of mortality, but the lack of a topography-related pattern in mortality (which would have suggested an effect of wave force), heat stress and desiccation stress are the most probable causes of mortality in the present study. In terms of experimental technique, the most enduring problem with studies of post-settlement mortality is the separation of settler migration from actual mortality (Gosselin & Qian 1997, Cole et al. 2000). None of the methods outlined in the introduction are able to make this separation, and the method used in the present study is no exception. Although it is argued (according to Assumption 3) that rates of migration to and from

experimental substrata are equal, under field conditions the complex dynamics of settlement behaviour and spatio-temporal variability of settlement make this assumption tenuous. This has the frustrating implication that most studies of early mortality, including the present one, could either under- or overestimate mortality depending on relative rates of emigration/immigration. This issue is particularly relevant given the potentially strong effects of emigration on final population size in organisms like mussels (Bayne 1964).

The method of out-planting calcein-stained settlers, as done by Phillips (2002, 2004), is an improvement insofar as it allows immigrant settlers to be distinguished from original settlers. The loss of stained settlers however, can still not be categorically ascribed to either mortality or emigration, and the method may not be easily practicable on a large scale. Perhaps the most viable option for future research is an adaptation of the method of Walters (1992); here silicone vacuum grease was applied to experimental plates thereby permanently attaching settling larvae. In a post-settlement mortality context this method would allow the use of mapping or photographic techniques to follow settlement and mortality at short intervals without the confounding factor of emigration. The silicone grease does not affect settlement or attachment, and has been successfully used in a recent recruitment study by Dobretsov & Wahl (2008). The application of practical methods for accurately establishing post-settlement mortality should allow future work to focus on better integrating information from all life stages.

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