VARIABILITY IN AND COUPLING OF LARVAL AVAILABILITY AND SETTLEMENT OF THE MUSSEL PERNA PERNA: A SPATIO-TEMPORAL APPROACH.

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By

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Abstract

Population dynamics of many intertidal organisms are highly influenced by the abundance and distribution of planktonic larvae in the water column and their arrival on the shore. The brown mussel, *Perna perna* was used to investigate two of the primary processes that affect population size and dynamics, larval availability and settlement, on the south coast of South Africa. *Perna perna* is a dominant species on rocky shores of the southern and eastern coasts of South Africa. It creates three-dimensional beds that provide habitats for many other species and hence promotes biodiversity.

Larval availability and settlement were examined at different spatial and temporal scales using a nested experimental design. To detect possible relationships between larval availability and settlement, the studies were simultaneous.

Two sites, 4km apart, were chosen to investigate mussel settlement patterns. Within each site, three locations (300m from each other) were selected. At each location, five artificial settler collectors were placed at approximately 20cm intervals. Collectors were replaced at a range of time intervals, from daily to seasonal, for 16 months. Each intertidal location was paired with an offshore station, 500m from the shore, where larval availability was measured. At each offshore station, three vertical hauls were collected twice a month using a plankton net. Plankton sampling lasted for 14 months and was designed to examine variability on three temporal scales: seasonal, lunar and daily.

The results showed no correlation between the distribution of larvae in the water and settlers on the shore. While larvae were abundant in the water at the start of sampling, they became very rare throughout the rest of the study at both sites and all locations. In contrast, distinct peaks of settler abundance were observed during the seasonal settlement study.

In addition to the expected, strong temporal variation that emerged from both studies at all time scales, spatial patterns of variability were also observed. While no spatial effect was detected for the larvae in the water column, there was distinct spatial variation in settlement at the location level: some locations always showed higher settlement than others. These results suggest that, on scales of hundreds of meters to kilometers, larval availability and settlement are very unpredictable in time and that differential delivery of larvae occurs from nearshore waters to the shore.

Although the effect of the state of the moon (new or full) was not significant in either study, more settlers seemed to arrive on the shore during new moon. Wind direction did not correlate significantly with settlement. However, the dropping of offshore winds and the prevalence of onshore winds, which are characteristic of summer, may be linked to the start of settlement. Nevertheless, further investigations on tidal or lunar cycles and on the influence of wind on surface currents are required to clarify the effects of moon and wind on settlement.

Contents

Alla mamma, al babbo e alle sorelle.

Acknowledgments

This section could be amongst the longest of this thesis. I apologise to the readers for some unfamiliar words…unless they know Italian and some Florentine slang!

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Chapter 1

General Introduction

“ The best way to become acquainted with a subject
is to write a book about it”

Benjamin Disraeli

Onshore marine habitats can be distinguished from offshore environments in terms of their physical stability. In particular, onshore benthic systems, including intertidal habitats, are exceptionally unstable, disturbed, and more subject to unpredictable physical changes than offshore environments (McKinney, 1986; Jablonski & Botjer, 1990).

From an evolutionary point of view, since onshore environments are highly variable at different spatial and temporal scales, one would expect selection to produce a larger diversity of marine invertebrates in onshore than in offshore systems (Jablonski & Botjer, 1990). If this was so, there should be higher concentrations of onshore genera and families, but this does not seem to apply to all invertebrate taxa (Jablonski and Botjer 1990).

Much debate about the evolution of marine invertebrate species has focused around the onshore-offshore pattern of species' distribution. The suggestion is that many higher taxa originated in onshore environments, followed by offshore expansion with many onshore-originated groups moving into offshore areas. Finally, some higher taxa that moved offshore would have permanently settled in the offshore environments (Jablonski & Botjer, 1990).

Support for this theory comes from McKinney (1986). He suggests that more onshore species exhibit a progenetic reproductive strategy, in which organisms become sexually mature while still morphologically juvenile. The organisms exhibiting this strategy also exhibit shorter generation times and smaller body size. These characteristics make onshore organisms more prone to rapid evolution and more resistant to extinction than offshore ones (McKinney, 1986).

The onshore-offshore theory could then be applied to intertidal environments that, being part of the onshore systems, can also be considered to be highly unpredictable and variable, especially with respect to disturbance by physical factors. Species living intertidally could be better adapted to physical stresses. Indeed, globally, rocky shore habitats have been colonised and exploited by an extremely resistant, successful and ancient invertebrate group; the mytilid mussels (Berry, 1978; Suchanek, 1985).

Mussels occupy a relatively narrow band in the intertidal. Physical stresses such as salinity, temperature, wave action, wave-generated storms and exposure to air limit the distribution of mussels intertidally and affect the mortality of adults considerably. For example, temperature and the risk of desiccation limit the distribution of mussels at the upper tidal levels (Suchanek, 1985). Subtidally, their distribution is often limited by predators, such as starfish, dogwhelks and subtidal fish (Seed, 1976; Suchanek, 1985; Dame, 1996). Also, the availability of suitable substrata can be a limiting factor for the distribution of mussels. Even within the narrow band that is inhabitable for mussels, they are subjected to the stresses induced by intra- and interspecific competition for space (Seed, 1976; Suchanek, 1985; Roughgarden *et al*., 1988; Dame, 1996).

Despite the factors that delimit the distribution of the mytilid family, mussels are still among the dominant organisms on rocky shores and provide ideal habitats and refuges for many other invertebrate species (Harris *et al*., 1998). Indeed, mussel beds form a packed matrix that decreases the effect of wave action, temperature and sunlight (Suchanek, 1985). Mussel assemblages increase the amount of habitat suitable for other organisms by modifying the substratum itself. In addition, mussel presence increases water retention and the deposition of feacal and pseudofeacal material (Suchanek, 1985; Seed *et al*., 2000). As a result, mussels can be considered to be " physical ecosystem engineers" (Jones *et al*., 1997), able both to modify and to maintain the environment and to create the perfect habitat for many other species (Jones *et al*., 1997; Seed *et al*., 2000).

The success of mussels can partly be found in their evolutionary history. Indeed, the neotenous retention of the post-larval byssus, used for attachment during metamorphosis, facilitated their expansion from soft sediments to hard substrata, by allowing a firm fastening of mussels to rocks (Suchanek, 1985; Seed *et al*., 2000). The heteromyarian shell shape, which is characteristic of mussels, also provides an ideal form for space occupancy and offers an excellent design for gregarious behaviour, for living in high densities and, again, for strong attachment to the substratum (Runnegar & Pojeta, 1985; Suchanek, 1985; Morton, 1992; Guinez & Castilla, 1999; Seed *et al*., 2000).

Mussels have solved the problem of survival under the unstable and variable conditions typical of the intertidal zone by securing themselves to the rocks and by being gregarious.

Mussel beds are extremely dynamic communities with levels of productivity that can be compared to those of tropical rain forests and kelp beds (Suchanek, 1985; in Seed *et al*., 2000). The genus *Mytilus* is found in the cold waters of both the northern and southern hemispheres, while in the tropics and subtropics *Mytilus* is replaced by the genera *Perna* and *Septifer* (Suchanek, 1985; Seed *et al*., 2000). *Perna perna* is the dominant species on rocky shores of the south, east and west coasts of Africa (Berry, 1978; Van Erkom Schurink & Griffiths, 1990) and has become invasive in the Gulf of Mexico (Hicks *et al*., 2001).

The great success of mussels on the shore would appear to come in spite of the limitations of their reproductive strategies and larval histories. Mussels are highly sedentary and, in contrast to barnacles, exhibit external fertilization. This involves an enormous amount of energy loss or wastage. With the release of sperm and eggs into the water, most gametes are lost and, even after external fertilization has occurred, almost 99% of zygotes die (Thorson, 1950; Bayne, 1976; Suchanek, 1985; Underwood & Fairweather, 1989; Levitan, 1995; Underwood & Keough, 2001). In addition, dispersal of larvae in the water column results in a further loss of potential recruits to the final adult populations (Underwood & Keough, 2001).

The energetic investment required by mussels for their reproductive strategy appears to be very high. Although the total energy available to each individual is limited, mussels seem to spend lots of energy in three processes that involve great loss: external fertilization, long-lived larval dispersal and final settlement (Todd, 1985). However, life histories are generally phylogenetically constrained and can evolve only in certain directions. Also organisms can only select amongst a limited set of habitats. Therefore, the alternatives for habitat selection and reproductive strategy are not unlimited (Todd, 1985; Scheltema, 1986; Begon *et al*., 1990).

The colonisation of the highly disturbed rocky shore environment and reproduction through external fertilization, with its attendant risks, along with the associated risks of dispersal and settlement, may be the best evolutionary option that mussels have had.

Classical models of life history selection, such as r-K selection and bet-hedging theories, refer to parameters like adult size, size and abundance of offspring, mortality and habitat stability. None of these models alone explains the life histories and reproductive strategies of mussels. Some of the characteristics exhibited by mussels match one model and some another (Calow, 1983; Todd, 1985; Begon *et al*., 1990; Halliday, 1993).

The r-model is characterised by small adult size, short life span, early reproductive maturity, semelparity and production of many small offspring whose survival depends on generally unpredictable environmental factors. The K-model is characterised by the production of few, high quality offspring with a high input of adult energy for parental care (Todd, 1985; Begon *et al*., 1990; Hallam, 1990; Halliday, 1993).

The bet-hedging model is based on relative levels of mortality and on the predictability of adult and juvenile mortality. r-selected species are those in which adult mortality is more unpredictable than that of the juveniles. K-selected species are organisms for which juvenile mortality is more unpredictable (Todd, 1985).

A third view emphasises habitat type and size of adult organisms. Size-beneficial habitats favour species in which large adult size is beneficial for the survival of the species (Begon *et al*., 1990; Lincoln *et al*., 1997).

Mussel life histories show a mixture of patterns from all three of these models. The life span of these organisms is relatively long: *Perna perna* can live up to 5-6 years (McQuaid & Lindsay, 2000) and the adults can reach up to 20 cm in length (pers.obs.), though this is very rare. These characteristics agree with both size-beneficial habitat and K-models. The large adult size could be beneficial against predators as well as in intraspecific competition for space, and may help in resisting and surviving the severe conditions experienced on the shore (Begon *et al*., 1990). Individuals of *P. perna* can also be considered precocious as they can become sexually mature when they exceed only 20mm in length (Lasiak, 1986; Phillips, 1994; Ndizpa, 2002; Lawrie & McQuaid, in prep). This is a characteristic that agrees with size-neutral habitats and r-models. Also, in accord with the K-model is the fact that mussels are highly iteroparous and can reproduce frequently

(Berry, 1978; Ndizpa, 2002; Lawrie & McQuaid, in prep). Yet, they also produce huge quantities of gametes leading to the production of many, small pelagic larvae, a characteristic of an r-model species (Thorson, 1950).

The habitat in which these mussels occur is highly unpredictable (distinctive of r-selected species for the bet hedging model and for the classic r-model), due to strong wave action, currents and the harsh conditions typical of the intertidal zone, especially in subtropical regions (Suchanek, 1985). Although the mortality of larvae is very high, it is more predictable than the mortality of adults, which are more subject to the unstable conditions of the shore (Thorson, 1950; Widdows, 1991). This last pattern is considered characteristic of K-selected species in the bet-hedging model.

The production of large quantities of gametes is certainly a great advantage for mussels, considering the precarious conditions the pelagic larvae have to withstand during dispersal (Underwood & Keough, 2001). The small size of larvae could also be considered an advantage for the survival of the larva itself. In fact, it is believed that mussel larvae are transported like passive particles in the water column (McQuaid & Phillips, 2000). Their characteristic small size allows them to float and to be carried in the water instead of sinking to the sea bottom, away from rich food areas or settlement sites (Underwood & Keough, 2001).

During dispersal, pelagic larvae can potentially be carried over large distances (Scheltema, 1986; Widdows, 1991; McQuaid & Phillips, 2000; Poulin *et al*., 2002). Larval transport in the water, associated with settlement on the shore of competent larvae, allows the colonisation of empty patches of rocks or even new areas and regions (Todd, 1985; Underwood & Fairweather, 1989). Dispersal and settlement strongly shape population structure and, therefore, affect the entire community associated with the adult beds (Gaines & Bertness, 1992; Morgan, 2001).

Since larvae remain in the water column for quite some time (the average period for a mussel larva to become competent is between 3 and 4 weeks; Bayne, 1965), their transport and survival chances are exceptionally variable. In fact, the nature of dispersal and settlement can make larval and settler abundances particularly variable, as they are strongly influenced by unpredictable environmental conditions (Underwood & Fairweather, 1989; Gaines and Bertness, 1992; Bertness *et al.*, 1996; Eckman, 1996). Thus, the availability and settlement of mussel larvae are highly variable because of mussel reproductive strategies and also because of the general characteristics of the environment in which the larvae are found. Variability of larval availability and of settlement intensifies even further when different spatial and temporal scales of the environmental and biological components are considered (Gaines & Bertness, 1993; Jenkins *et al.*, 2000; Morgan, 2001; Drouin *et al.*, 2002). In particular, those abiotic factors that influence the transport and delivery of larvae can vary considerably, both at fine and large temporal and spatial scales. Of these factors, currents are among the most important (Eckman, 1996). The variability of currents changes dramatically at different spatial and temporal scales and, therefore, so too will the transport and delivery of larvae onto the shore (Pineda, 2000). For example, the transport and survival of larvae is mainly determined by large offshore oceanographic currents, then, closer to the shore, by finer-scale, more local currents and, finally, by the microhydrodynamics typical of inshore coastal areas, which characterise the movement of water around rocks and crevices (Underwood & Fairweather, 1989; Pineda, 2000).

On the east coast of South Africa, the warm Agulhas current is the major offshore current (Hunter, 1981; Goschen & Schumann 1990; Goschen & Schumann, 1994). It flows parallel to the coast at a maximum speed of 2.5 m*sec^{-1}, roughly following the 200m isobath and diverging from the coast towards the south-west. Approaching Port Elizabeth it flows approximately 70km offshore (Fig.1, chapter 2), but it can show dramatic onshore/offshore meanders, on occasions coming virtually into the intertidal (Goschen & Schumann, 1990; Goschen & Schumann, 1994). This current is likely to influence the survival and settlement of mussel larvae dramatically as it undergoes a major offshore retroflection south of South Africa (Goschen & Schumann 1990) and larvae taken up by the Agulhas current would have virtually no chance of returning to the shore.

The Agulhas is an oceanically forced western boundary current. Inshore currents on this coast (between the Agulhas front and the coastline) are wind forced, vary at different spatial scales and are affected by factors acting at both large and fine temporal scales. For example, current speed and direction can vary on an hourly, daily, tidal and seasonal basis. The different temporal scales on which currents can change are greatly influenced by the action of the wind. It has often been observed that the direction and speed of the main coastal winds directly influence inshore currents, and consequently, the transport and delivery of pelagic invertebrate larvae (Pineda, 1991; Gaines and Bertness, 1992; Bertness *et al.*, 1996; McQuaid & Phillips, 2000; Poulin *et al*., 2002). The impact of wind action on water decreases with increasing water depth, and therefore the maximum and most direct effect of this environmental feature occurs at the water surface (Field *et al*., 1980).

Currents, and the other physical factors influencing water movement at different spatial and temporal scales, are closely related to the transport of invertebrate larvae and to their delivery to the shore. Seasonal and geographical differences in the reproductive strategies selected by many sedentary benthic invertebrates also influence larval dispersal and settlement at different spatial and temporal scales. These physical and biological factors act jointly to enhance variability in the structuring and maintenance of adult populations and, therefore, the intertidal community as a whole (Connell, 1985; Lewin, 1986; Young, 1987; Underwood & Fairweather, 1989; Minchinton & Scheibling, 1991; Grosberg & Levitan, 1992; Alexander & Roughgarden, 1996; Pulfrich, 1996; Morgan, 2001; Underwood & Keough, 2001; Jeffery, 2003).

However, biological processes alone, such as larval supply and settlement, can also be considered as key aspects for an understanding of population dynamics. In particular, settlement can be referred to as the bridging factor, the connecting path between two crucial phases of many sedentary marine invertebrates' life histories: dispersal and permanent recruitment to adult populations. Hence, this study focused on the variability of larval supply and settlement of mussels at different spatial and temporal scales, with specific attention to possible coupling between these two processes.

Structure of the thesis

This thesis is divided into 6 main chapters.

Chapter 1 forms the general introduction, highlighting the causes of variability in the availability of mussel larvae and their settlement. Chapter 2 discusses spatial and temporal variability in the availability of *Perna perna* larvae, monitored in the water column at two sites along the south east coast of South Africa. Chapter 3 deals with spatial and temporal variability of *Perna* settlement, measured at the same sites. Chapter 4 considers possible relationships between larval availability and settlement of *Perna perna*. Here the data presented in Chapters 2 and 3 are combined and analysed together. Chapter 5 examines the particular effect of one abiotic factor, the wind, on the transport and delivery of *Perna perna* larvae onto the shore. The settlement data analysed during this study are the same as those collected for the survey in Chapter 3. Finally, Chapter 6 concludes the thesis with a general discussion.

Chapter 2

Nearshore larval availability of *Perna perna* on the south coast of South Africa: spatial and temporal variability

"All truths are easy to understand once they are discovered;
the point is to discover them"
Galileo Galilei

Introduction

Intertidal organisms with external fertilization produce pelagic larvae that are forced to disperse in the water column. The amount of time that larvae spend in the water before they come back on the shore varies, depending on the species, on the larval stages involved and on the duration of each stage (Widdows, 1991). However, regardless of the duration of the typical larval period, some larvae can also delay metamorphosis and therefore the time of arrival on the shore, if conditions are not optimal for the larvae to settle (Thorson, 1950; Bayne, 1965; Sprung, 1984; Lutz & Kennish, 1992; Seed & Suchanek, 1992; Marshall *et al.*, 2003). Of course, spending longer in the water could increase the risk of mortality due to predation (Widdows, 1991), even though it has been recently suggested that pelagic larvae undergo little planktonic predation (Baldwin *et al.*, 1995; Tamburri & Zimmer-Faust, 1996; Johnson & Shanks, 1997; Johnson & Brink, 1998; Johnson, 1999; Johnson & Shanks, 2003).

The postponement of settlement can be induced by cues that influence the behaviour of larvae. For example, some larvae can achieve postponement by actively controlling their position in the water column (Mileikovsky, 1973; Norkko *et al.*, 2001; Satumanatpan & Keough, 2001) and thus, the timing of arrival on the substratum. It has been observed that environmental cues drive the settlement of megalops crab larvae to the substrata in estuaries, after fertilization of eggs has occurred in open coastal waters. Different cues stimulate an endogenous response resulting in active searching for the substratum. In general, the nature of these cues is not always known, but in a few cases it has been shown that re-entering an estuary is triggered by a specific phase of the tide or cues from the light cycle (Paula *et al*., 2001; Ross, 2001; Tankersley *et al*., 2002; Forward *et al*., 2003).

Tankersley *et al*. (2002) have observed that, at night, megalops larvae keep swimming during flooding tides and settle just before low tide. In this case, the behavioural response is triggered by changes in salinity. However, light also inhibits larval transport: more larvae arrive in the estuary during morning than evening low tides.

In general, offshore dispersal of larvae is a very complex and variable process. It is unclear from which adult populations larvae originate, and it seems highly improbable that they will settle back into the population from which gametes were spawned (Minchinton & Scheibling, 1991), especially if the dispersal phase is relatively long (Graham & Sebens, 1996). In fact, larvae can potentially be transported over large distances, both offshore and along the coast (Van Dover *et al*., 2001; Widdows, 1991; Poulin *et al*., 2002). Therefore, it is extremely difficult to follow the dispersal phases of marine invertebrates. However, although difficult, it is important to assess dispersal in a quantitative way, in order to understand population dynamics and model predictions of final population sizes (Wiens *et al*., 1993; Eckman, 1996; Norkko *et al*. 2001). The present study has addressed mussel dispersal without considering the potential offshore spreading of larvae, trying instead to estimate the abundances of mussel larvae just off the coast, in nearshore waters. In fact, it is in the inshore waters that larvae are likely to be found in higher densities (Pulfrich, 1997). Thus, this study was focused on the abundance of larvae that are available for settlement, in other words, close to the shore and to potential settlement sites.

However, the availability of larvae in inshore waters is closely related to dispersal, therefore those processes that affect dispersal can influence the abundance of larvae too. As already mentioned, larval behaviour can change the position of organisms in the water and, consequently, the availability of larvae. The output of gametes can also be variable and can affect the spatial distribution of larvae in the water (Hurlbut, 1991; Jenkins *et al*., 1999) and finally determine spatial variability of sedentary organisms (Reed *et al*., 2000). Indeed, gamete availability may vary, depending on the seasonality of the reproductive cycle, and can change if gametes are released in different amounts in different areas, due to the patchy distribution of adults on the shore. Physical parameters, such as hydrodynamics, water temperature, internal waves and tides, geography and topography can also affect the abundance and transport of larvae in the water (Gaines *et al*., 1985; Roughgarden et al., 1987; Pineda, 1991; Gaines & Bertness, 1992; Bertness *et al*., 1996; Jenkins *et al*., 1999; Drouin *et al*., 2002; Poulin *et al*., 2002). All these factors operate on larval availability at different spatial and temporal scales (Eckman, 1996; Stoner & Davis, 1997; Dobretsov & Miron, 2001; Poulin *et al*., 2002). In fact, the variability of larval abundance can be influenced by fine or large-scale spatial patterns of currents: large-scale oceanographic currents or fine-scale local hydrodynamics (Stoner & Davis, 1997; Satumanatpan & Keough, 2001). It also happens that different quantities of larvae are found at different geographic sites, hundreds of kilometers apart, or even at different positions on the same shore, separated by distances of only hundreds of meters (Satumanatpan & Keough, 2001). Ross (2001) showed that barnacle larvae from a temperate mangrove forest are transported in a passive way at large spatial scales and that their distribution is influenced by hydrodynamics. However, it has been suggested that, at finer spatial scales, marine larval settlement can be driven by active larval behaviour, which can influence habitat selection, settlement and the final distribution of adult organisms (Meadows & Campbell, 1972; Strathman *et al*., 1981).

In addition to spatial variability, temporal variation should also be considered when exploring larval availability in the water column (Beukema *et al*., 2001; Lipcius & Stockhausen, 2002). In fact, in such a variable system, the abundance of larvae collected from the water column at a particular place, can change dramatically with time (Pulfrich, 1997; Stoner & Davis, 1997; Ross, 2001; Poulin *et al.*, 2002; pers obs). Larval abundance can differ on very fine temporal scales. For example, patches of larvae can be found at one place at a specific time, but not seconds or minutes later (pers. obs.). But also, availability of larvae can change on a seasonal scale: because of a seasonal reproductive cycle, or even seasonal patterns of water currents (Van Dover *et al*., 2001).

Because of the extraordinary variability and patchiness of organisms in this system (McQuaid & Phillips, 2000; Natunewicz & Epifanio, 2001; Zeidberg & Hammer, 2002), not many studies have examined larval dispersal and availability in a quantitative way (Norkko *et al*. 2001). The present study has tried to quantify larvae of *Perna perna* in the water column and is one of very few studies to inspect larval availability in nearshore waters, i.e. just behind the surf zone. In fact, most studies on zooplankton abundance, even those done in coastal waters, have traditionally considered sampling areas that are at least kilometers offshore.

Finally, there are very few investigations that have simultaneously considered spatial and temporal variability in the abundance of zooplankton in the water, especially abundances of mussel larvae. The present survey examines the variability of *Perna perna* abundance, considering both spatial and temporal variability at different scales.

Materials & Methods

Study sites: The study was conducted between March 2000 and April 2001 at two sites, chosen near Kenton-on-Sea (33° 41'S, 26° 40'E), on the south coast of South Africa (Fig. 1). The sites were in Kenton: Middle Beach and High Rocks, which lies about 3km east of Middle Beach (Figs 2 & 3).

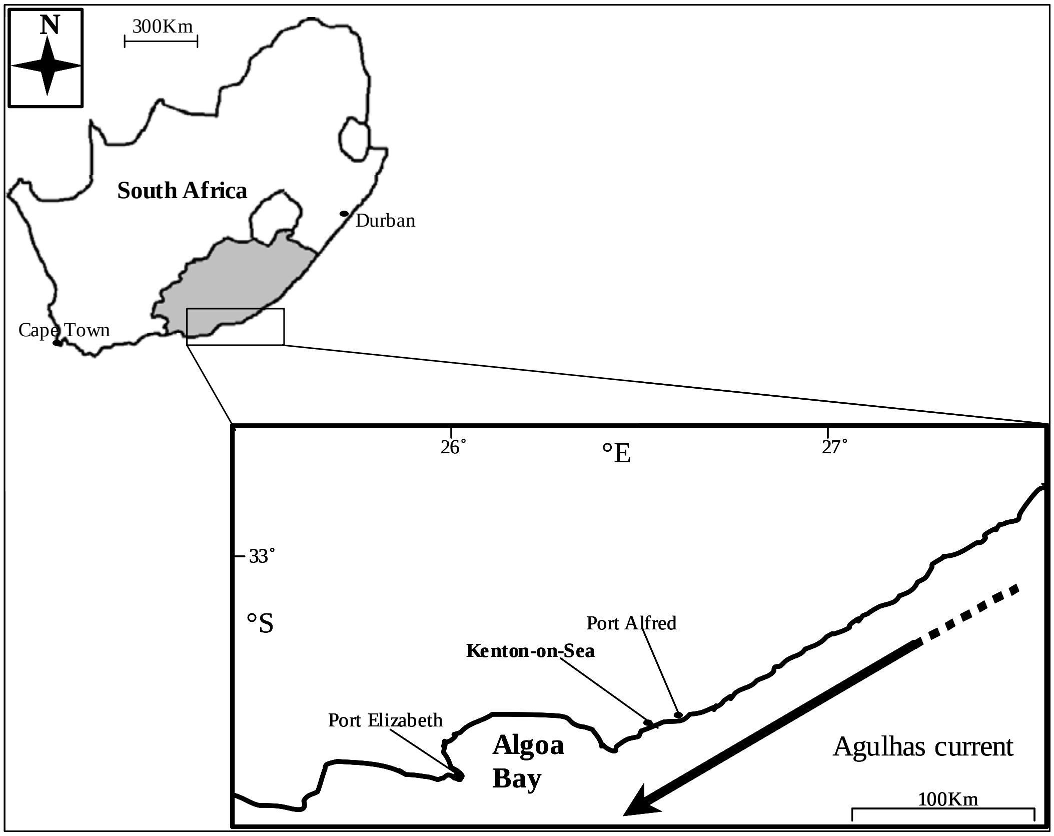

Fig.1 Map of South Africa and study area.

Fig. 2 Sampling site: Middle Beach in Kenton (KE).

Fig. 3 Sampling site: High Rocks (HR).

Sampling was conducted at offshore sites that were opposite intertidal sites on rocky shores formed of Aeolian dune rock and exposed to similar levels of wave action. Wave exposure was estimated during each of the four seasons by measuring weight loss of 3 cement balls attached to the rocks at each intertidal location for two days. Larval availability in the nearshore water column was investigated, approximately 500 meters offshore, at KE (Kenton) and HR (High Rocks), using a nested approach. At each site, three locations were identified, about three hundred meters apart (Fig. 4).

Sampling at the offshore locations was done just behind the surf zone. The distance from the shore changed with tide level and sea conditions, but was always within 500 meters of the shore.

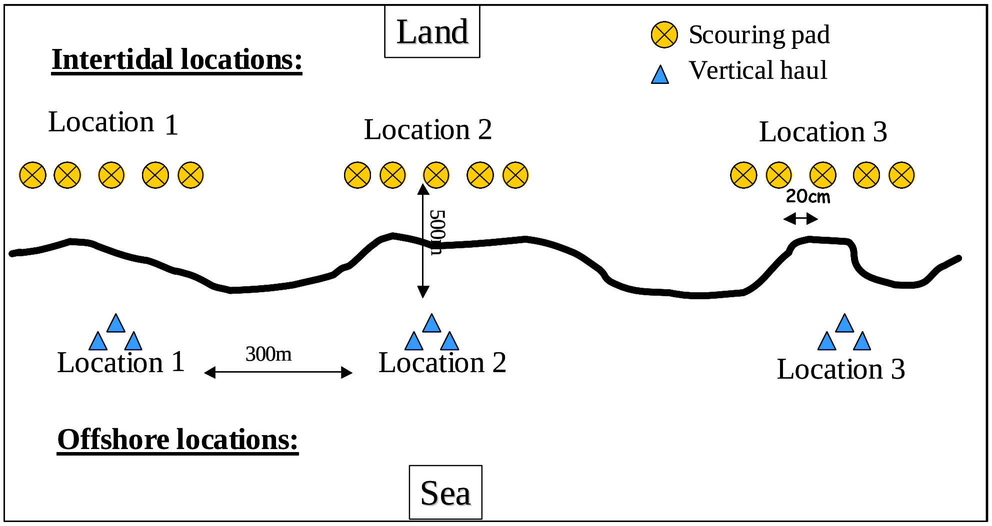

Fig. 4 Experimental design for the offshore larval availability study. One site only (detailed description in text).

<u>Larval availability</u>: Abundance of *Perna perna* larvae in the water column was examined by collecting plankton samples just behind the surf zone. Due to possible larval stratification within the water column (McQuaid & Phillips, 2000), sampling was done by

means of vertical hauls using a plankton net. The net had a mesh of 80µm and a mouth of 30X30cm; it was pulled from approximately 1m above the seabed (to minimise sand collection) to the surface at a rate of about $0.5m*s^{-1}$. The depth of the hauls ranged between 7 and 10 meters. The volume of water filtered was calculated from the area of the mouth of the net and the depth of the haul.

At each location, three replicate plankton samples were taken (Fig. 4) and wind direction, speed and time of collection were recorded. The position of each location was determined during every sampling event using GPS (Global Positioning System).

The samples were preserved in a solution of 40% formalin and 60% sea water and returned to the laboratory for analysis. Samples often contained considerable amounts of coarse sand, with particles approximately the same size as larvae. To make the identification of larvae easier (Garland & Zimmer, 2002), samples were stained with a few drops of 0.38% Rose Bengal (Acid red, C.I.45440) in 95% alcohol, which stains only organic material. The contents of each sample were examined under a dissecting microscope and *Perna perna* larvae were identified (Fig. 5), counted and measured under 25X magnification to the nearest 0.05mm using an eyepiece graticule (1mm = 25units). All measurements were transformed to µm for analysis. Since most of the time the number of larvae did not vary amongst replicates, only two out of the three replicates were processed and used for statistical analysis.

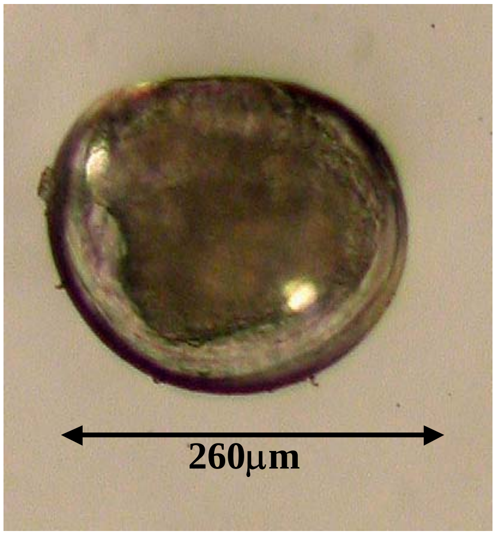

Fig.5 *Perna perna* larva collected from the water column

Periodicity: Temporal variation in larval abundance was inspected at different scales, from fine to relatively coarse.

There were three main studies: 1) seasonal; 2) lunar; 3) daily (two separate studies in 2000 and 2001).

1) Seasonal study. Large-scale temporal variation was examined so that information on abundance of larvae due to seasonality could be obtained. For this purpose, sampling continued throughout the year from March 2000 to April 2001 (14 months), with a total of 23 plankton collections. The reduced number of sampling (23 collections instead of 28) was due to bad weather conditions, when plankton sampling could not be performed. During this time larval availability was examined by sampling fortnightly, as close as possible to spring tide. Thus, plankton sampling was done for 14 consecutive months, twice a month: one collection was at new moon spring tide and the other at full moon spring tide.

2) Lunar study. In order to monitor the possible effect of lunar phase on larval availability, a subset of data from the seasonal study was analysed. Because of missing samples, for the lunar study, the data set needed to be balanced and reduced. Therefore, fewer sampling events (16) were used here than for the seasonal study.

3) Daily 2000 and 2001 studies. In order to detect fine-scale temporal variability, daily plankton collection was performed during two independent periods: the first from 20th of March to 10th of April 2000 (21 days), the second from 6th of February to 11th of March 2001 (34 days). From previous studies, February, March and April (late austral summer) are expected to be months of high larval availability and settlement for *Perna perna* (Lawrie & McQuaid, in prep). During these two cycles, plankton collection was carried out every day except when sea conditions were too dangerous (approximately 25% of total number of days).

Statistical analysis. All statistical analyses were performed using the software package Statistica 6. All data were analysed using parametric tests and since the experimental design was balanced, analysis of variance (ANOVA) was chosen to test the hypotheses.

Three separate analyses were performed to investigate the different temporal scales of variability (seasonal, lunar and daily). The data set varied for each analysis, therefore independent analyses for each temporal scale could be run.

Mixed model, nested ANOVA was used to examine scales of variation due to temporal and spatial factors. All independent variables and the possible interactions among factors were considered to assign variability. Location was nested within site and crossed by time. The spatial factors were treated as random, since the chosen sites were "ecological replicates" of the nearshore area and were close enough to allow simultaneous collection of data. Likewise location was treated as a random factor, nested within site (Zar, 1984). In the seasonal study, the temporal factor, date, was treated as fixed. This choice was made because, although some sampling collections were missed due to bad weather conditions, sampling was initially planned for regular fortnightly collections, which would have allowed the detection of variability of larval availability due to seasonal differences. In the two daily studies (daily 2000 and daily 2001), the temporal factor day was treated as random. The daily studies were important to investigate variation in larval abundance at fine temporal scales (day), but the actual day of sampling was of no interest. An additional analysis was run to look at the effects of the phase of the moon (full or new moon) on the availability of *Perna* larvae in the water. In this case, the factor moon was treated as fixed and crossed by date, site and location (nested in site). The ANOVA tables for random and mixed models were made manually following Zar (1984) in order to calculate the degrees of freedom, mean squares and F-ratios.

All types of interactions for the different studies are summarised in Table 1. Normality was examined using the Kolmogorov-Smirnov test and homogeneity of variances determined using Cochran’s test. When the homogeneity assumptions could not be satisfied, the dependent variable was logarithmically transformed (Winer, 1971; Lindman, 1974; Sokal & Rohlf, 1981; Zar, 1984; Underwood, 1997). For the larval availability study no post-hoc test was run because the significant interactions observed in most of the Anova tables would have made the post-hoc tables difficult to interpret.

Table 1 Summary of analyses of variance used for the different studies on availability of *Perna perna* larvae in the water. Independent variables, interactions within factors, type of effect and transformation choice are listed for each study.

ANALYSIS	EFFECT (Random/Fixed)	TRANSFORMATION
1) Seasonal study: variation of larval abundance due to season		Log transformation, Log (X+1)
Site	Random	
Location (Site)	Random	
Date	Fixed	
Date *Site	Random	
Date *Location (Site)	Random	
2) Lunar study: variation of larval abundance due to lunar phase		Log transformation, Log (X+1)
Site	Random	
Location (Site)	Random	
Date	Fixed	
Moon	Fixed	
Date * Moon	Fixed	
Date *Site	Random	
Moon*Site	Random	
Date *Location (Site)	Random	
Moon*Location (Site)	Random	
Date *Moon*Site	Random	
Date *Moon*Location (Site)	Random	
3) Daily study, analysed separately for 2000 & 2001: variation of larval abundance due to day of collection		2000-Log transformation, Log (X+1) 2001-NOT transformed
Site	Random	
Location (Site)	Random	
Day	Random	
Day*Site	Random	
Day* Location (Site)	Random	

Results

The size of *Perna perna* collected 500 meters offshore varied between 200µm and 1.3mm (one individual). However, the size range was heavily dominated (99.87%) by the 200 – 400µm size class (Fig.6). This was the class used for the analysis of larval abundance in the water column at all three temporal scales: seasonal, lunar and daily. This size class almost overlapped the size range of animals collected during the settlement study and was therefore considered to represent the size of competent larvae, still in the water column, but ready to settle on intertidal substrata. In addition, in most cases, the morphology of these larvae was very similar to that of individuals collected during the settlement study (see chapter 3).

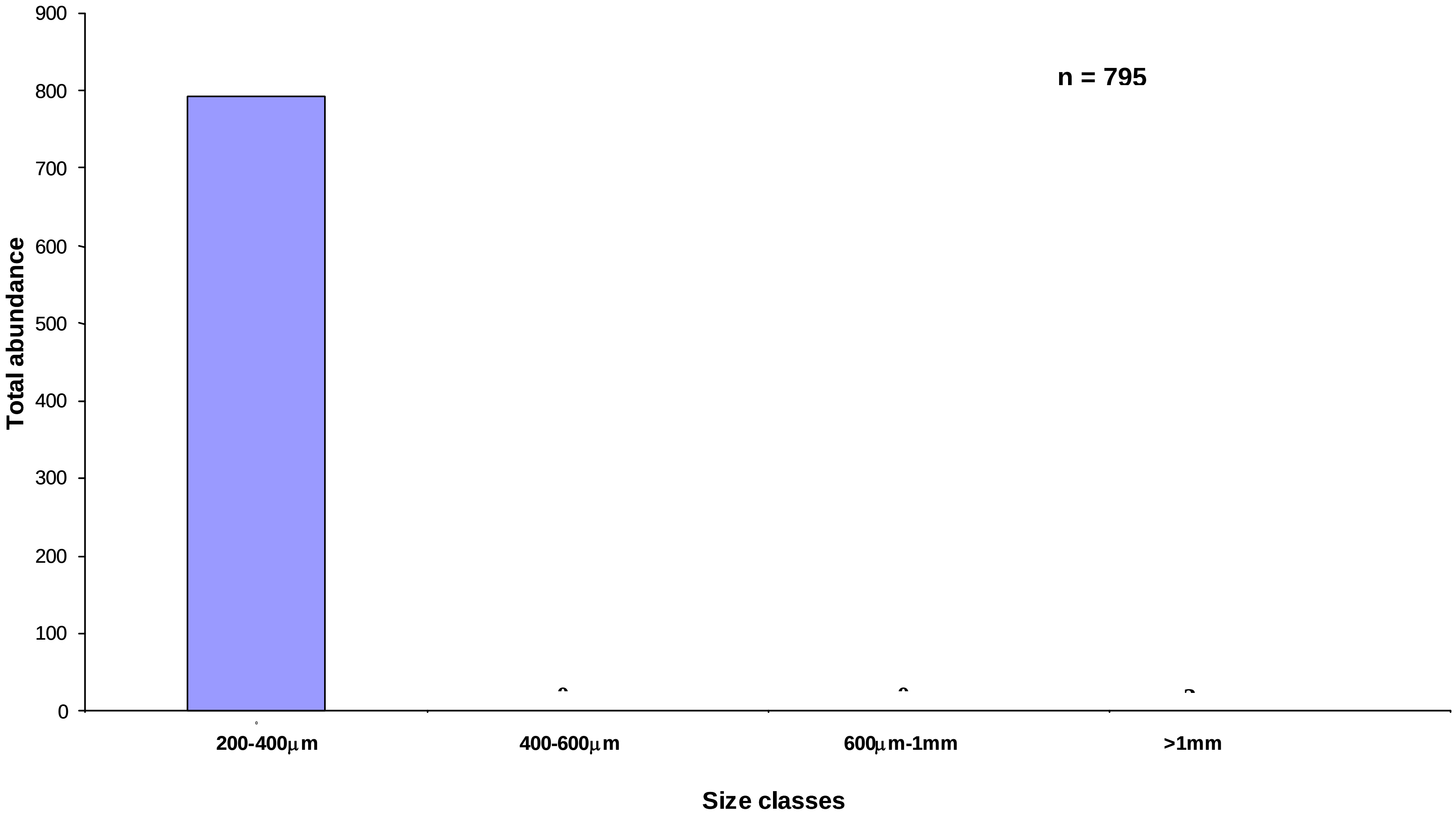

Fig. 6 Size distribution of *Perna perna* in the water column

1) Seasonal study

The water column was sampled twice a month, around spring tides, for 14 months from March 2000 to April 2001. Seasonal collections showed peaks in larval distribution at the beginning of the sampling period, on very specific occasions: the first two dates of collection (20th of March and 3rd of April 2000). For the rest of the study, larvae were extremely rare. This was observed at both sites, High Rocks (HR) and Kenton (KE) (Fig. 7). In particular, larvae were abundant at HR on the first sampling day, 20th of March, reaching mean values up to 307.14 ± 4.51 larvae per cubic meter at HR location "D" (HR D) (Fig. 7).

Variances of all the data were found to be homogeneous after logarithmic transformation using Cochran's test for homogeneity ($p > 0.05$). The ANOVA table for the seasonal study showed that neither site nor location, nested within site, affected the variability of larval abundance significantly ($p > 0.05$ in both cases; Table 2). Nevertheless, there were strong significant interactions between date and location and date and site ($p < 0.00001$). Temporal patterns of variability in larval abundance differed drastically among different sites and different locations. Although site itself did not have a significant effect on the number of larvae in the water, histograms of cumulated larvae per site and location (Figs.8 & 9) indicate obvious differences between HR and KE, with total values at HR more than three times greater than at KE. Possibly, the effect of site was masked by the low numbers of larvae collected throughout the rest of the study and the high temporal variability (effect of date, $p < 0.00001$). However, when an ANOVA was run only on those dates with high numbers of larvae, site did not have a significant effect.

Finally, even though the main factor location did not have a significant effect on the abundance of larvae, the histograms of total cumulated larvae per location showed that total numbers of larvae differed strongly between locations. HR D and HR E had total values an order of magnitude higher than the others (Fig.9).

Table 2 ANOVA for seasonal study. df = degrees of freedom; MS = Mean Square; F = F-Ratio; p = probability value; n.s. = p > 0.05; **** = p < 0.00001

	df	MS	F	p
Date	22	9.07101	49.83076	****
Site	1	0.49264	5.38565	n.s.
Location (Site)	4	0.09147	0.502	n.s.
Date*Site	22	1.63829	3.82697	****
Date*Location	88	0.42809	2.35169	****
Residual	138	0.18204		

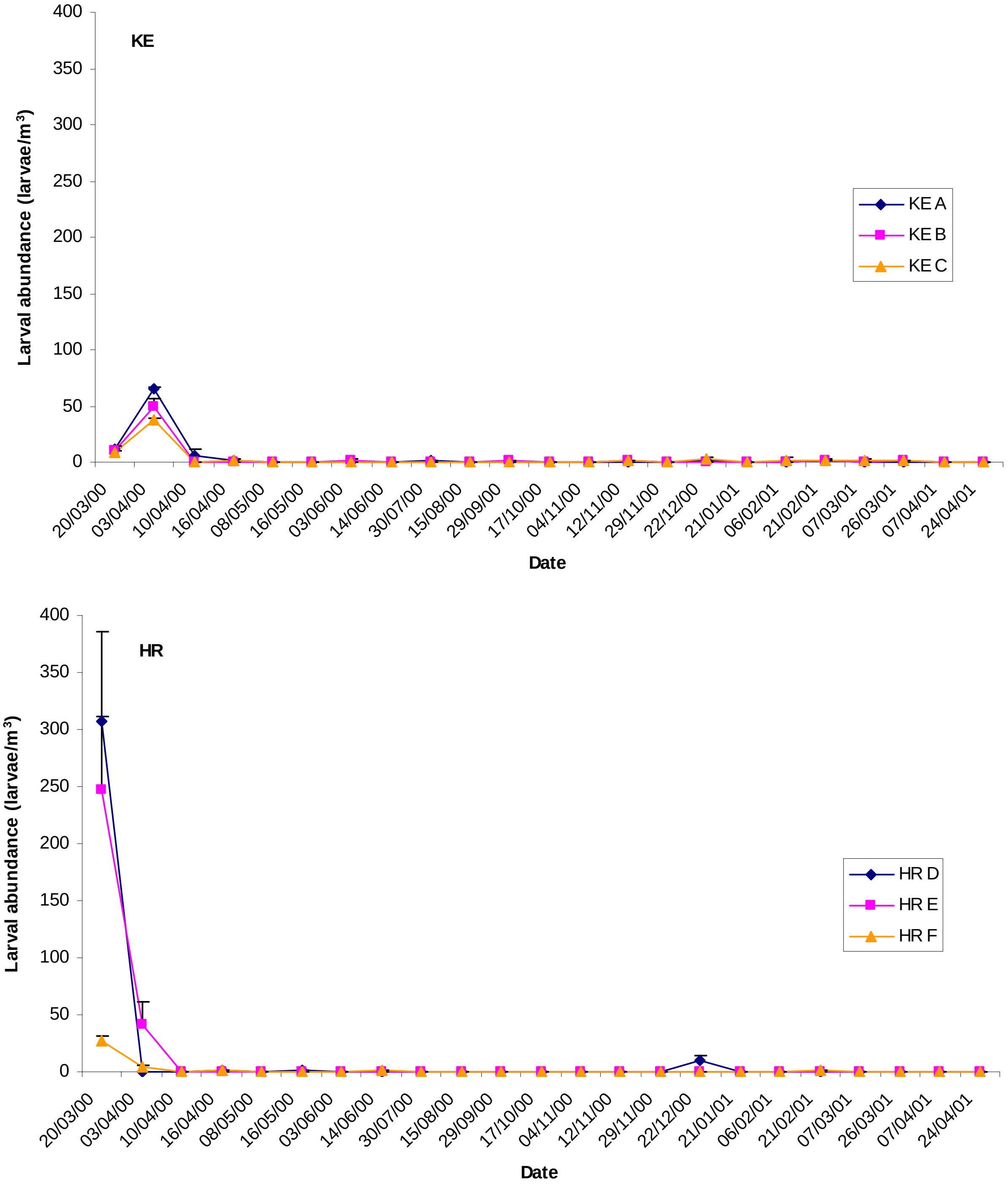

Fig. 7 Seasonal larval availability at KE (locations A, B, C) and HR (locations D, E, F). Points indicate mean values and error bars show standard errors (SE).

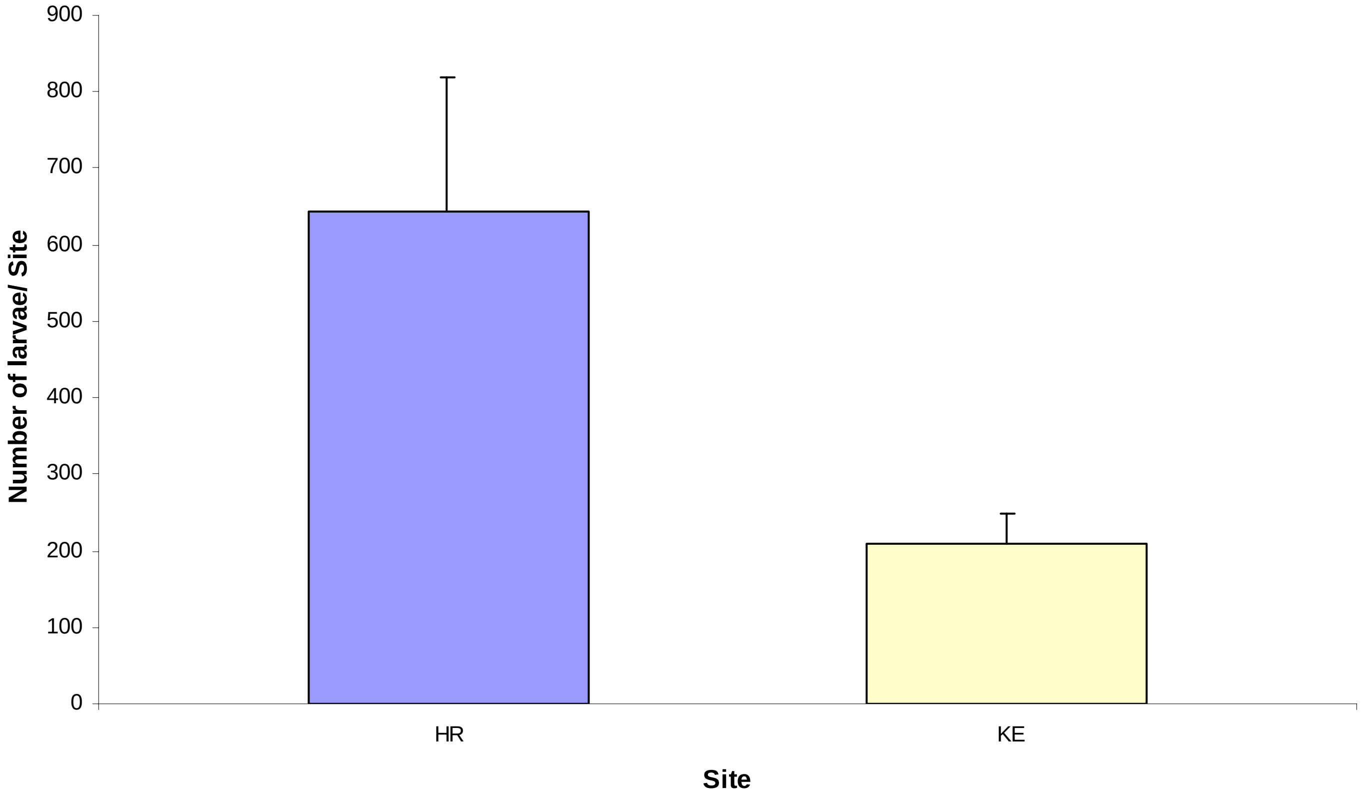

Fig. 8 Larval availability for the seasonal study - cumulated means for site (HR and KE). Error bars show standard errors (SE).

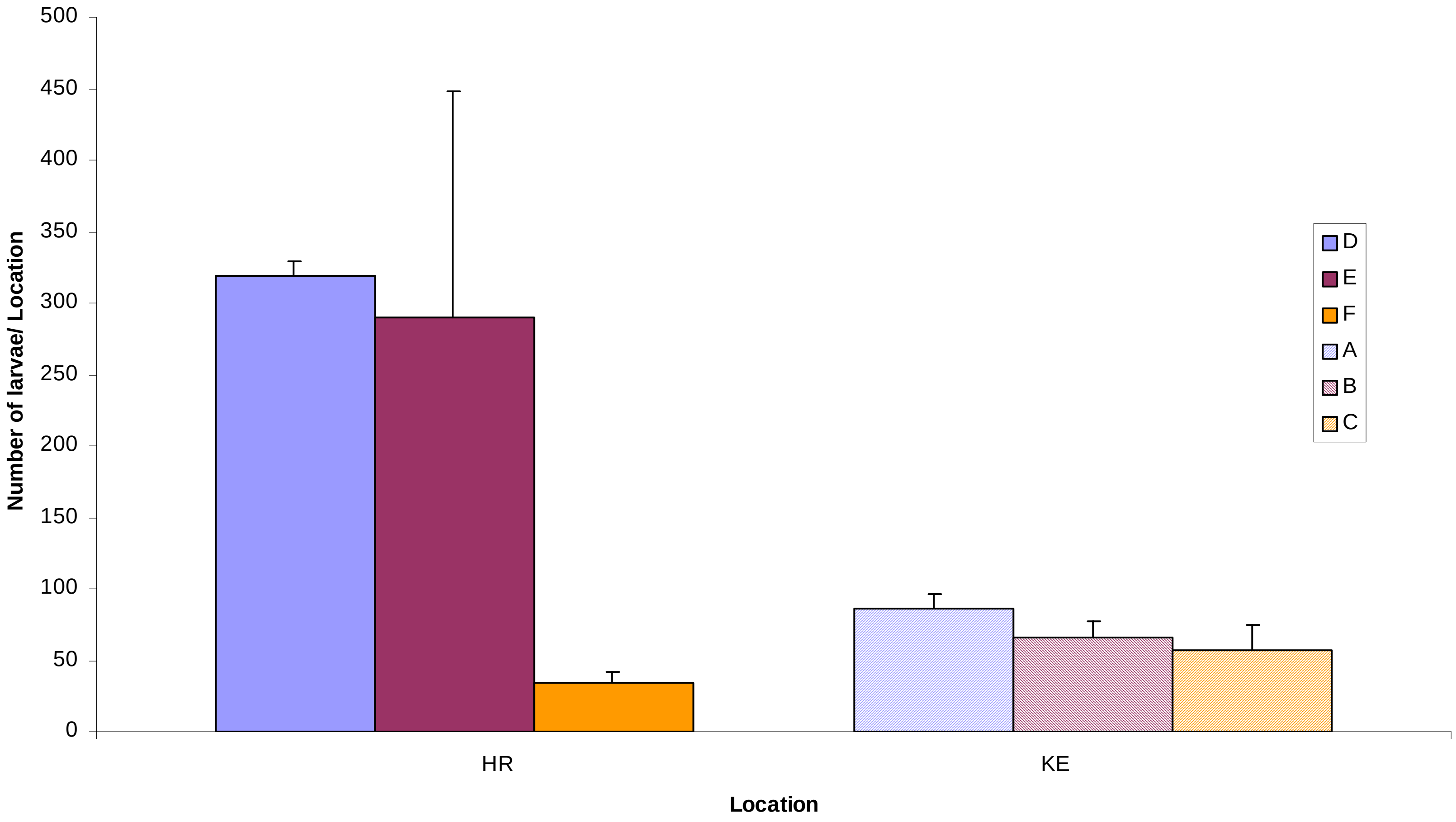

Fig. 9 Larval availability for the seasonal study at HR and KE - cumulated means for location (A, B, C, D, E, F). Error bars show standard errors (SE).

2) Lunar study

The data used for this study were logarithmically transformed and homogeneity of transformed data was confirmed by with Cochran's test ($p > 0.05$). The lunar study was performed by sampling the water column at new and full moon for 14 months, to investigate the possible influence of the phase of the moon on larval availability. This was difficult to assess because there were only two events when larvae were found in the samples and each event showed a peak at only one of the two sites. On 20th of March 2000, a full moon period, many larvae were collected at HR (Fig. 10). On the other hand, the 3rd of April 2000 was a new moon period and this was the day when most larvae were found at KE (Fig. 11).

The results of the ANOVA (Table 3) showed that date significantly influenced the variability of larvae in the water ($p < 0.00001$). Moon, the two spatial factors, site and location and the interaction between date and site did not show any significant effect on the abundance of *Perna* ($p > 0.05$.). Two other significant sources of variability were the interactions of the temporal variables, moon and date, with site and also with location ($p < 0.00001$, for both interactions). Thus, the effect of the moon differed among dates and locations and also between sites (Figs. 10 & 11).

The interaction between moon and site also showed a significant effect on the variability of larvae in the water ($p < 0.05$).

Table 3 ANOVA for lunar study. df = degrees of freedom; MS = Mean Square; F = F- Ratio; p = probability value; n.s. = p > 0.05; * = p < 0.05; **** = p < 0.00001;

	df	MS	F	p
Date	7	27.21100	599.452	****
Moon	1	0.71348	0.136	n.s.
Site	1	0.01748	0.156	n.s.
Location (Site)	4	0.11219	0.618	n.s.
Date*Moon	7	0.31761	0.0775	n.s.
Date*Site	7	0.04539	0.2510	n.s.
Date*Location	28	0.18087	0.9957	n.s.
Moon*Site	1	5.24715	18.7296	*****
Moon*Location	4	0.28015	1.5423	n.s.
Date*Moon*Site	7	4.09789	7.2726	********
Date*Moon*Location	28	0.56347	3.1021	********
Error	96	0.18164		

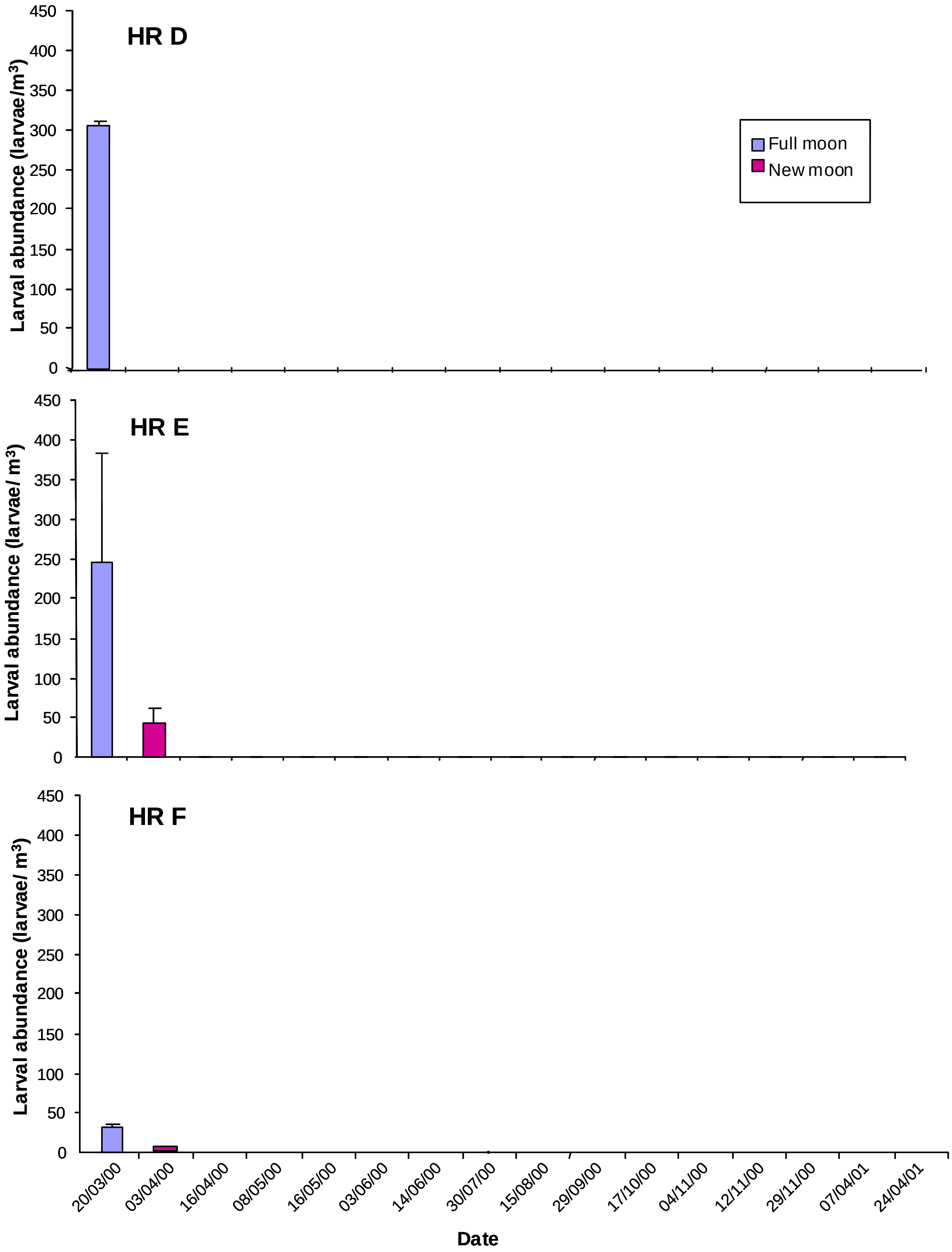

Fig. 10 Lunar larval availability at HR. Bars indicate means at full and new moon. Error bars show standard errors (SE)

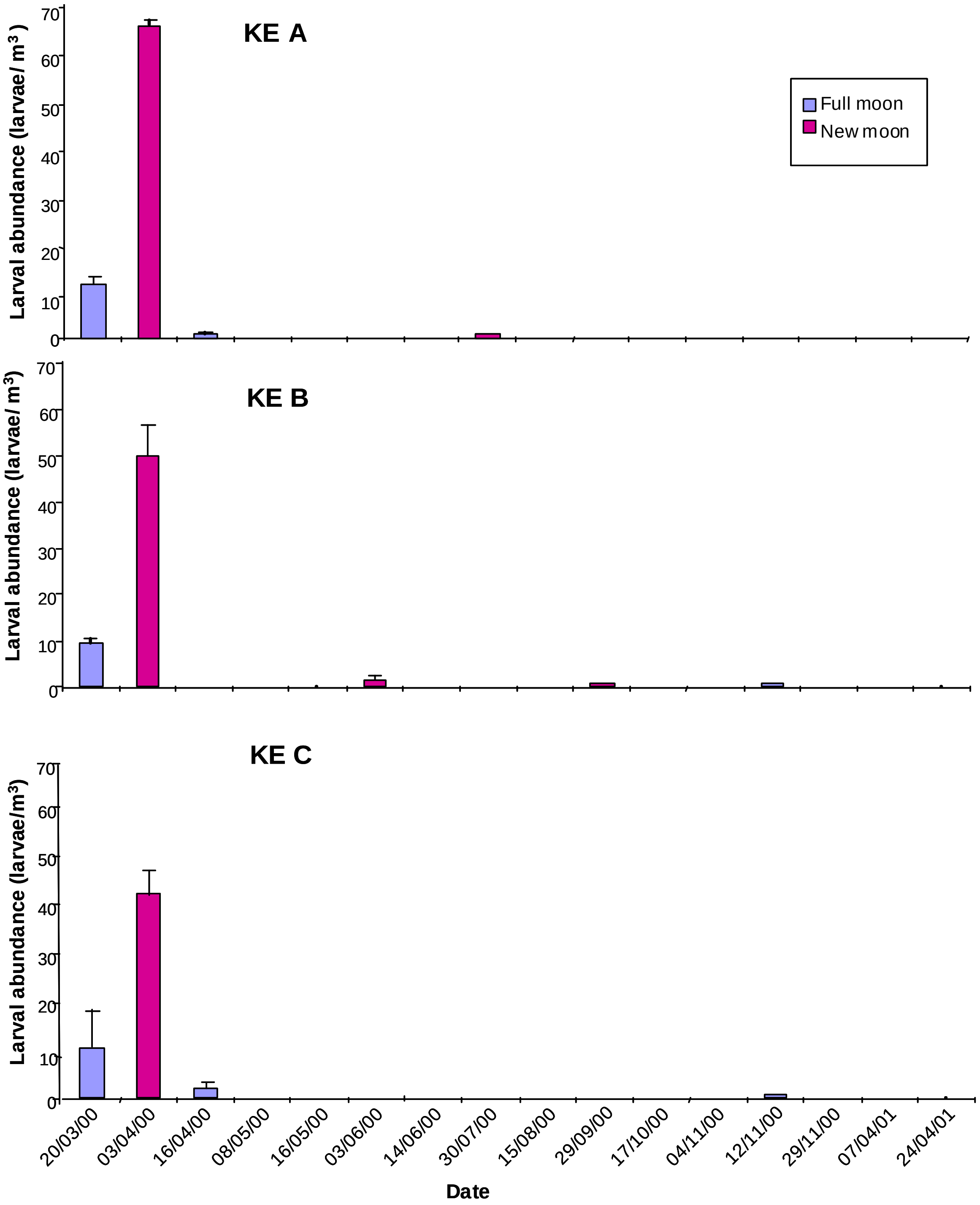

Fig. 11 Lunar larval availability at each location at KE. Bars indicate means at full and new moon. Error bars show standard errors (SE).

3) Daily study, 2000

Fine temporal scale variability of larvae in the water was investigated through two sessions of daily sampling, in 2000 and 2001.

The data from the 2000 study were logarithmically transformed and homogeneity of transformation was confirmed using Cochran's test ($p > 0.05$).

The numbers of larvae found in the water during the 2000 daily sampling (20th March to 10th April) varied considerably from one day to the next, at both sites (Fig. 12).

Two-way nested ANOVA showed that the variation in larval abundance from daily collections was significantly affected by three factors: day, the interaction between day and site and the interaction between day and location ($p < 0.00001$ in all three cases). The other main factors, location nested in site and site alone did not have significant effects on larval distribution ($p > 0.05$; Table 4). Again, the lack of site and location effect was possibly due to very low values on most days. Time proved again to be an important factor, influencing the availability of larvae in the water column on a daily basis; in fact, the number of larvae could change drastically, often from one day to the next, at both sites and some locations. There were also clear differences in larval abundance between one site and the other on the same sampling day and between one location and another at the same site (Fig. 12). Hence, the significant interaction between day and site and also between day and location ($p < 0.00001$). For example on the 20th of March and on the 24th of March on average, many more larvae were collected at HR rather than at KE (Fig. 12).

Differences in larval availability between sites and locations are also represented in two cumulative graphs for site and location (Figs. 13 & 14).

Table 4 ANOVA for daily 2000 study. df = degrees of freedom; MS = Mean Square; F = F-Ratio; p = probability value; n.s. = p > 0.05; **** = p < 0.00001

	df	MS	F	p
Day	16	15.7708	4.7789	****
Site	1	2.3344	1.7858	n.s.
Location (Site)	4	1.3072	0.3939	n.s.
Day*Site	16	3.3001	3.4414	****
Day*Location	64	0.9589	2.4113	****
Error	102	0.3977		

The interaction between day and location was significant. However, all days when the number of larvae was high, showed peaks at all locations simultaneously, with the single exception of the 24 March 2000 at KE. Thus, the interaction between day and location reflects the weak spike on 24 March and also the fact that some locations showed strong peaks, while some weak and others none on the same day.

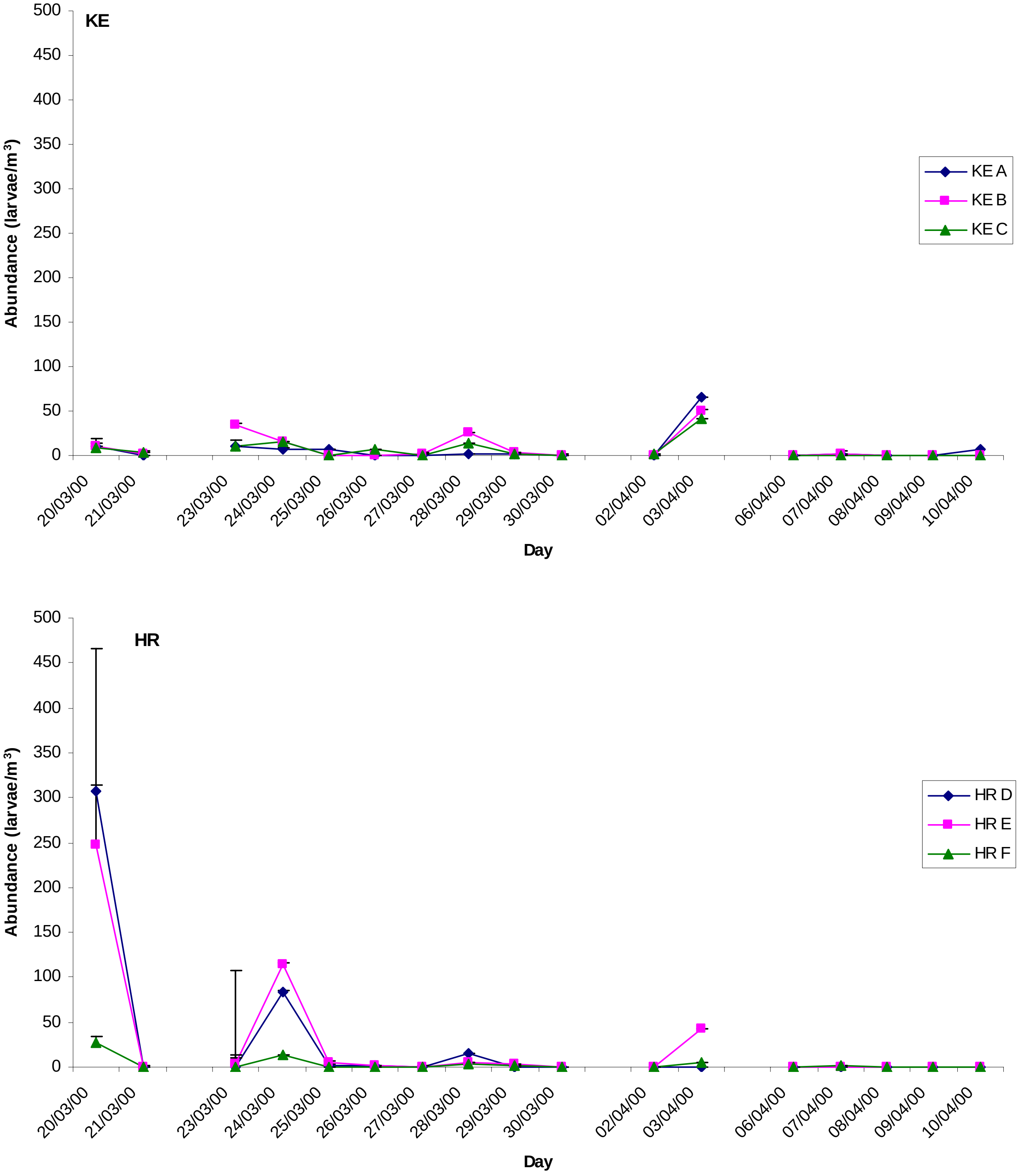

Fig. 12 Daily 2000 larval availability at KE (locations A, B, C) and HR (locations D, E, F). Points indicate mean values and error bars show standard errors (SE).

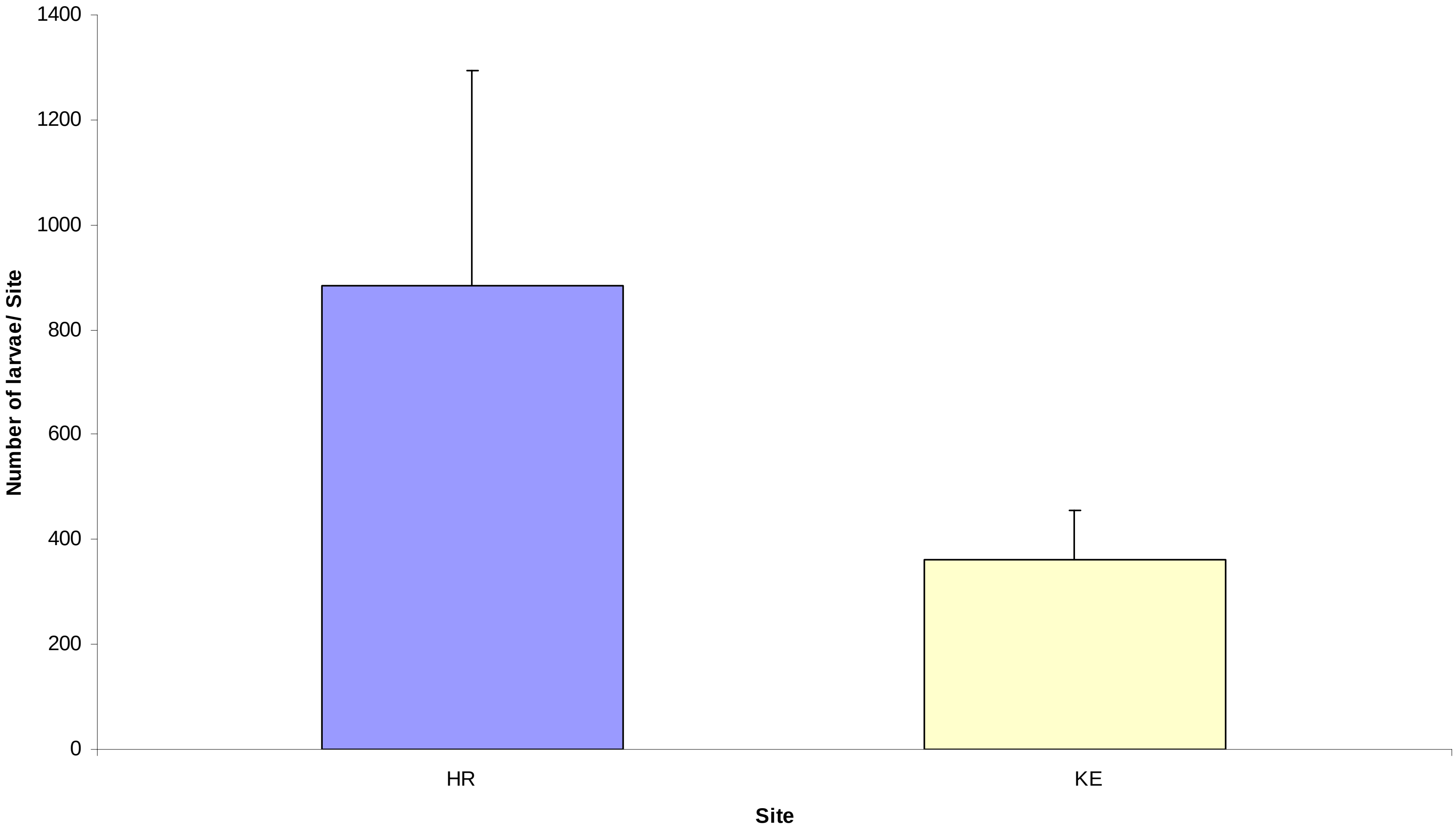

Fig. 13 Larval availability for Daily 2000 study - cumulated means for each site (HR and KE). Error bars show standard errors (SE).

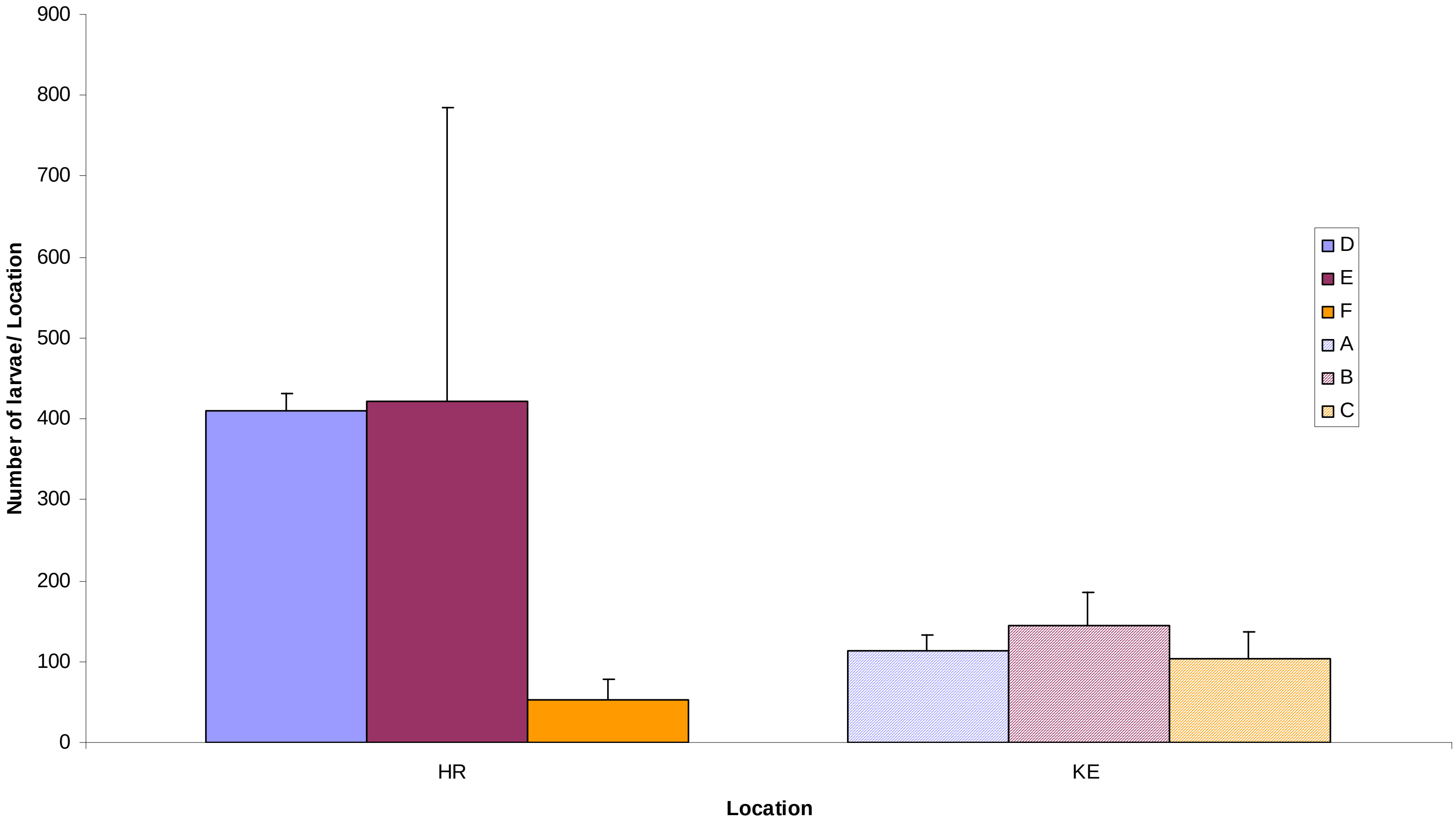

Fig. 14 Larval availability for Daily 2000 study at HR and KE - cumulated means for each location. Error bars show standard errors (SE)

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3') Daily study, 2001

Cochran's test indicated that the raw data exhibited homogeneity of variances (p >0.05) and thus the daily 2001 data were analysed without transformation.

The processing of samples collected during the 2001 daily collection, from 7th February to 9th March, revealed generally small numbers of larvae at both sites and all locations. However, there was extreme variability in abundances on a daily scale (Fig. 15). For instance, on the 20th and 25th of February, there were peaks at HR, but the numbers dropped to zero on each of the following days (Fig. 15). The same scenario occurred at KE, with larvae showing peaks on several occasions, with numbers falling to zero on the subsequent days (Fig. 15). Differences in larval availability between sites and amongst locations are represented in two cumulative graphs for site and location (Figs. 16 & 17).

In contrast to the results obtained from the 2000 daily study, the only factor that showed a significant effect in the 2001 analysis was day. The factors site, location nested within site, and all possible interactions did not show significant effects (p > 0.05) (Table 5).

Table 5 ANOVA for daily 2001 study. df = degrees of freedom; MS = Mean Square; F = F-Ratio; p = probability value; n.s. = p > 0.05; **** = p < 0.00001

	df	MS	F	p
Day	21	13.32968	4.2980	****
Site	1	0.36292	0.0798	n.s.
Location (Site)	4	4.54464	1.4653	n.s.
Day*Site	21	3.10130	0.8964	n.s.
Day*Location	84	3.45956	1.2855	n.s.
Error	132	2.69105		

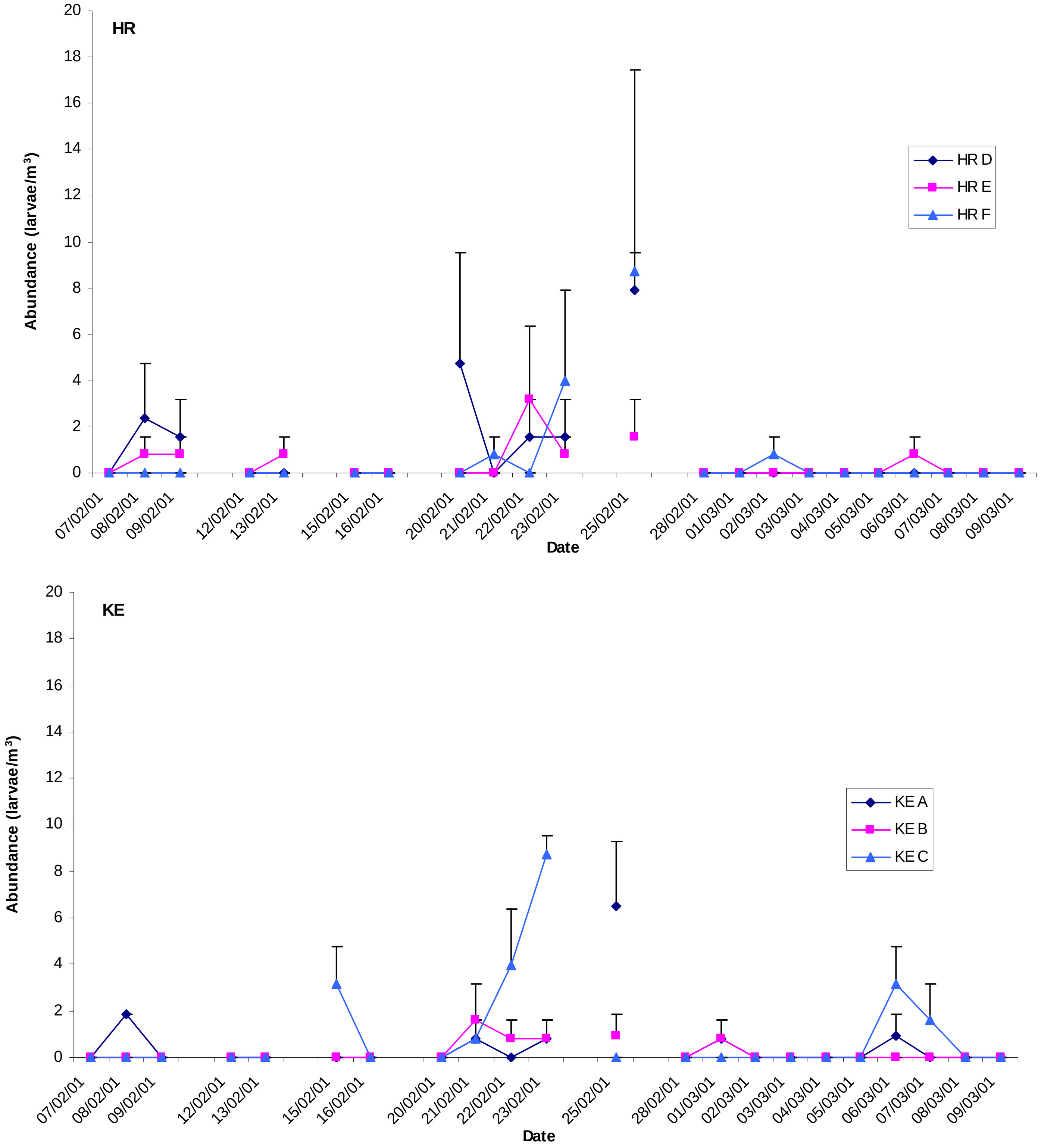


Fig. 15 Daily 2001 larval availability at KE (locations A, B, C) and HR (locations D, E, F). Points indicate mean values and error bars show standard errors (SE).

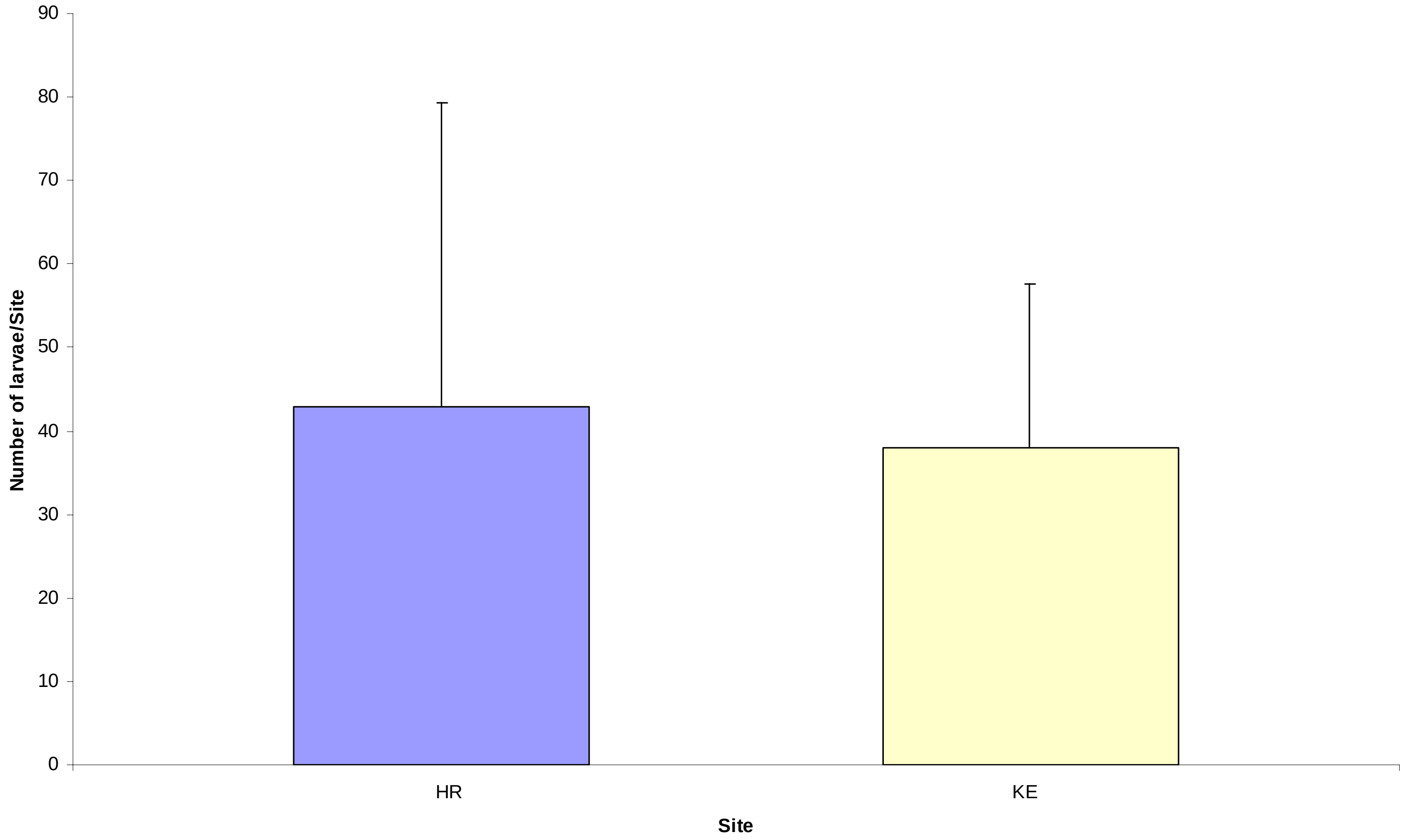


Fig. 16 Larval availability for Daily 2001 study - cumulated means for each site (HR and KE). Error bars show standard errors (SE).

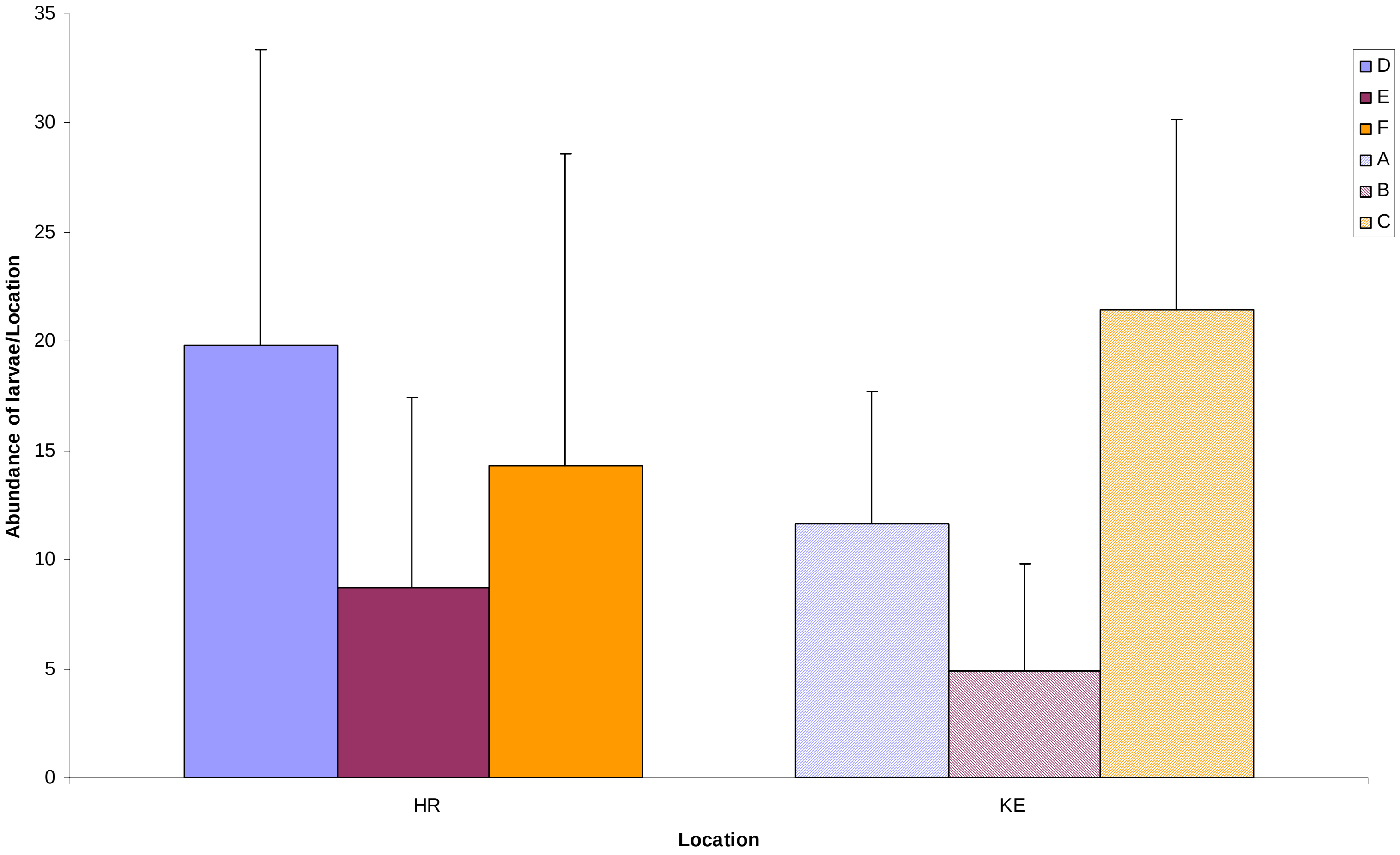


Fig. 17 Larval availability for Daily 2001 study at HR and KE - cumulated means for each location. Error bars show standard errors (SE)

Summmary of larval availability results

Time was the only factor that affected the variability of larvae in the water column, in all studies. Interactions between time and site also significantly affected the variability of larvae in the water column on seasonal and daily (2000 only) scales. Finally, the interactions between date, moon and site and the interaction between date, moon and location were also very strong components of variability for the lunar study. The main factors site, location and moon did not have significant effects at any temporal scale. All the main sources of variation for larval abundance in the nearshore waters are summarised in Table 6 and will be discussed in detail in the following section.

In general, none of the spatial variables alone, site nor location, had a significant effect in any of the analyses, but both interacted significantly with the temporal components in most analyses. This indicates that temporal patterns of larval abundances in the water column differed between site and amongst locations at all the three scales examined (season, moon, day), with no prevailing geographical or spatial effect.

Table 6 Larval availability. Summary of effects of the independent variables and interactions in different studies of larval abundance using nested analysis of variance. NS= not significant; **** = $p < 0.00001$; ** = $p < 0.05$; -- = not applicable.

	SEASONAL STUDY	LUNAR STUDY	DAILY 2000 STUDY	DAILY 2001 STUDY
Site	NS	NS	NS	NS
Location	NS	NS	NS	NS
Time	****	****	****	****
Time*Site	****	NS	****	NS
Time*Location	****	NS	****	NS
Moon	--	NS	--	--
Time*Moon	--	NS	--	--
Moon*Site	--	**	--	--
Moon*Location	--	NS	--	--
Time*Moon*Site	--	****	--	--
Time*Moon*Location	--	****	--	--

Discussion

Size and abundance

Bayne (1964) proposed a theory of primary-secondary settlement of mussels, suggesting that initial settlement occurs on filamentous algae, followed, after a period of growth, by detachment and secondary occupation of the adult mussel beds. This theory has been supported by several studies (Eyster & Pechenik, 1987; Pulfrich, 1996). However, others have refuted it (Petersen, 1984; McGrath *et al.*, 1988; Lasiak & Barnard, 1995), finding mussel larvae able to settle on filamentous algae, hard substrata and adult mussel beds. In all cases the focus of study has been settlement only, with little attention being given to the size of larvae while still in the water column (Kautsky, 1982; Caceres-Martinez & Figueras, 1998). During the present study, almost all larvae collected from the water column had a maximum shell length ranging from 200 to 400µm. Larger mussels (> 400µm) occurred, but were extremely rare (0.13% of total). The 200 - 400µm size class corresponds approximately (Caceres-Martinez & Figueras, 1998; Phillips & Gaines, 2002) to the size of animals ready to settle on the rocks and the absence of bigger larvae shows that very few potential secondary settlers were collected from the water column during this study.

Studies of the abundance of planktonic stages of different intertidal invertebrates have used different techniques, including bongo nets (bivalves: Pulfrich, 1997), pumps (barnacles: Miron *et al.*, 1995; Satumanatpan & Keough, 2001; ascidians: Hurlbut, 1991; bivalves: Caceres-Martinez & Figueras, 1998), towing (bivalves: Stoner & Davis, 1997;

barnacles: Drouin *et al*., 2002; Pineda *et al*., 2002), vertical hauls (bivalves: McQuaid & Phillips, 2000; Dobretsov & Miron, 2001; Lawrie & McQuaid, in prep.) or traps specifically designed for collections integrated in time and space (bivalves: Dobretsov & Miron, 2001).

Integration of sampling, over the water column for example, could reduce the method sensitivity (Miron *et al.*, 1995), so the sampling procedure must be designed carefully with an understanding of how larvae are distributed. In fact, depending on the position of larvae in the column, one could make predictions on settlement of larvae in the benthos (Miron *et al.*, 1995). If larvae usually stay at the surface, then settlement follows tidal oscillations; if larvae are uniformly distributed in the water column, settlement decreases from low to high shore, depending on the immersion time. Finally, if larvae are usually at or near the seabed, maximum settlement should occur on the low shore (Miron *et al.*, 1995). McQuaid & Phillips (2000) found that mussel larvae, collected from the same area as the present study, are distributed randomly throughout the water column. Therefore, bottom to surface hauls seemed to be an effective and simple technique for a realistic estimation of the abundance of mussel larvae in nearshore waters. The bottom to surface sampling method excludes the possibility that some larvae could have been missed due to stratification as this procedure integrates sampling through the whole water column.

Although fecundity was not investigated during this study, Jenkins *et al*. (2000) suggest that fecundity in barnacles does not influence recruitment to the final adult population: it seems rather that it is variability during larval stages in the water that will lead to either poor or good recruitment. Variability in abundance of larvae in the water will change at large and fine temporal and spatial scales. One of the possible reasons for such variability could be the irregularity of the phytoplankton availability; phytoplankton forms the main food for larvae during the planktonic stage (Jenkins *et al*., 2000).

Spatial variability

Larval supply of marine invertebrates living intertidally can be influenced by adult abundance, hydrodynamics and larval behaviour (Hurlbut, 1991). Spatial variability in the abundance of planktonic larval stages of benthic organisms has largely been studied in order to understand the processes and the scales that affect their distribution in the water and their arrival on the shore (Pineda, 1991; McQuaid & Phillips, 2000; Dobretsov & Miron, 2001; Natunewicz & Epifanio, 2001; Norkko *et al*., 2001). Larval supply and, in some cases, behaviour, vary greatly depending on the species examined, on the geographic position of the study sites and on the different spatial scales examined (Satumanatpan & Keough, 2001). For example Satumanatpan & Keough (2001) believe that passive transport, due to oceanographic processes, regulates the distribution of barnacle larvae at large scales, while active behaviour would influence their movement at finer scales, when they are closer to the shore. Ross (2001) also describes the same type of effect at large and fine spatial scales on the distribution of barnacle larvae in a mangrove forest; however, the ability of larvae to influence their position by active behaviour is much greater in a calm habitat like mangroves, than on wave-exposed rocky shores.

Modeling of larval fish distribution also suggests the importance of passive transport due to hydrodynamics, with passive transport being enhanced by strong wind action (Jenkins *et al*., 1999).

The results of the present study show that abundance of larvae in the water column does not show strong spatial effects at the spatial scales examined during this study. The effects of site (km scale) and location (100m scale) on larval abundance were not significant. Although the number of larvae collected at HR was larger than that sampled at KE, and despite the fact that some locations presented more larvae than others, the signal provided by the spatial variables was not strong enough to give a significant spatial effect of space in the ANOVA.

This seems to support the idea that while larvae are still in the water, the processes that affect the variability of their distribution operate on scales larger than those investigated, while micro-hydrodynamics influence settlement (Pineda, 2000).. In the case of the present study, currents may affect the distribution of larvae on scales larger than a few kilometers.

Nevertheless, the spatial signal alone could have been masked for two main reasons. First, numbers of larvae were generally very low, frequently zero, and this could have hidden the effect of the spatial factors alone. Second, the spatial effect could have been expressed through the strong interactions between spatial and temporal variables. In fact, temporal patterns in larval abundance differed between sites and amongst locations.

Patchiness in the distribution of zooplankton in the water column has been attributed to several physical and biological processes such as water temperature, salinity, internal and interaction between prey and predators (Stoner & Davis, 1997; Natunewicz & Epifanio, 2001). Vertical migration would probably depend on light, gravity or food concentration; these are all factors that can change over spatio-temporal scales that are relevant to this study (Dobretsov & Miron, 2001). Some hypotheses maintain that there are offshore regions where larvae accumulate, where it would be possible to find an available pool of larvae (Pineda, 2000). But we need to consider where the larvae come from and how they would reach these pools. It is difficult to measure all the possible variables that could affect larval distribution and therefore to estimate the position, the size (Natunewicz & Epifanio, 2001) and the variability within these plankton patches. Offshore dispersal, due to large oceanographic processes, could strongly affect the distribution of larvae and transport them over long distances (Widdows, 1991; Stoner & Davis, 1997). However, physical factors (and for some species, behaviour) can control retention and trap larvae with a strong effect on the scales of dispersal (Mariani *et al*., 2000; McQuaid & Phillips, 2000; Caceres & Soluk, 2002; Drouin *et al*., 2002; Zeidberg & Hammer, 2002). VanDover *et al.* (2001) suggest that there could be a trophic explanation for local dispersal and larval retention at hydrothermal vents, as it would be advantageous for larvae to be retained locally where there are symbionts that can contribute to larval growth. Strathmann *et al*. (2002) find larval retention to be advantageous for larval survival. In fact, since the parental and juvenile habitats are very favourable, larvae would do better if retained locally rather than being dispersed over large distances (Strathmann *et al*., 2002).

Although some bivalve larvae show active swimming behaviour, controlling their position in the water (Dobretsov & Miron, 2001; Norkko *et al.*, 2001), some studies on mussel larvae and other molluscs seem to support the idea that they are transported passively, without even showing the patterns of vertical migration that barnacle larvae do (Scheltema, 1995; McQuaid & Phillips, 2000; Satumanatpan & Keough, 2001). On oceanic scales, dispersal would be limited by large-scale hydrodynamics. More locally the direction and the degree of dispersal would depend completely on hydrography, and retention within nearshore waters would be influenced mainly by changes in wind direction (McQuaid & Phillips, 2000).

From the points discussed above, there is reason to believe that larvae distributed so far offshore as to reach the Agulhas current would be lost and irrelevant, even though the present study did not consider spatial scales larger than a few kilometers along the coast and hundreds of meters offshore. Roberts (unpubl. data) has recently found that an oceanographic drifter deployed 2km offshore in this region was transported approximately 770km south to the region of the subtropical convergence in just 52 days.

Temporal variability

In addition to spatial variability, the abundance of pelagic larvae is strongly influenced by time (Beukema *et al.*, 2001; Drouin *et al.*, 2002; Poulin *et al.*, 2002). This was obvious during the present study, though the abundance of *Perna* larvae in the water column was very limited throughout almost the whole study period.

The occurrence of large peaks of larval abundance in March and April 2000 and, thereafter the almost complete absence of larvae from the water column do not agree with other studies done on the same species, either in the same area or along the same coast. In these studies it seemed that *P. perna* larvae were continuously present throughout the year, although densities fluctuated considerably (Lasiak, 1986; Lawrie & McQuaid, in prep.). These different findings could be due to geographical differences (Lasiak's study was run 400km farther east) or, more probably, temporal differences (Lasiak's study was done between 1982 and 1983 and Lawrie & Mc Quaid's between 1998 and 1999). In this study, time had a very strong influence on larval abundance, both at coarse and fine scales (from seasonal to daily observations).

The system in which larvae were found is physically extremely unpredictable at many scales and that is why the abundance of larvae changed not only seasonally, but also dramatically from day to day. For example, larvae were often abundant at a particular location on a particular day, but not the day after or before. Hence, the concept of sampling on the "right" day, at the "right" place appears to be very important. Daily sampling was the finest scale at which sampling was done, but variability in the distribution of larvae might occur at even finer temporal scales. However, in order to test variability of larval availability at fine temporal scales, abundance of larvae should be assessed with a different experimental design. Sampling in the water column should be done at much more frequent intervals than in the present study (McQuaid & Phillips, 2000).

Nevertheless, the sampling interval chosen for this investigation was intended to assess variability of larvae from month to month. In fact, although the precise day and time in which most larvae were present in the water could have been missed, the fortnightly sampling procedure should assure the collection of new larvae following potential successive spawning events. Pulfrich (1997) supports this design, considering monthly sampling of bivalve larvae to be a poor estimation of real larval densities while fortnightly sampling can track spawning events. In fact the development time for a bivalve larva varies between three to four weeks, from the appearance of the veliconch to disappearance as a pediveliger (Pulfrich, 1997). In this way, fortnightly sampling is frequent enough to identify the appearance of new larvae while also detecting the arrival of larvae from different areas.

Although a few studies have considered the possible effects of lunar or tidal cycle on the abundance and distribution of planktonic larvae offshore (Stoner & Davis, 1997; Paula *et al*., 2001; Ross, 2001; Satumanatpan & Keough, 2001), the present investigation showed that moon did not affect the densities of mussel larvae. During this study larvae were caught in abundance in nearshore waters on only two occasions: one at full moon and the other at new moon. The lack of significance of lunar effect in this study emphasises the high variability of this system where oceanographic currents and finer scale hydrodynamics seem to be the factors that most strongly influence larval availability of *P. Perna* in the water column. However, the same sampling design, applied when larvae occurred at high densities, could lead to results different from the present one.

The next chapter discusses how the effects of spatial and temporal factors that operate on larval settlement are different from those affecting larval distribution in nearshore waters.

Chapter 3

Settlement of *Perna perna* on the south coast of South Africa: spatial and temporal variability

"There are three kinds of lies: lies, damned lies,
and statistics"
Mark Twain

Introduction

Sedentary intertidal organisms with a pelagic larval stage, like mussels, can be considered as forming open populations, to distinguish them from species with closed populations, in which adults and offspring belong to the same community and where limited migration occurs across the perimeter of the population (Roughgarden *et al*., 1985). Gametes of external fertilizers are released into the water where larvae will spend some time before returning to the rocky shore. Consequently, larvae generally have little chance of returning to the adult population from which the gametes were initially released. In addition, migration among such populations takes place not over a perimeter, but through the water column, and therefore occurs on much larger spatial scales than a two dimensional area (Roughgarden *et al*., 1985).

The process that describes the return of larvae to the shore is settlement, which is considered one of the main processes that regulates the dynamics and structure of benthic populations (Roughgarden *et al*., 1988; reviewed by Underwood & Fairweather, 1989; Olafsson *et al*., 1994; Caley *et al*., 1996; Hunt & Scheibling, 1998; Balch & Scheibling, 2000; Fraschetti *et al*., 2003). The literature describes this process in several ways, but there has also been some confusion through a failure to distinguish settlement from recruitment. This ignores post-settlement mortality, which is often a very important phenomenon (Keough & Downes, 1982; Connell, 1985; Minchinton & Scheibling, 1993; Rodríguez *et al*., 1993; Gosselin & Qian, 1997; Balch & Scheibling, 2000; Jenkins *et al*., 2000; Fraschetti *et al*., 2003). Therefore, there is a need to redefine these two distinct processes, which are important determinants for the structuring of intertidal communities.

Settlement is the permanent, reversible or irreversible contact that planktonic larvae establish with the substratum (Bayne, 1964; Keough & Downes, 1982; Connell, 1985; Lasiak and Barnard, 1995; Jenkins *et al*., 2000; Fraschetti *et al*., 2003). As this contact is made the larvae may or may not go through a phase of metamorphosis (Seed & Suchanek, 1992). Poulin *et al*. (2002), consider settlement to be the transition from the planktonic larval stage to life in the benthos.

Recruitment of many sessile marine juveniles into the adult population, in general, is more difficult to define, as the difference between settlers and recruits is not as clearly defined as the simple arrival of new individuals on the shore. However, regardless of the species under examination, recruitment is generally described as the number of individuals that have recently settled and that have survived for a certain period after settlement, during which time some degree of post-settlement mortality may have occurred (Bayne, 1964; Keough & Downes, 1982; Connell, 1985; Lasiak & Barnard, 1995). Pineda (2000) defines recruitment rate as the rate at which juveniles join the population.

Most studies have used morphometric analysis to define settlement and to distinguish this factor from recruitment. In the case of bivalves, specific size classes (not always completely corresponding) have been given at which the larvae are considered to be competent for settlement (Bayne, 1964; McGrath *et al*., 1988; Widdows, 1991; Caceres-Martinez *et al*., 1993; Lasiak & Barnard, 1995; Buchanan & Babcock, 1997; Pulfrich, 1997; Hunt & Scheibling, 1998; Ramirez & Caceres-Martinez, 1999). Categorisations have been made in accordance with measurements of laboratory-reared larvae (Siddall, 1980), descriptions of larval morphology (Hanyu *et al*., 2001; Garland & Zimmer, 2002), or measurements of animals found on artificial substrata (Lasiak & Barnard, 1995).

In the present study, it is mainly settlement of *Perna perna* on the shore that has been considered. In this context settlement is described as the first arrival of larvae on the rocks, while recruits are considered to be those individuals that had gone through morphometric changes having already settled on the rocks for a period longer than two days. The two days is the minimum period that ensures no morphometric larval change and no loss of larvae from the pads. (pers. obs.; Lawrie, pers. comm.; Bownes, pers. comm.). In this study, another means of distinguishing settlers from recruits is from a description of the shell. The size and characteristics of the shells of recruits clearly differ from those of both settlers and larvae found in the water column, which are very similar (pers. obs.). Settlement is the process associated with the first encounter of the substrata and therefore, it is also the process most closely related to larval availability in the water column.

Bayne (1964) suggested a different model for the interpretation of settlement in bivalves: the primary-secondary settlement theory. His studies on mussels showed that larvae were able to attach initially to filamentous substrata like algae and then to detach from them. After being suspended again in the water the larvae were able to reattach on adult mussel beds (Bayne, 1964). Bayne considered this process an adaptive mechanism: larvae settling onto mussel beds only during secondary settlement would reduce competition between larvae and adults. This theory has been supported (Eyster & Pechenik, 1987; Pulfrich, 1996) or refuted (McGrath *et al*., 1988; Caceres-Martinez *et al*., 1993; Lasiak & Barnard, 1995) by various studies. Huxham & Richards (2003) support the theory that postlarval active substratum selection determines the final adult distribution of *Macoma baltica* and *Cerastoderma edule*. Still, many of these studies have concentrated on the definition of this process, without really considering the importance of settlement itself in determining the structure of the final adult population (Gaines & Bertness, 1992). In order to be able to make predictions about the dynamics and variability of the adult population, settlement rates should be determined and the possible causes that affect this process and its variability should be identified. Only relatively recently have investigations attempted to quantify settlement and to establish which are the factors that can influence it (Jenkins *et al*., 2000). For example, Connell (1985) proposed the idea that a quantification of settlement is necessary to determine what mechanism affects the final size of an intertidal population. He observed that variation in settlement rates affected mortality of recently settled larvae and therefore, the final size of the population. If there was generally high settlement of barnacle larvae, then post-settlement processes influenced and gave shape to the adult population. In contrast, if sparse settlement occurred, then the spatial and temporal variability of settlement itself influenced the size structure and the distribution of adults. Underwood & Denley (1984) also found that settlement of barnacles was very important in determining variability and the composition of intertidal populations when settlement rates were low. Smaller numbers of larvae occupy less space and, therefore, have more chance of surviving and so structuring the final community. Navarrete & Wieters (2000) found that at least 65% of mortality of recently settled barnacles could be attributed to the adults and their predation of larvae during low recruitment periods. However, the negative effect of adult predation on settlement disappeared during high recruitment years.

Different studies have highlighted several possible features that can influence settlement in marine and freshwater systems, and they can be either physical or biological (Delafontaine & Flemming, 1989; Pineda, 1994; Hills & Thomason, 1996; Walters & Wethey, 1996; Marsden & Lansky, 2000; Pineda, 2000; Kobak, 2001). Among the main factors that control the abundance of invertebrate larvae settling on the rocks are the effects of the hydrodynamics (Abelson & Denny, 1997; Archambault & Bourget, 1999). In addition to these physical factors, larval behaviour can have a strong impact on the arrival of larvae. Some larvae can actively swim or control their position in the water column and determine where and when to settle (Scheltema, 1974; Scheltema, 1986; Bourget, 1988; Hurlbut, 1991; Jenkins *et al*., 2000; Pineda, 2000; Tankersley *et al*., 2002). Once larvae are competent to settle, there can be some sort of cue that indicates to them the presence of the "right" substratum (Raimondi, 1988). Again, these cues can be either biotic or abiotic, such as surface chemical characteristics, biofilm formation on the rock surface, substratum orientation, sunlight, texture, magnetism and the presence of conspecifics, especially if the species considered shows gregariousness (Cooper, 1981; Stamps & Krishnan, 1990; Morse, 1991; Roberts *et al*., 1991; Hills & Thomason, 1996; Yulianda, 1998; Marsden & Lansky, 2000; Olivier *et al*., 2000; Kobak, 2001; Lau & Qian, 2001; Harder *et al*., 2002; Kingsford *et al.*, 2002; Lagersson & Høeg, 2002; Pineda *et al*., 2002).

All these factors operate at different spatial and temporal scales (Gaines & Bertness, 1992; Bertness *et al*., 1996). Therefore, the high variability frequently observed in settlement rates can be explained on the basis of variation in such features: in fact, they change at large and fine scales over time and space (Noda *et al*., 1998; Balch &

Scheibling, 2000; Jeffery & Underwood, 2000; Jenkins *et al*., 2000). In particular, variability of hydrodynamics is inevitable: large and fine-scale changes in current patterns over time and space may profoundly influence settlement processes.

However, most of the studies performed on variability of settlement have concentrated on only one aspect, the study of either spatial or temporal variation (Bayne, 1964; Rojas, 1969; Acuna, 1977; Denley & Underwood, 1979; Caceres-Martinez *et al*., 1993; Caceres-Martinez & Figueras, 1997; Balch & Scheibling, 2000). Those surveys that have investigated both forms of spatial and temporal variation, have not usually measured both large and fine scales of variability.

Thus, there is a great need for studies in which as many variables as possible are considered together, at different scales, in order to get a more complete picture of the dynamics that regulate the arrival of pelagic larvae onto the shore (Minchinton & Scheibling, 1991; Hunt & Scheibling, 1998; Jeffery & Underwood, 2000; Jenkins *et al*., 2000).

The present study is one of the few examples of the concurrent investigation of different scales of temporal and spatial variation in settlement of benthic invertebrates. Larval settlement was studied in *Perna perna*, on the south coast of South Africa. In this study, settlement rates were measured in several temporal studies, involving seasonal to daily sampling, at different spatial scales, from kilometers to hundreds of meters.

Materials and Methods

Study sites: The sites chosen for the settlement investigation were the same as those used for the larval availability study: Middle Beach in Kenton (KE) and High Rocks (HR), 3km from Kenton-on-Sea (see Figs. 2 & 3, chapter two).

For the settlement study, each intertidal site had three locations, opposite the offshore ones, approximately 300m apart, all at the same level on the low shore (Fig. 2). All locations were at the edge of wave-cut platforms of Aeolian dune rock. The six locations all experience similar exposure to wave action, mostly receiving the influence of secondary waves. In this context, secondary waves refer to waves that roll onshore, after the main wave force has been partially dissipated within the surf zone. Thus, closer inshore, waves are less energetic, due to offshore reefs that break the maximum force of the waves. Intertidal sand movement was seasonal and quite substantial on the highest level of the shore, but none of the locations was ever covered or strongly affected by sand during the study.

On the low shore, mussel beds show very patchy distribution, with areas of high and low densities. However, all the locations were chosen where adult mussel cover was densest and similar (approximately 80% cover). All locations were characterised by the presence of the foliose red alga *Gelidium pristoides*, with which mussel patches were often interspersed.

Settlement: Larval settlement on the shore was investigated from March 2000 to June 2001 at intertidal sites and locations. The arrival of *Perna perna* larvae on the shore was monitored at each location, at regular intervals, using kitchen scouring pads as artificial larva collectors. Pads were made of plastic, circular, about 10-11cm in diameter and 2 cm thick (Fig.1). Prior to use on the shore, new pads were left in sea water for one or two weeks, to develop a natural biofilm and leach any surface chemicals.

Fig. 1 Example of plastic scouring pad secured to the rocks.

Five artificial larva collectors were secured about 20cm apart at each location, using screws attached to the rocks (Fig. 2). Pads were secured to the screws and replaced regularly using three plastic cable ties (Fig. 1). Since the places where pads were attached needed to be replaced quite frequently, due to the loss of screws, the exact positions of the pads changed.

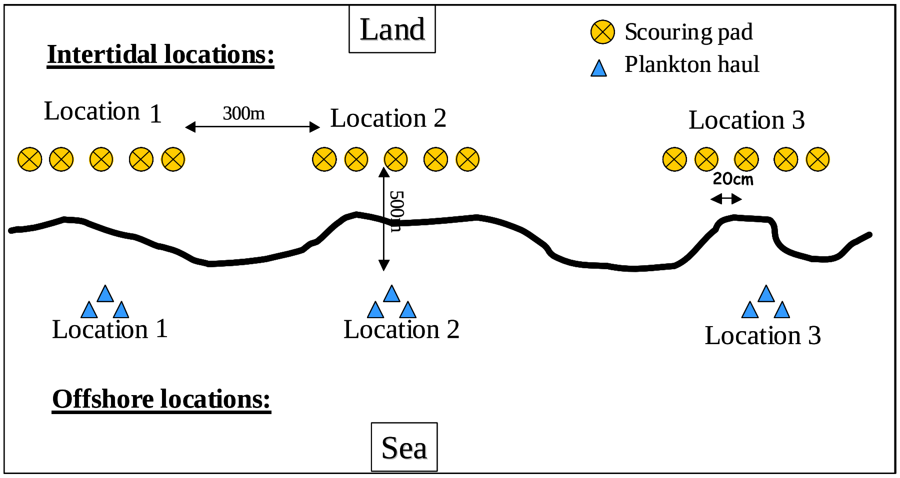


Fig. 2 Experimental design for the intertidal settlement study. One site only (detailed description in text).

After collection, pads were returned to the laboratory. The samples were usually processed immediately after collection, but on some occasions pads were frozen before analysis. Pads were rinsed thoroughly in fresh water that was filtered through a 75µm sieve. The contents were collected in a Petri dish and examined under a dissecting microscope, at 12X magnification. Settlers of *Perna perna* were identified (Fig. 3), counted, measured to the nearest 0.08mm, using an eye - piece graticule (1mm = 12 units), and preserved in 70% alcohol.

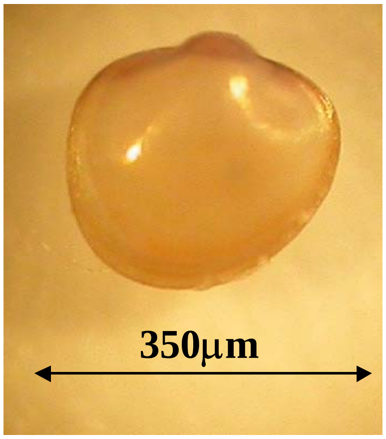


Fig.3 *Perna perna* settler trapped in a scouring pad.

Periodicity: as for larval abundance, temporal variation was inspected at different scales. In order to understand how settlement changes from season to season, sampling was performed throughout the duration of the study, for 16 months. In addition, the influences of the lunar and tidal cycles on settlement were also examined. To inspect these aspects, sampling took place fortnightly, throughout the study: around full moon and new moon spring tides. At each spring tide, pads were collected and replaced at two day intervals: pads were collected and replaced two days before spring tide, on spring tide (at the lowest tide) and two days after spring tide.

In order to detect possible differences in daily settlement rates (fine-scale variability) pads were replaced daily. This scale of variability was investigated during periods of expected high settlement (Lawrie & McQuaid, in prep.), which coincided with the dates of the daily plankton sampling.

Statistical analysis: The use of a repeated measures design was inappropriate because the placement of pads changed frequently due to the frequent loss of screws from the rocks. As a result, the approach used for the settlement study was analysis of variance and the nested design was the same as described in the previous chapter on larval availability. Significant results were tested by post-hoc comparisons using the Student Newman-Keuls' test (Zar, 1984). Post-hoc tests were run only when the outcome of the Anova did not reveal any significant interaction amongst factors: otherwise this would have made the post-hoc tables difficult to interpret.

Settlement was investigated at a variety of temporal scales, using different subsets of data. A single analysis considering all different scales simultaneously would have lead to an unbalanced design, therefore, distinct analyses for each temporal study were required.

In fact, during the settlement study, because pads were occasionally lost, especially during storms, it was necessary to balance the data set. Data were balanced choosing one of two options. When up to two out of the five pads were lost, the mean from the remaining pads was used for the missing values (this occurred in between 2% and 5% of sampling events, from daily to seasonal studies). Those dates when three or all five pads were lost at one or more locations were eliminated from the analysis. This is why, especially during the lunar and daily studies, some sampling dates are missing.

There were five separate analyses for this study: 1) seasonal; 2) lunar; 3) tidal; 4) daily (two separate studies for 2000 and 2001); 5) neap tide recruitment (to examine the influence of the neap tide on recruitment rates).

Settlement was monitored over 29 fortnightly sampling events. Each pad, used to quantify settlement, was collected after 2 days on the shore. During each fortnightly sampling event, pads were collected two days before spring tide, 2 days after spring tide and at spring tide. Different subsets of these data were used in each of the following analyses.

1) Seasonal study. This was based on samples collected at spring tide only. Using these samples, settlement was monitored over 29 sampling events.
2) Lunar study. This study was based on the same samples used for the seasonal study, using only pads collected at spring tide. However, the data set for this analysis was reduced to 24 events, due to the need to balance for missing data.
3) Tidal study. The tidal study examined the effect of the state of the tide, around spring tide. In particular, this study examined the possibility that settlement differed before, during and after spring tide. In contrast to the above analyses, this study used data from pads collected two days before spring tide, two days after

spring tide and at spring tide. In this way, settlement was followed over 12 spring tides, for a total of 36 sampling events.

4) Daily studies. There were two separate daily sampling events, in 2000 (March/April) and 2001 (February/March), when pads were replaced daily for 16 and 20 days respectively.
5) Neap tide recruitment study. This study considered the abundances of larvae that accumulated between two consecutive spring tide cycles, for a total of 26 sampling events. The pads considered for this analysis were collected on the first day of a spring tide cycle, after being on the shore for the duration of the previous neap tide period. The neap tide recruitment study examined recruitment rather than settlement, since pads were left on the shore from the last day of a spring tide cycle to the first day of the following one (for a total of 7 to 9 days). Data were averaged to one day.

For the lunar study, the factor moon was crossed by the factors date, site and location (with location nested within site). The same design was applied to the tidal study in which tide was crossed by date, site and location (with location nested within site).

All temporal factors, with the exception of day in the daily studies, were considered to be fixed. The two spatial variables, location and site were treated as random factors. The explanation for the choice of the type of effect is described in the methods section of the previous chapter under “statistical analysis” (see chapter two).

The seasonal, lunar and tidal studies were conducted separately so that the data set would be maximised and balanced. In fact, by examining the effect of date, moon and tide within the same analysis, the whole data set for ANOVA would have been reduced to only one complete sampling event. Table 1 shows a summary of how factors were treated in each study and the type of interactions considered.

Table 1 Summary of analyses of variance used for the different studies on settlement of *Perna perna* larvae. Independent variables, interactions within factors, type of effect and transformation choice are listed for each study.

ANALYSIS	**EFFECT** (Random/Fixed)	**TRANSFORMATION**
1. Seasonal study: variation of settler abundance due to date/season		Log transformation, Log (X+1)
Site	Random	
Location (Site)	Random	
Date	Fixed	
Date *Site	Random	
Date *Location (Site)	Random	
2. Lunar study: variation of settler abundance due to the lunar phase		Log transformation, Log (X+1)
Site	Random	
Location (Site)	Random	
Date	Fixed	
Moon	Fixed	
Date * Moon	Fixed	
Date *Site	Random	
Moon*Site	Random	
Date *Location (Site)	Random	
Moon*Location (Site)	Random	
Date *Moon*Site	Random	
Date *Moon*Location (Site)	Random	
3. Tidal study: variation of settler abundance due to state of tide		Log transformation, Log (X+1)
Site	Random	
Location (Site)	Random	
Date	Fixed	
Tide	Fixed	
Date *Tide	Fixed	
Date *Site	Random	
Tide*Site	Random	
Date *Location (Site)	Random	
Tide*Location (Site)	Random	
Date *Tide*Site	Random	
Date *Tide*Location (Site)	Random	
4. Daily separate studies for 2000 & 2001: variation of settler abundance due to day of collection		NOT transformed
Site	Random	
Location (Site)	Random	
Day	Random	
Day*Site	Random	
Day* Location (Site)	Random	
5. Neap tide recruitment study: variation of recruit abundance between two consecutive spring tides		Log transformation, Log (X+1)
Site	Random	
Location (Site)	Random	
Fortnight	Random	
Fortnight *Site	Random	
Fortnight *Location (Site)	Random	

Results

The sizes of *Perna* settlers collected on scouring pads during the settlement study were more variable than for the larval availability study. Occasionally, individuals of up to 2.5mm shell length or greater were found on the pads (23% of collected larvae), but the most common size of larvae collected on the shore ranged between 200 and 400µm (almost 60% of all collected larvae) (Fig. 4). The 200 - 400µm size class was used for the analysis of abundance of larvae settling onto the rocks at different spatial and temporal scales, as animals falling in this range were considered to be primary settlers, arriving on the shore for the first time. On most occasions, the morphology and the morphometrics of settlers were also similar to those of larvae collected from the water column. However, in some cases, morphological differences could also be detected when comparing larvae from the water column and settlers (see Fig. 5 chapter 2 and Fig.3 in chapter 3). For the neap tide study, organisms from all size classes were taken into account, since this analysis considered recruit rather than only settler abundance.

Each of the following sections discusses the results of the different temporal studies, dealing first with the raw data and figures and then with the ANOVA results.

1) Seasonal study

The data for this study were obtained from scouring pads collected twice a month during spring tide for 16 months. In particular, pads were collected during lowest spring tides, after being left on the shore for two days.

The abundance of settlers collected on the pads was generally low throughout the whole study period. Although there were clear differences in abundances of settlers collected at different locations, on the whole, settlers collected at the two sites, HR and KE, showed similar patterns with a small peak in abundance on the 29th of September 2000 and a bigger one on the 27th of December 2000 (Fig. 5). The absolute maximum value was recorded at HR D, where, on the 27th of December, a mean of 16.8 ± 4.79 larvae per pad was found (Fig. 5).

The assumption of homogeneity of variance for the ANOVA was satisfied when the logarithmically transformed data were tested with the Cochran's test ($p > 0.05$).

On a seasonal basis, variability in abundance of larvae arriving on the shore seemed to be due to different factors. Date and location as main factors had significant effects on settler variability ($p < 0.00001$; Table 2). The interaction between date and location also explained much of the variability in the abundance of settlers ($p < 0.00001$). The effect due to the interaction between date and site was also a significant source of variability ($p<0.01$). This reflects the fact that, on the whole, similar numbers were found at the two different sites (no significant effect of site), but that the timing differed. The numbers of larvae varied significantly depending on the date and from one location to the next.

Table 2 ANOVA for seasonal study. df = degrees of freedom; MS = Mean Square; F = F-Ratio; p = probability value; n.s. = $p > 0.05$; **** = $p < 0.00001$; ** = $p< 0.01$

	df	MS	F	p
Date	26	3.84789	22.1956	****
Site	1	4.10988	1.2072	n.s.
Location (Site)	4	3.40421	19.6366	****
Date*Site	26	0.30177	1.7406	**
Date*Location	104	0.41396	2.3878	****
Error	648	0.17336		

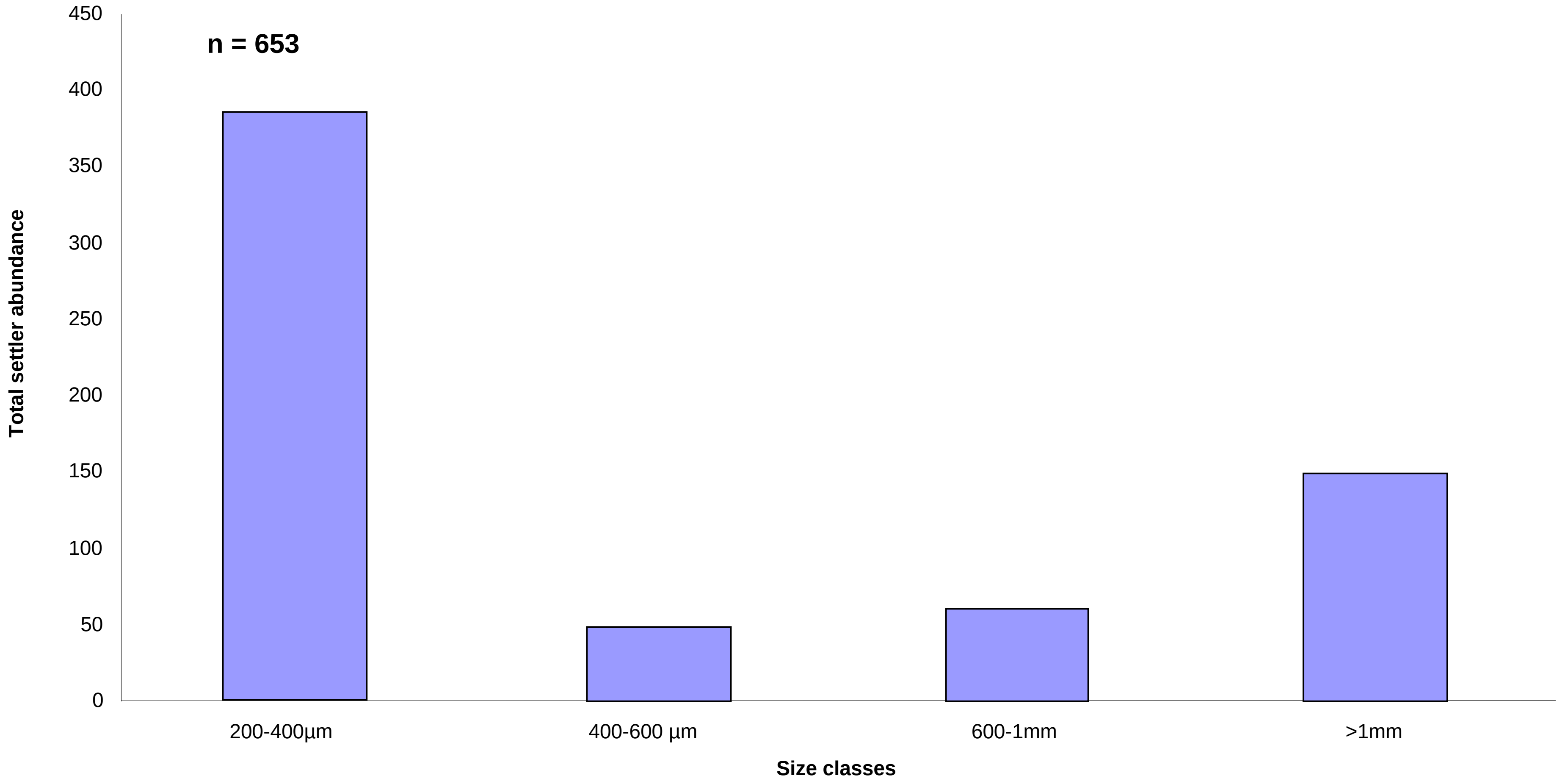
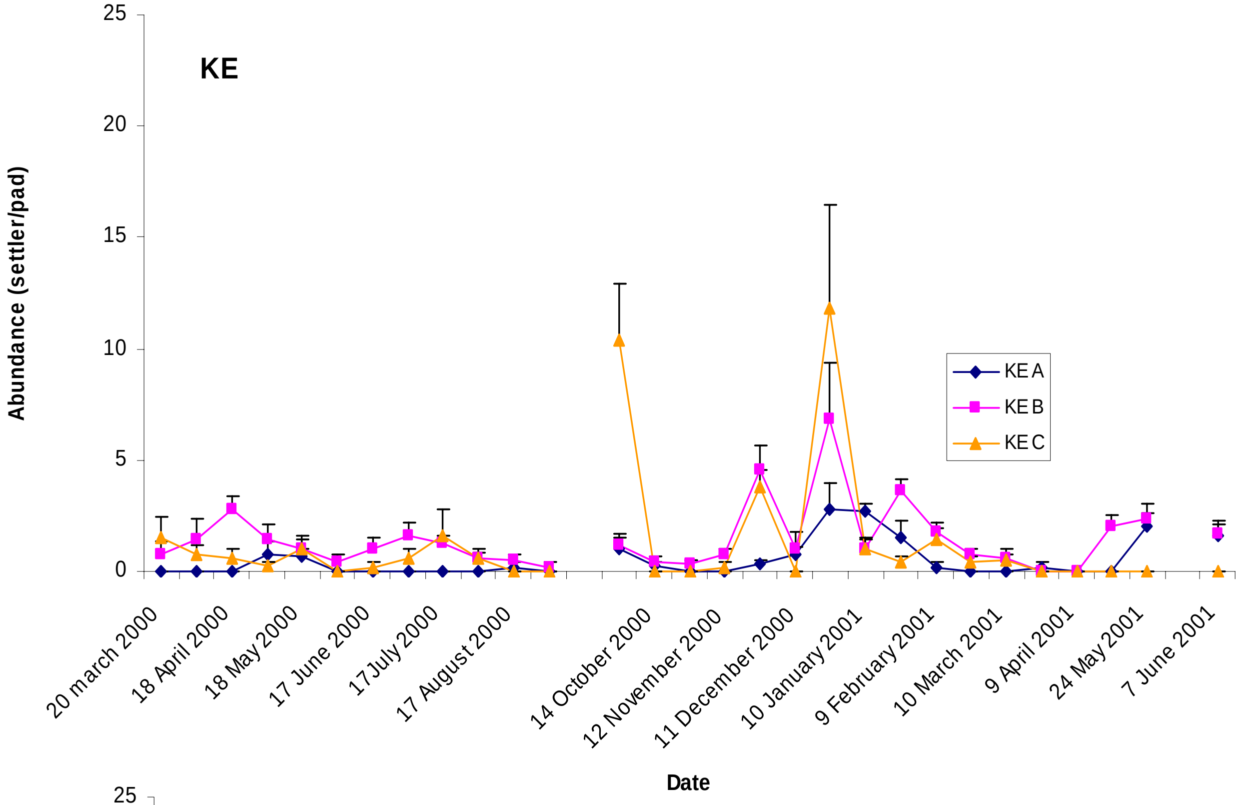


Fig. 4 Size distribution of settlers (HR and KE pooled).



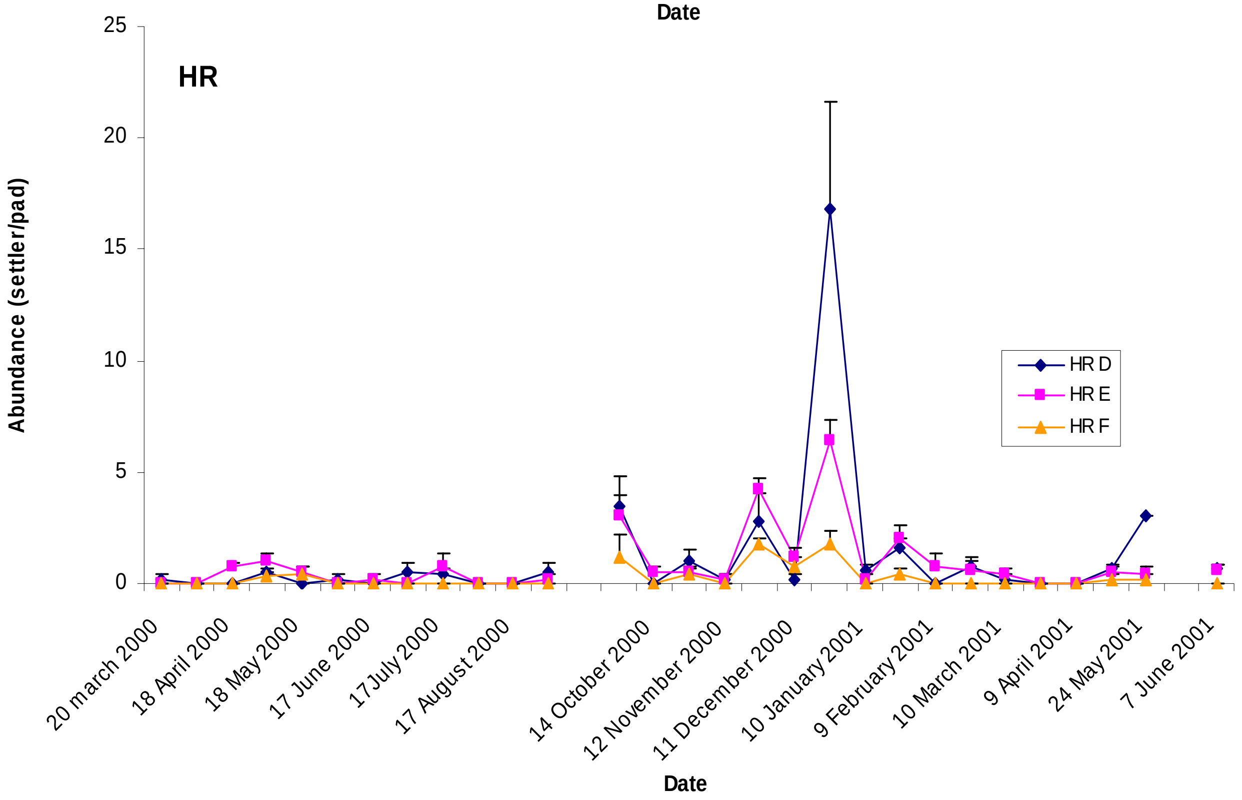


Fig. 5 Seasonal settler availability at KE (locations A, B, C) and HR (locations D, E, F). Points indicate mean values and error bars show standard errors (SE).

2) Lunar study

Possible effects of the phase of the moon on settlement were examined in this analysis. In this study the assumption of homogeneity necessary for ANOVA was satisfied for logarithmically transformed data (Cochran's homogeneity test; $p > 0.05$).

It appeared that, at both sites, more larvae generally arrived at new moon than at full moon, although there were clear differences in abundances at the various locations (Fig. 6). Nevertheless, the analysis of variance did not show a clear effect of moon as a main source of variability for the larvae coming from nearshore waters onto the rocks ($p > 0.05$; Table 3). The non-significant effect of moon as main factor could have been masked by the significant interaction between date and moon ($p < 0.00001$). The interaction between date, moon and location was also significant ($p < 0.00001$). This reflects the fact that, on those occasions when large numbers of settlers were collected on the pads, the phase of the moon influenced the arrival of larvae to the shore (Fig. 6). Date and location, nested within site, also had a significant effect on the variability of abundance of larvae ($p < 0.00001$). The p-values for the factor site and the interaction between date and site, and moon and site, were not significant ($p > 0.05$; Table 3).

Table 3 ANOVA for lunar study. df = degrees of freedom; MS = Mean Square; F = F-Ratio; p = probability value; n.s. = p > 0.05; **** = p < 0.00001

	df	MS	F	p
Date	12	5.5809	17.47017	****
Moon	1	11.8994	9.38660	n.s.
Site	1	3.3522	0.74625	n.s.
Location (Site)	4	4.4921	26.09016	****
Date *Moon	12	2.9955	10.94390	****
Date*Site	12	0.3195	1.34766	n.s.
Moon*Site	1	1.2677	257.44365	n.s.
Date*Location	48	0.2370	1.37676	n.s.
Moon*Location	4	0.0049	0.02860	n.s.
Date*Moon*Site	12	0.2737	0.39779	n.s.
Date*Moon*Location	48	0.6881	3.99650	****
Error	624	0.1722		

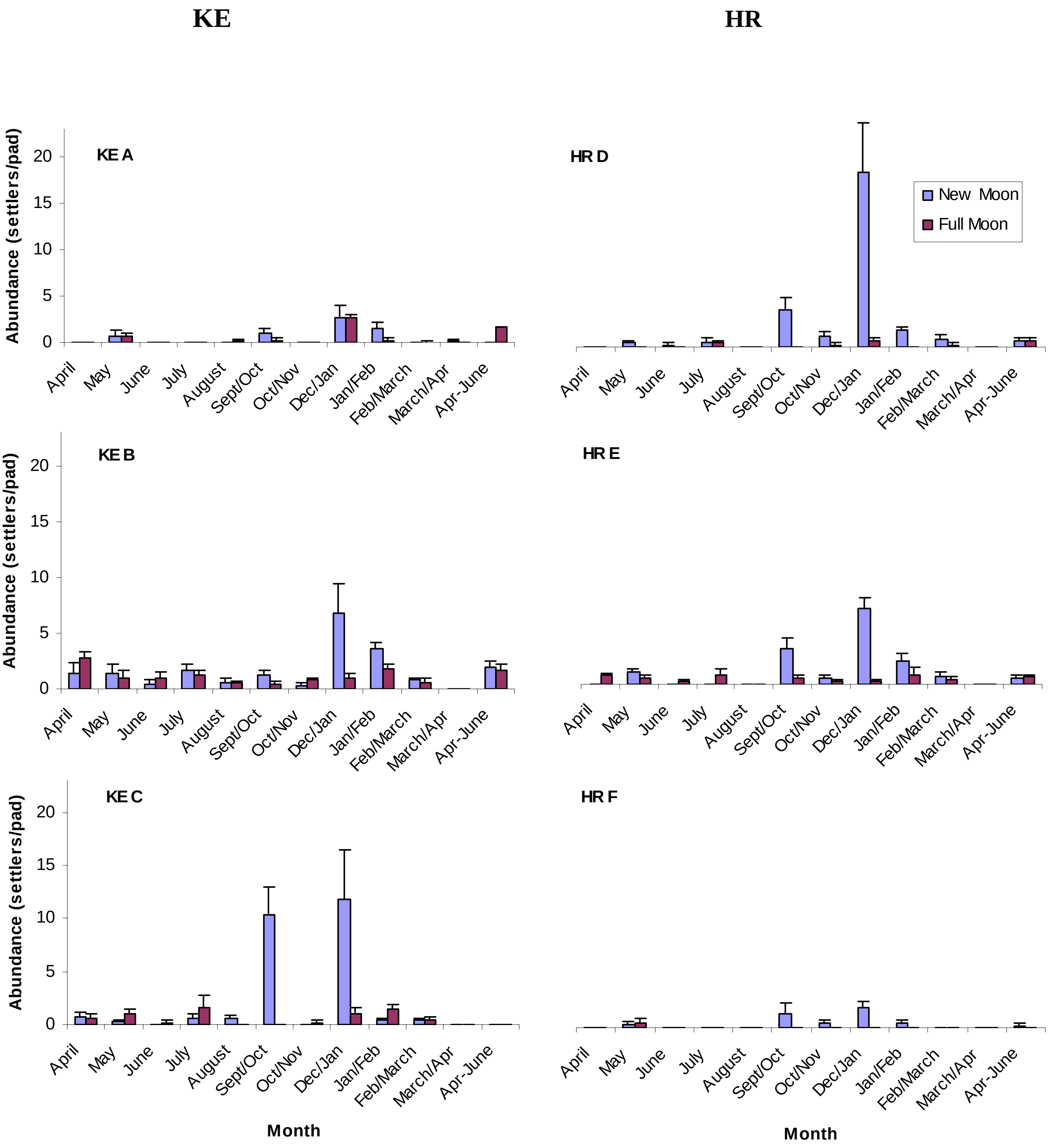


Fig. 6 Lunar settlement at KE (locations A, B, C) and HR (locations D, E, F). Histograms indicate mean values and error bars show standard errors (SE).

3) Tidal Study

The effect of tide on settlement was also investigated, for the entire study period. Days 1, 2 and 3 of each tide represented, respectively, two days before full spring tide, spring tide and two days after spring tide. The graphs did not show clear patterns, with no obvious influence of state of the tide on the abundance of larvae arriving on the shore (Fig. 7). Cochran’s test showed homogeneity of transformed data ($p > 0.05$).

The results of the analysis of variance, confirmed these observations: there was a specific non-significant effect of state of the tide (state of the tide in this analysis indicates which day of tide: 1, 2, 3) (Table 4). Only date and location had significant effects as main factors ($p < 0.00001$ for both components), while the factor site did not (Table 4). The only interactions that influenced the variation of the settler abundance in a significant way involved those two variables that were significant as main effects: the interactions between date and location and that among date, location and tide ($p < 0.00001$). In other words, tide affected locations differently and this effect differed among dates. This suggests that the effect of tide was in fact due to an effect of day, rather than state of the tide *per se*.

Table 4 ANOVA for tidal study. df = degrees of freedom; MS = Mean Square; F = F-Ratio; p = probability value; n.s. = p > 0.05; **** = p < 0.00001

	df	MS	F	p
Date	9	22.1163	45.0525	****
Tide	2	0.6383	1.3002	n.s.
Site	1	7.1337	0.88788	n.s.
Location (Site)	4	8.0345	49.7396	****
Date*Tide	18	0.9716	1.4918	n.s.
Date *Site	9	0.4909	1.2466	n.s.
Tide*Site	2	0.0075	0.0544	n.s.
Date *Location	36	0.3938	2.4379	****
Tide*Location	8	0.1381	0.8547	n.s.
Date*Tide*Site	18	0.6513	0.8526	n.s.
Date*Tide*Location	72	0.7639	4.7290	****
Error	716	0.1615		

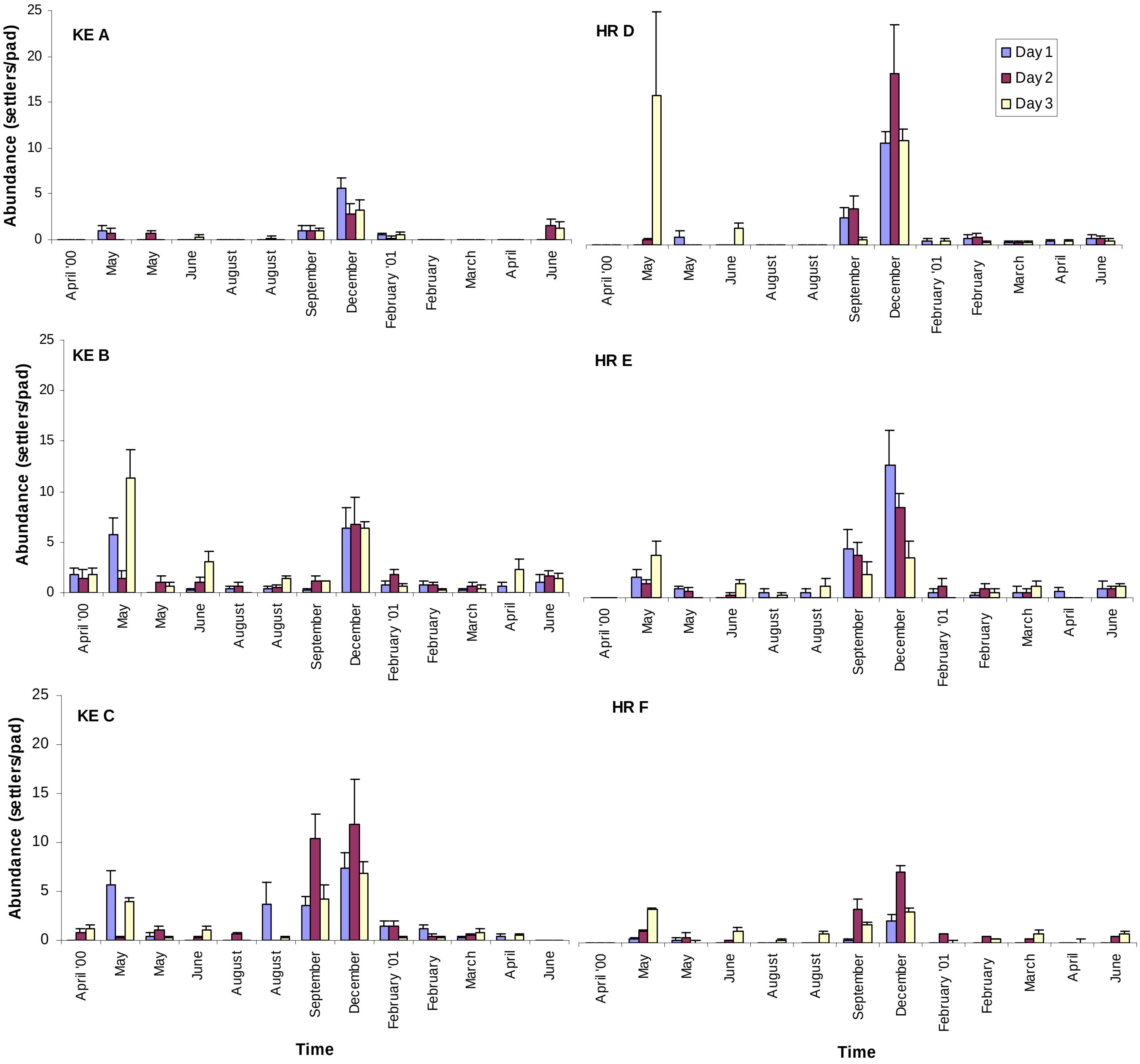


Fig. 7 Tidal settler availability at KE (locations A, B, C) and HR (locations D, E, F). Histograms indicate mean values for locations and error bars show standard errors (SE).

4) Daily study (2000)

To look at variability of numbers of settlers on fine temporal scales, pads were collected at daily intervals, on two separate occasions during 2000 and 2001.

Daily investigations of settlement from 20th March to 10th April 2000 (a total of 16 days) again revealed very low numbers of settlers at both High Rocks and Kenton with maximum mean values of about 2 larvae/pad/day (Fig. 8). Although the values were low, the numbers of settlers changed from one day to the next and from one location to another at both sites (Fig. 8).

Cochran's test confirmed the homogeneity of the untransformed data ($p > 0.05$).

Analysis of variance revealed that only location had a significant effect on the variability of larvae ($p < 0.00001$), while the components site, day, and all possible interactions showed no significant effects (Table 5).

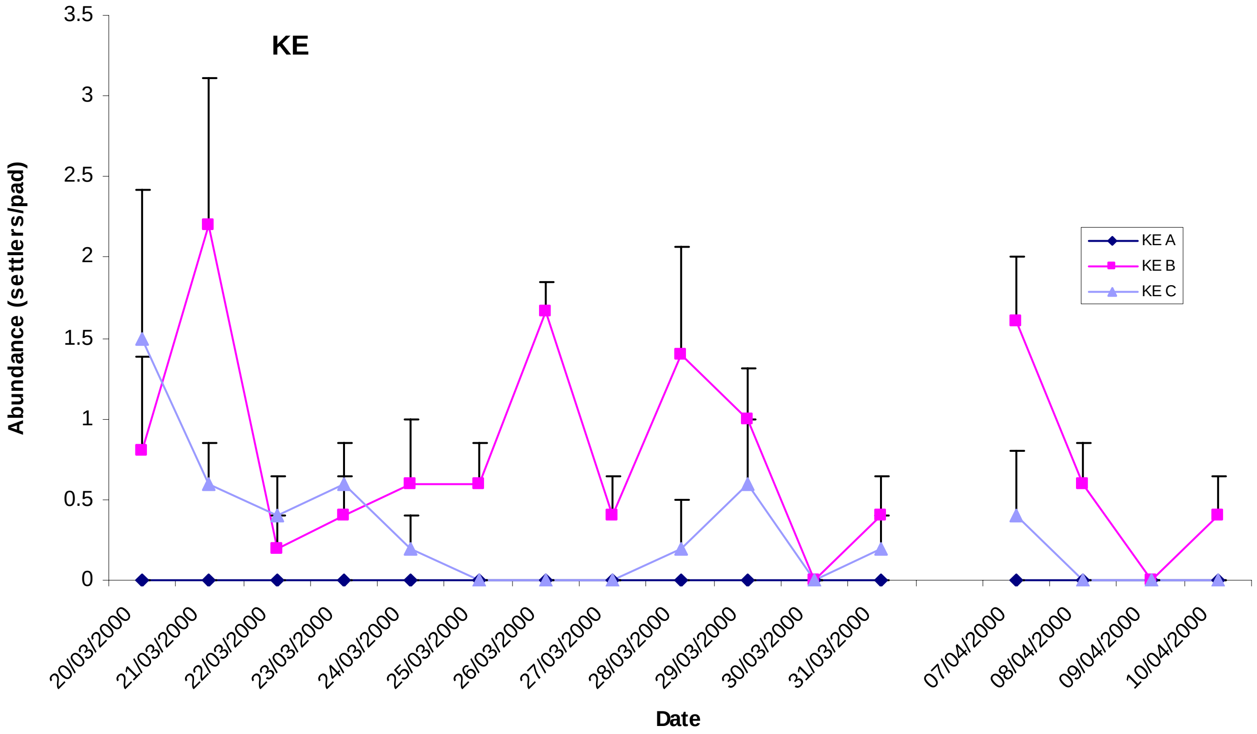
Table 5 ANOVA for daily 2000 study. df = degrees of freedom; MS = Mean Square; F = F-Ratio; p = probability value; n.s. = $p > 0.05$; **** = $p < 0.00001$

	df	MS	F	p
Day	15	0.63160	0.90889	n.s.
Site	1	8.02988	1.32186	n.s.
Location (Site)	4	6.07467	11.71388	****
Day*Site	15	0.69491	1.34000	n.s.
Day*Location	60	0.51859	1.84986	n.s.
Error	384	0.28034		

The Student Newman Keuls post hoc test for locations revealed significant differences between KE B and all the other locations (Table 6). KE B was the location where maximum numbers of settlers were collected, while at all other locations the values were generally very low (Fig. 9).

Table 6 Student Newman Keuls post hoc test on locations, homogeneous groups for 2000 daily study

Site	Location	Tot larvae Mean per pad per day	Group		
			1	2	3
KE	A	0	****		
HR	F	0.054	****		
HR	E	0.083	****	****	
HR	D	0.147	****	****	
KE	C	0.294		****	
KE	B	0.767			****



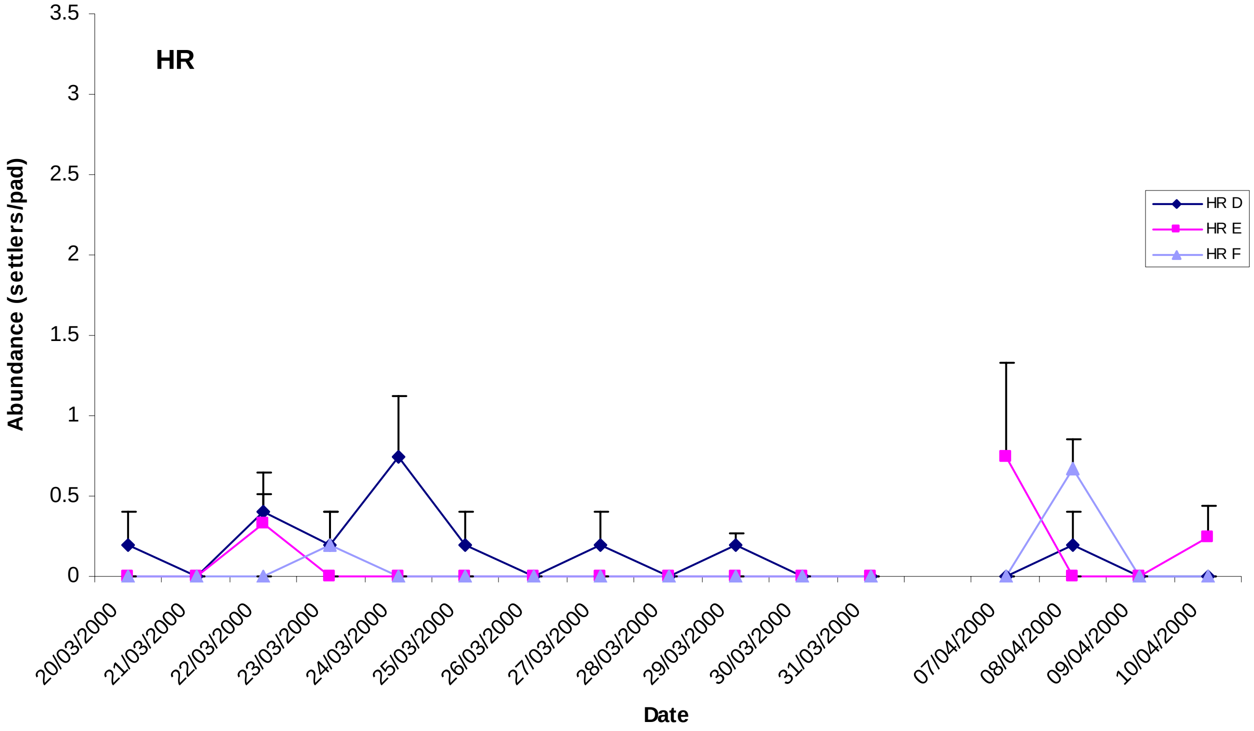


Fig. 8 Daily 2000 settler availability at KE (locations A, B, C) and HR (locations D, E, F). Points indicate mean values and error bars show standard errors (SE).

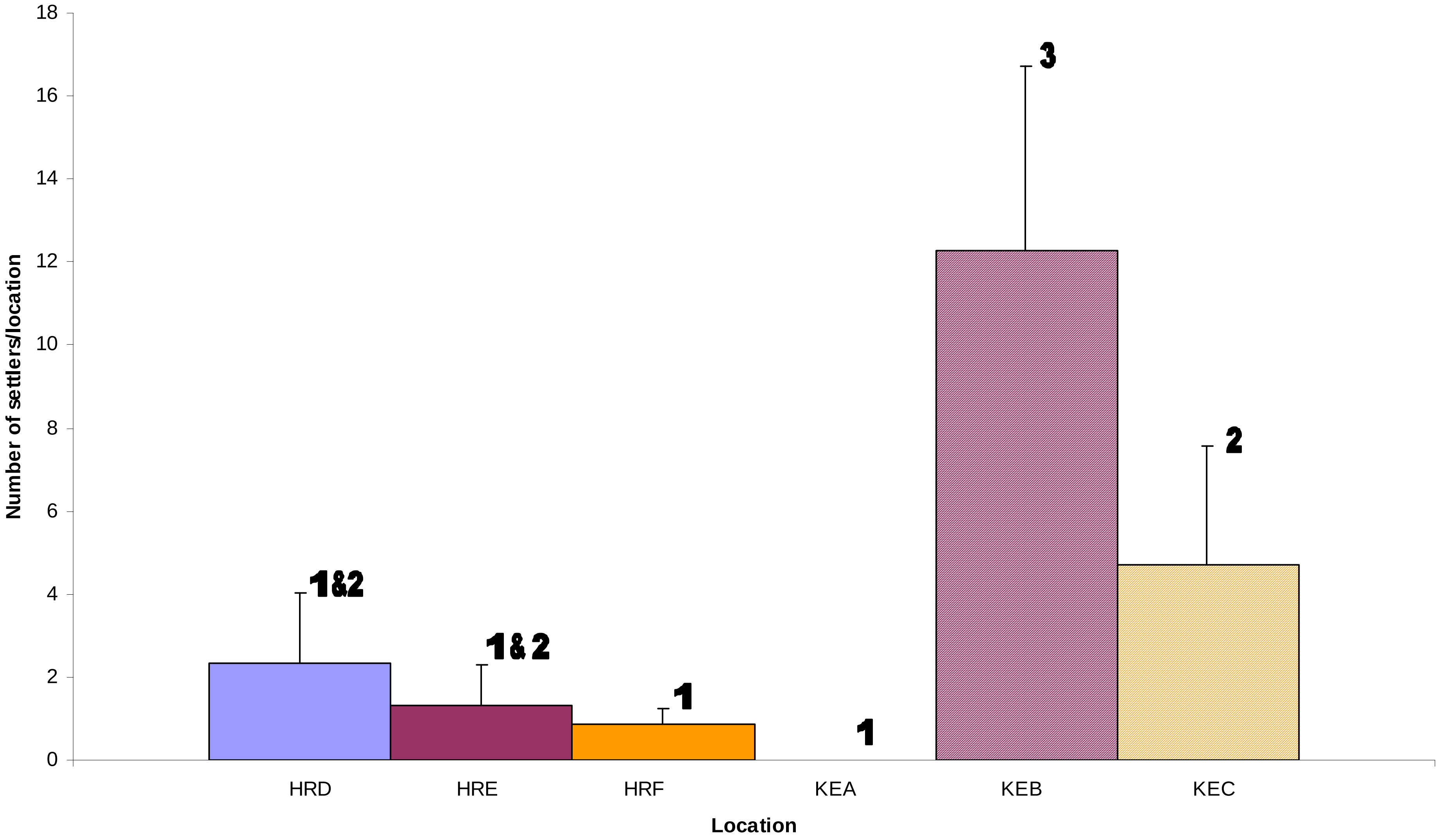


Fig. 9 Daily study (2000). Histograms indicate cumulated means for locations and error bars show standard errors (SE). Numbers in bold show the homogenous groups from the Student Newman Keuls' post hoc test.

4’) Daily study (2001)

The second daily collection was performed over 19 days, in 2001, from 7th February to 11th March.

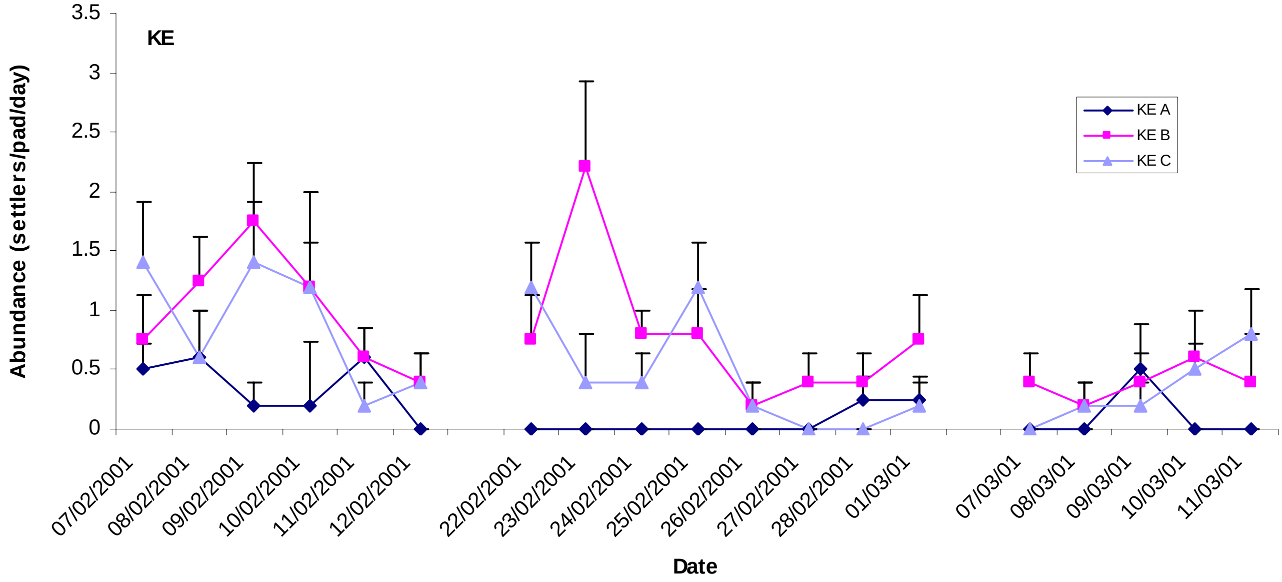
As in 2000, numbers of settlers collected at the two sites during this daily sampling were very low. In this case as well, there were differences in abundances between one day and the next one and differences between one location and another within a site (Fig. 10). Homogeneity of untransformed data was tested, and confirmed, using Cochran’s test ($p > 0.05$).

Analysis of variance confirmed the strong spatial variability at location level, already observed by simply plotting the data (Fig.10). In fact, the variation in abundance of settlers seemed to be affected by the interaction between day and location ($p < 0.001$) and location ($p < 0.00001$). The other factor that had a significant effect was day ($p < 0.05$) (Table 7).

Settler abundance at each location (Fig.11) seemed to be quite similar to the patterns of the daily 2000 collections (Fig.8). In 2000 and 2001, KE B was the location where maximum numbers of settlers were collected, while at all other locations the values were generally very low (Fig. 11).

Table 7 ANOVA for daily 2001 study. df = degrees of freedom; MS = Mean Square; F = F-Ratio; p = probability value; n.s. = p > 0.05; **** = p < 0.00001; *** = p < 0.001; * = p < 0.05

	df	MS	F	p
Day	17	1.11232	1.77211	*
Site	1	10.02686	1.72725	n.s.
Location (Site)	4	5.80511	9.99080	****
Day*Site	17	0.62768	1.080264	n.s.
Day*Location	68	0.58104	1.616127	***
Error	432	0.35953		



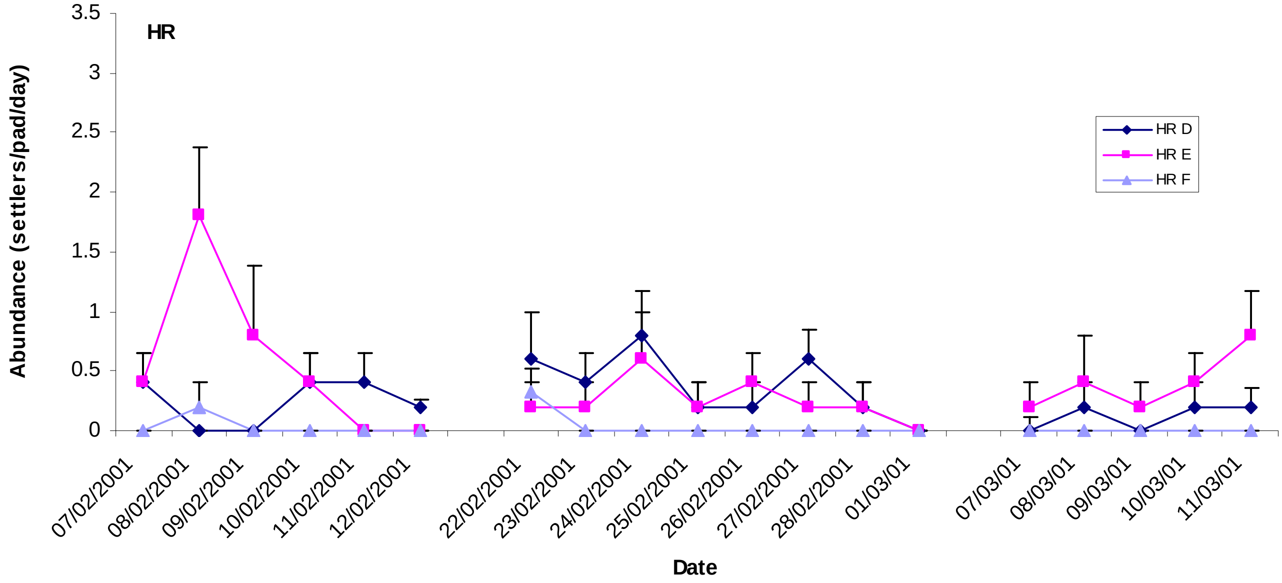


Fig. 10 Daily 2001 settler availability at KE (locations A, B, C) and HR (locations D, E, F). Points indicate mean values and error bars show standard errors (SE).

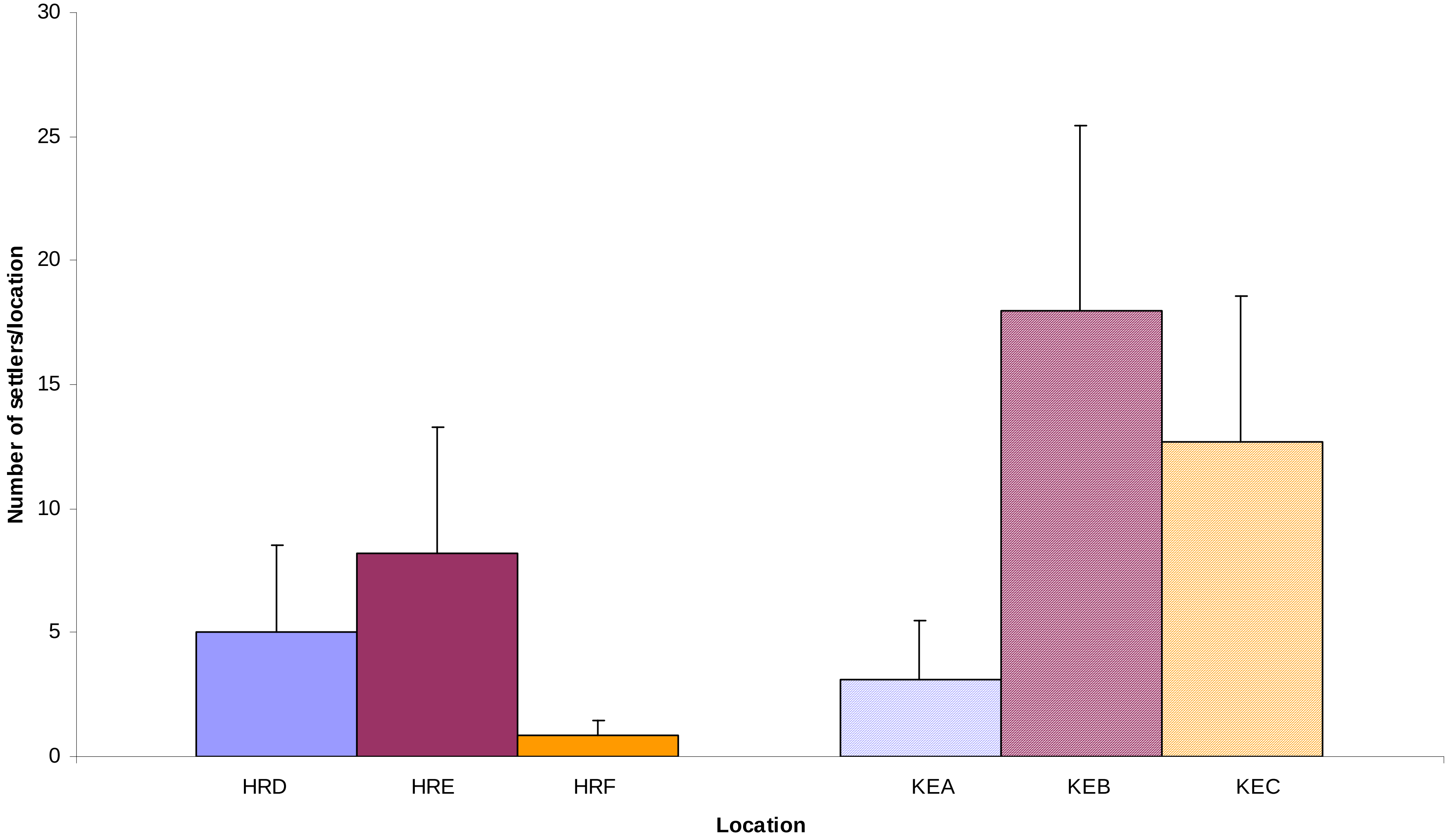


Fig. 11 Daily study (2001). Histograms indicate cumulated means for locations and error bars show standard errors (SE).

5) Neap tide recruitment study

In order to collect information on the numbers of larvae arriving on the rocks during neap tides, pads were left on the shore from the last day of collection of one spring tide cycle to the first day of the following cycle (in other words 7 - 9 days), throughout the whole settlement study. Since pads were left on the shore for a period longer than two days, this study considered the abundance of recruits rather than settlers.

Again, recruits were scarce even when allowed to accumulate over a longer period. Similar patterns were observed, with main peaks occurring at both sites on the 1st of May 2000 and 23rd of December 2000, and generally fewer recruits at HR than at KE (Fig.12). Homogeneity of data, tested using Cochran's test, was achieved after logarithmic transformation ($p > 0.05$).

ANOVA produced very similar results to the analysis of seasonal settlement data.

Variability in settler numbers appeared to be due to three components: fortnight, location and the interaction between fortnight and location ($p < 0.00001$ in all cases) (Table 8). However, while in the seasonal study the interaction between date and site was significant ($p < 0.01$), for the neap tide recruitment study the interaction between fortnight and site was not significant ($p > 0.05$). If we take the 1st of May and the 23rd of December as examples, we can see that the numbers of recruits differed quite considerably between different locations (Fig. 12), but peaks of abundance appeared at almost all locations on those dates. As it was already observed for the daily studies, the neap study revealed again that KE B was the location where most settlers were collected. Again, the significant interaction seems to be due to the magnitude of temporal effects, rather than to actual differences in timing between locations (Fig. 12).

Table 8 ANOVA for neap tide-recruitment study. df = degrees of freedom; MS = Mean Square; F = F-Ratio; p = probability value; n.s. = p > 0.05; **** = p < 0.00001

	df	MS	F	p
Fortnight	25	17.5639	18.02598	****
Site	1	18.2126	0.64213	n.s.
Location (Site)	4	28.3627	13.83646	****
Fortnight * Site	25	0.9744	0.47533	n.s.
Fortnight * Location	100	2.0499	8.26745	****
Error	624	0.247943		

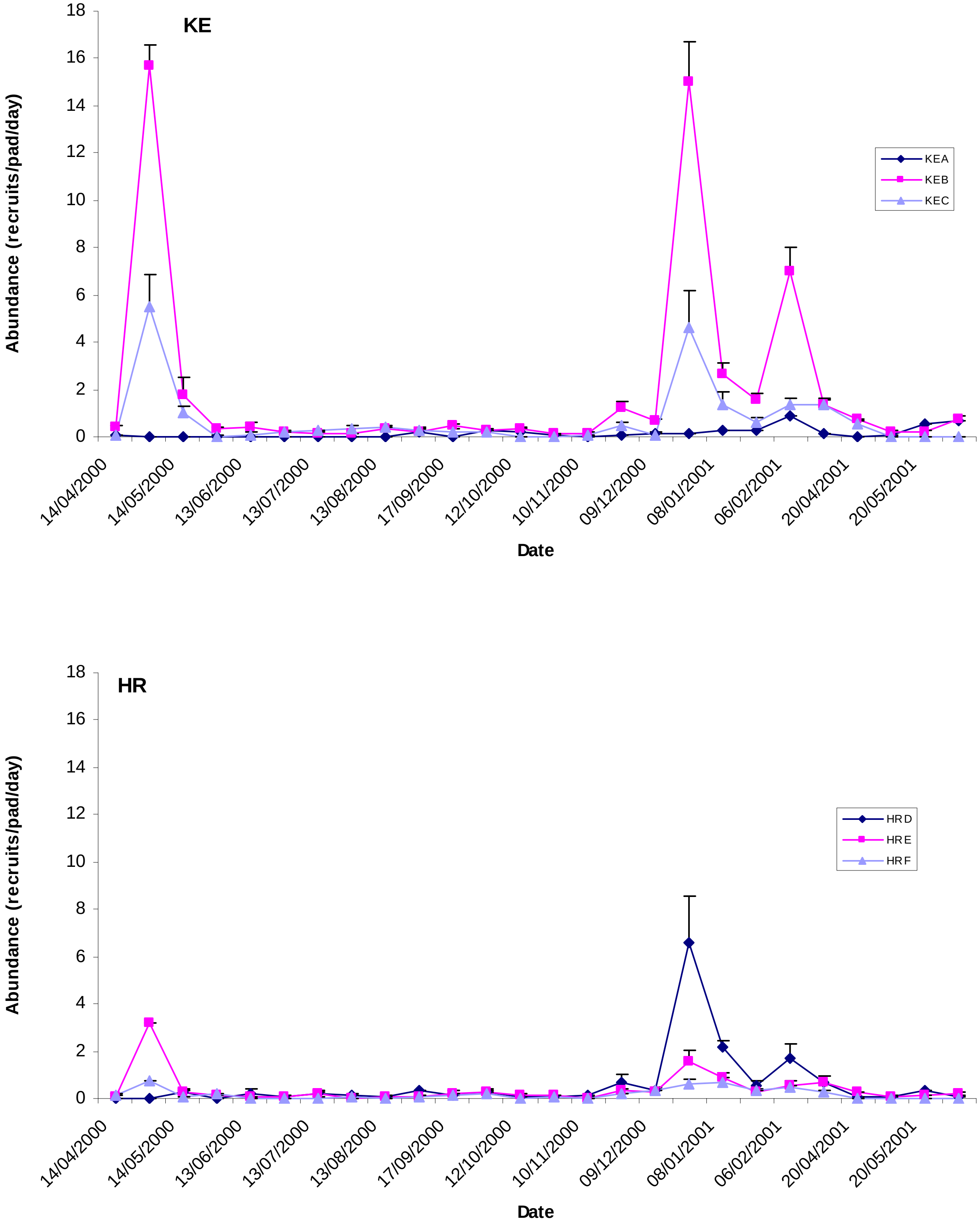


Fig. 12 Neap tide recruit availability at KE (locations A, B, C) and HR (locations D, E, F). Points indicate mean values and error bars show standard errors (SE).

Summary of settlement/recruitment results

The results (summary table, Table 9) obtained through the analysis of variance emphasised an obvious, common trend in the variation of settlement: the strong, highly significant effect of location, at all temporal scales. The large spatial scale variable, site, never influenced the abundance of *Perna perna* settlers significantly. The only exception was in the seasonal study when there was an interaction between date and site. Time also had significant effects in all analyses, with the single exception of the 2000 daily study, in which day had no significant effect. The lunar study suggested that the moon may affect the numbers of settlers, because, although the effect of moon was not significant as a main effect, there was a significant interaction of moon and date.

The effect of tide could only be investigated in the tide study, but the analysis indicated that this component did not affect the arrival of larvae on the shore.

Finally, the neap tide recruitment study highlighted the significant effects of temporal and fine spatial factors, both as main factors and as interaction between location and fortnight.

Table 9 Larval settlement/recruitment. Summary of effects of the independent variables and interactions in different studies of settler abundance using nested analysis of variance. NS = not significant; **** = p < 0.00001; *** = p < 0.01; ** = p < 0.05; -- = not applicable.

	SEASONAL	MOON	NEAP TIDE RECRUITMENT	TIDE	DAILY 2000	DAILY 2001
Site	NS	NS	NS	NS	NS	NS
Location	****	****	****	****	****	****
Time	****	****	****	****	NS	**
Time*Site	**	NS	NS	NS	NS	NS
Time*Location	****	NS	****	****	NS	****
Moon	--	NS	--	--	--	--
Time*Moon	--	****	--	--	--	--
Moon*Site	--	NS	--	--	--	--
Moon*Location	--	NS	--	--	--	--
Time*Moon*Site	--	NS	--	--	--	--
Time*Moon*Location	--	****	--	--	--	--
Tide	--	--	--	NS	--	--
Time*Tide	--	--	--	NS	--	--
Tide*Site	--	--	--	NS	--	--
Tide*Location	--	--	--	NS	--	--
Time*Tide*Site	--	--	--	NS	--	--
Time*Tide*Location	--	--	--	****	--	--

Discussion

Size and abundance

The choice of an appropriate method is of basic importance when estimating abundances of planktonic larvae for the study of intertidal population dynamics. For example, while investigating settlement, some studies have highlighted the preference of some species for various materials and textures over others (Petersen, 1984; Dobretsov & Wahl, 2001; Devakie & Ali, 2002). Eyster and Pechenik (1987) have shown that mussel larvae prefer to settle on natural filamentous substrata, such as algal material or byssal threads rather than on eyebrow hair, cotton thread, scouring pad fiber or glass fiber. On the other hand, the use of artificial collectors is ideal for standardising the quantification of settlement, by sampling at frequent time intervals in order to minimise migration, predation and mortality of larvae. Collectors also help in the distinction of settlement from post-settlement processes. In addition to this, the use of artificial larval collectors facilitates good replication in space and time (Balch & Scheibling, 2000) and collectors have been largely employed for the estimation of abundance of larval stages of invertebrates (sea urchins: Rowley, 1989; Harrold *et al.*, 1991; Keasing *et al.*, 1993; Ebert *et al.*, 1994; megalops crab: Tankersley *et al.*, 2002; ascidians: Hurlbut, 1991). In the present study, the utilisation of scouring pads seemed to be the only option for exploring larval abundances over a long period of time (16 months). Traditionally, in this field, the estimation of abundances of intertidal settlers or recruits has been done through destructive methods, by scraping replicates of small patches of natural substrata from the shore (Todd, 2003). There are three problems with this approach. First, this method would have been too destructive for a long term investigation. Second, scouring pads offer a uniform and constant area and texture, unlike natural substrata. Lastly, using destructive samples it is not possible to tell when larvae arrived.

Pineda & Caswell (1997) consider the so called intensification effect which produces overestimations of settlement rates for barnacles, especially if there is little ideal substratum available and if the species considered shows gregarious behaviour. Thus, variability in the availability of the “right” substratum can be very important for variation in settlement. Also it is better to use traps or collectors that are not too large: settlement magnitude could decrease if settlement area increases, unless, again, the species under investigation does not show gregariousness (Pineda, 2000). Larval traps should also be tested for their efficiency, at fine temporal and spatial scales, for the species they are targeting and specifically for the type of environment in which they will be placed (Todd, 2003).

In the present study, the choice of artificial collectors seemed to be ideal since the homogenous surface of the pads minimises variability of the substratum. The efficiency of scouring pads has been tested in at least three other studies, which measured the settlement rates of *Mytilus edulis* (McQuaid, pers. comm.), *Perna perna* and *Mytilus galloprovincialis* (Lawrie *et al*., in prep; Bownes and McQuaid, pers. comm.). All investigations proved the success of these collectors. In conclusion, the choice of scouring pads made of filamentous material and left in sea water to develop a biofilm, seemed to be the best option to measure the abundance of mussel settlers over large temporal scales.

60% of all larvae collected from the scouring pads were in the range of 200 - 400µm and were considered settlers. This choice is based on previous studies in which 230µm (Siddall, 1980) or the class 250 – 470µm (Ramirez & Caceres–Martinez, 1999) were considered the size for competent pediveligers. The remaining 40% of all larvae collected in this study were larger than 400µm, with 17% being 400µm – 1mm and 23% larger than 1mm. Another study on settlement of *Mytilus galloprovincialis* showed that, although the majority of pediveligers were small, a minor proportion was larger than expected. This study suggested that a few postlarvae exhibit secondary dispersal (Ramirez & Caceres–Martinez, 1988). Thus, the results from the present study could support the theory of primary and secondary settlement proposed by Bayne (1964), since different sizes of larvae were found on collectors specifically sampled for settlement. However, the theory also predicts that two different size classes of larvae should be found on different substrata: smaller individuals on filamentous material, and larger ones on adult mussels beds (Buchanan & Babcock, 1997). This did not correspond with the present findings. Although settlement onto mussel beds was not investigated here, pads collected both very small competent larvae and larger sized individuals. McGrath *et al*. (1988) also found that competent larvae of *Mytilus edulis* settle both onto filamentous algae and adult beds.

Another point against the primary – secondary settlement theory is the fact that no larvae larger than 400µm were found when collecting information on the availability of *Perna* larvae in nearshore waters. A simpler explanation for the occurrence of large individuals on settlement pads is that larvae, after settling for the first time on the shore, can be dislodged, actively or passively, on local scales (up to a maximum of 500 meters offshore) and reattach locally without following the secondary dispersal processes suggested by Bayne (1964) and other supporters of his theory (Eyster & Pechenik, 1987; Pulfrich, 1996).

Spatial variability

The main objective in this study was not to investigate possible secondary dispersal, but rather to evaluate the spatial and temporal scales that influence the variability of settler abundance.

There is a general tendency, in the study of settlement of intertidal species, to find that spatial and temporal variables operating at different scales affect the distribution and abundance of larvae in different ways. Jenkins *et al*. (2000) believe that the large-scale processes that could influence barnacle settlement range from upwelling to local wind patterns, while fine-scale factors would depend more on local hydrodynamics, larval behaviour and substratum characteristics.

The delivery of larvae can indeed operate differentially at different spatial scales. Many studies suggest that large spatial scale variation controls the distribution and abundance of larvae offshore, while larval settlement is more influenced by finer scale variability (Connell, 1985; Pineda, 2000).

Offshore and onshore processes control larval transport and delivery in different ways. Offshore transport operates on large spatial scales and on larger numbers of larvae, while the larvae are still relatively far offshore. On the other hand, onshore transports work on fewer larvae, when they are closer to the shore (Pineda, 2000). Possible processes that could influence the distribution of larvae on large spatial scales are oceanographic hydrodynamics, possibly controlled by the moon, wind direction, speed and wave height (Jeffery & Underwood, 2000). In contrast, the arrival of larvae onshore, is more affected by local hydrodynamics, influenced by coastal morphology, local topography, wave action on the shore (Connell, 1985; Gaines *et al*., 1985; Delafontaine & Flemming, 1989; Hills & Thomason, 1996; Jeffery & Underwood, 2000) and by finer scale local micro-hydrodynamics, influenced by surface roughness, rock cracks, presence of sessile individuals, pools, channels, protected and exposed shelves, as well as the availability of horizontal or vertical substrata. Under such variable conditions, larvae face different conditions even at the same locations (Gaines *et al*., 1985; Pineda, 2000). Internal waves could also affect the differential delivery of larvae along the shore (Jeffery & Underwood, 2000). The results of this study show clearly that *Perna* larvae were delivered differentially at scales of hundreds of meters (locations), while consistent variability did not seem to be caused by larger spatial scale effects (sites, a few kilometers apart). In fact, all studies performed on settlement showed the strong effect of location on the variability of larvae arriving to the shore, while site was never a significant source of variation. Jenkins *et al*. (2000) also found high levels of spatial variation when looking at settlement of barnacles, but in that study the highest variation was found at the largest spatial scales. Pineda *et al*. (2002) found that there were consistent differences in barnacle settlement at sites one kilometer apart. Keasing *et al*. (1993), when investigating echinoderm settlement on coral reefs, found high variability in settlement at very fine scales (meters), rather than at hundreds of meters (where distribution of larvae was more homogenous).

Usually, the spatio-temporal variability of settlement has been attributed to post-settlement mortality, in other words differential mortality on the shore due to competition, predation or dislodgment. However, the supply and the differential arrival of larvae are also very important components influencing the final size of intertidal populations (Keough & Downes, 1982; Minchinton & Scheibling, 1991). In particular, it seems that, when settlement or recruitment rates are high, post-settlement processes determine the population structure (Connell, 1985; Minchinton & Scheibling, 1991); while, when settlement is low, the final adult abundance is influenced by spatio-temporal variation (Minchinton & Scheibling, 1991).

Few studies on mussel settlement have been carried out on the coast of South Africa and most have been in different areas of the east coast (Lasiak & Barnard, 1995; Lawrie *et al*., in prep; Bownes & McQuaid, pers. comm.). The results have been quite different in terms of abundances, and the main reason for such variability has been regarded as due to geographical differences. As it has been shown from the present study, variability in abundance occurs on scales of a few hundreds of meters. Therefore, it should come as no surprise that areas that are hundreds of kilometers apart, that may be on the open coast or in bays, and especially that show different biogeography, show different levels of settler abundance (Harris *et al*., 1998).

Active behavioural choice of substratum has not been demonstrated for mussels. However, the choice of settlement spots could dramatically influence the survival of the organisms. In fact, locations, and even finer spatial scales where settlement occurs, are very important because they determine the environmental conditions that the larvae will experience and whether they will eventually survive to become juveniles and adults (Hurlbut, 1991). On very fine spatial scales (cm to µm), the specific topography of the substratum (like crevices and pits) can enhance or decrease the survival of sessile organisms after settlement (Walters & Wethey, 1996).

Temporal variability

In addition to spatial components, the other main factor that influences variation in settlement is temporal variability. Measuring spatio-temporal variation through a hierarchical design is one of the best ways to investigate variability in settlement, which is otherwise difficult to measure in a quantitative and absolute way (Jenkins *et al*., 2000). Looking at temporal variability in settlement of marine invertebrates is important because time and space influence and regulate recruitment to the adult population and, therefore, these processes can help us understand the determination of the size and distribution of the final population.

The number of studies that have looked at temporal variability of settlement of intertidal organisms is considerable (for example: Rojas, 1969; Acuna, 1977; Roberts *et al*., 1991; Caceres *et al*., 1993; Lasiak & Barnard, 1995; Buchanan & Babcock, 1997; Hunt & Scheibling, 1998; Balch & Scheibling, 2000), but only a few have looked deeply at different temporal levels and tried to determine which scales most influence the variability of settlement (Bertness *et al*., 1996; Hunt & Scheibling, 1998).

Another consideration is that different scales of investigation also help in optimising the method for the study of a specific process in a particular species. In the case of settlement, sampling interval should be kept as short as possible because even fine-scale time intervals can influence estimates of settlement rates very distinctively (Minchinton & Scheibling, 1993; Pineda, 2000). Connell (1985) considers that the sampling interval closest to the ideal would be daily collections in order to minimise the loss of larvae that could detach and reattach over a certain period. In addition, it should be considered that, if collectors are left on the shore for a long time, the risk of predation, mortality, migration or more generally dislodgment, could influence estimates of abundances, usually resulting in underestimation. For example, Tankersley *et al*. (2002) showed that cumulative settlement of megalops larvae in collectors sampled at one-hour intervals was higher than settlement on collectors left in the water overnight. Nevertheless, it can also happen that long term colonisation (over the full period of study) is similar to accumulated short-term colonisation, for example, cumulative daily collections (Hunt & Scheibling, 1998).

Temporal variability was measured in this study at different scales, in order to see if variability of settler abundance varies more on coarse scales, such as seasonal, or lunar cycles or on finer scales, like tidal or daily cycles. Time was a very important factor in determining variability of larval abundance in almost all the analyses. In addition to time as a main factor, much of the variability of settler/recruit abundance was due to the interaction between time and the smallest spatial scale investigated here, location. This result again highlights the obvious importance of temporal variability in this type of study, and also the importance of synchrony of settlement between sites. In fact, most of the peaks of settler abundance occurred on the same dates at both sites and at most locations, even though the actual abundances differed amongst locations. Apart from spawning cycles, the reasons for such high temporal variability can be found, again, partly in hydrodynamics (Jenkins *et al*., 2000). Large temporal scales can influence currents, so that there can be seasonal patterns in water movement: currents can change from one month to another, on a seasonal basis. As in the case of spatial variability, local hydrodynamics influence temporal variation too, affecting, on fine scales, the arrival of larvae on the shore (Gaines *et al*., 1985). For example, certain localities may experience settlement at certain times, but not even seconds, minutes or hours later, when the water may be calmer, changes in sea levels could have occurred or the state of the tide could have changed (Pineda, 2000).

When looking at barnacle settlement, parameters such as water temperature, salinity, availability of food, illumination, reproductive output, mortality rates, presence of conspecifics, algal cover, and the presence of biofilm should be considered. All these factors change at different temporal scales and could therefore also influence the differential arrival of larvae (Gaines *et al*., 1985; Hills & Thomason, 1996). It has also been shown that changes in salinity, light and turbulence of water trigger a behavioural response in crab settlement. Megalops larvae remain swimming during flooding of the tide at night, while they settle just before low tide, when turbulence declines (Tankersley *et al*., 2002). Tidal amplitude can also control settlement of megalops larvae in a Mozambican mangrove system (Paula *et al*., 2001).

In the present study, the effect of tide (meaning the days prior to and immediately following spring tide) was investigated, but sampling was done only close to spring tide to observe whether tidal amplitude around the spring cycle would influence the settlement of *Perna*. The results were negative, probably because the differences in tidal range were minimal in this particular case.

Looking at seasonal collections, there were distinct peaks in larval abundance throughout the 16 months of study and within this period of high numbers of larvae, variability was specifically determined by the phase of the moon, with more larvae settling at new rather than full moons. However, it appeared that moon by itself did not significantly influence the rates of settlement. This lack of a moon effect, as a main factor, could have been because of clouding by the interaction between time and moon. In fact, it appeared that there was a strong influence of the moon only when there were peaks in larval abundance and only at specific locations. From these results it seems that the spatial and temporal factors examined become relevant in explaining settlement variability only when rates of settlement are relatively high.

The influence of lunar cycle on settlement of intertidal invertebrates has been suggested by other studies, but for some of them the influence of the moon probably reflects the influence of the tidal amplitude as flood tides are expected to be around a particular phase of the moon (Reyns & Sponaugle, 1999; Paula *et al.*, 2001). It has been shown that lunar cycle also influences the settlement of barnacles. Nevertheless, although temporal variability alone is generally very important for understanding settlement, the behaviour of some species could be triggered by physical or biological factors that vary periodically (Pineda, 2000). Finally, the influence of time on fine scales was clearly shown in one of the daily collections in the present study. Variability in larval abundance was very high with settler rates changing sharply form one day to the next.

This last result indicates that on this coast, the intertidal system is extremely unpredictable with respect to settlement rates and emphasises the importance of a short interval sampling design if one wishes to detect differences in settler abundance as this changes over very fine scales of time and space.

Chapter 4

Coupling of larval availability and settlement of *Perna perna*

"Anyone who has never made mistakes
has never tried anything new"
Albert Einstein

Introduction

In several studies it has been observed that the abundance of pelagic larvae and of settlers could certainly influence the final size of the adult population (Connell, 1985; Hurlbut, 1991; Pulfrich, 1996; Stoner & Davis, 1997; Mariani *et al*., 2000; Pineda, 2000; Beukema *et al*., 2001; Norkko *et al*., 2001; Drouin *et al*., 2002; Lipcius & Stockhausen, 2002; Pineda *et al*., 2002; Harii & Kayanne, 2003; Jeffery, 2003).

In particular, it is very important to look at possible relationships between the above mentioned life stages and the possible effects of larval availability and settlement on the adult population. In fact, it could be that links between larval availability and settlement could help to infer the population size and dynamics of the adults (Olson, 1985; Pineda, 1991; Miron *et al*., 1995; Pineda, 2000; Ross, 2001; Satumanatpan & Keough, 2001).

The ideal way to understand population dynamics of an intertidal species would be to quantify all different stages of the life cycle and to look at all possible relationships amongst these stages: from sexual maturity to spawning, from larval dispersal in the water column to final recruitment to the adult population. However, this seems unrealistic, as it is very difficult to follow and measure simultaneously all the phases of a life cycle, especially when allowing for spatial and temporal replication. Therefore, for the present study, only the relationships between two factors, involved in structuring intertidal populations, were inspected: larval availability and settlement (Harrold *et al*., 1991; Hurlbut, 1991; Minchinton & Scheibling, 1991; Jeffery & Underwood, 2000; Olivier *et al*., 2000; Pineda, 2000; Pineda *et al*., 2002).

The combined design of the studies discussed in chapters two and three allows us to examine possible relationships between the numbers of mussel larvae collected from the water column and the numbers of settlers found on the rocks. In fact, dispersal of *Perna perna* larvae, on the south coast of South Africa, appears to be unexpectedly limited in space (McQuaid & Phillips, 2000). So, it could be that larvae collected from the water are retained relatively locally and that the larvae collected from the rocks would originate from comparatively local larval stocks (Pulfrich, 1997; Stoner & Davis, 1997; Poulin *et al*., 2002; Zeidberg & Hammer, 2002). Certainly, the relatively fine scales used in this study should allow the identification of coupling (Hunt & Scheibling, 1998) between larval availability and settlement. So, we could expect to find a correlation between abundances of *Perna perna* larvae in nearshore waters and of settlers collected on adjacent intertidal stations.

Materials & Methods

Coupling patterns between larval availability of *Perna perna* in the water and settlement on the rocks were investigated.

The data used to examine possible relationships between the amount of larvae in the water and the abundance of settlers on the shore, were the same as the ones used for the larval availability (see chapter two) and the settlement (see chapter three) studies. Thus, only details of the statistical analysis description will be given.

Statistical analysis: Possible coupling between the abundance of *Perna* larvae in the water column and settlers on scouring pads was investigated by performing correlation analysis between the two factors. For this study, seasonal and daily abundances of larvae in the water were correlated with the abundances of settlers for the same daily periods and likewise for seasonal data. In addition to this, the data collected on larval availability and settlement (analysed in chapters two and three), were plotted against each other for each study respectively (seasonal, daily 2000, daily 2001) (Figs.1 to 3).

For each temporal survey, the means of larvae collected at the two sites, High Rocks (HR) and Kenton (KE) were plotted and the correlation coefficients (*r*) were calculated.

The package used for the statistical analysis was Statistica 6.0 (Statsoft).

Results

From the analysis of data performed in chapters two and three, a strong influence of location (fine spatial scale) was observed for settlement, but not for larvae in the water column. Therefore, at first, the mean of larval abundance from the three locations at each site was calculated. This was plotted against the numbers of settlers at each location at the same site. This did not show any obvious relationships. Secondly, since no spatial effect was observed for the larval availability study, the mean of larvae at each site was calculated, plotted and correlated against the mean of settlers at each location, and also at each site. This also did not show any correlation and the results of this analysis will not be shown in this context. Therefore, for each study, the mean of all three locations at each site was calculated for the larvae in the water and for the settlers on the pads and these values were correlated.

The plotting and analysis of data clearly showed that there was no correlation between larvae in the water and settlers arriving ashore. The graphs plotted from all three studies, but especially from the seasonal and the daily 2001 surveys, showed a complete lack of correlation (Figs.1 to 3).

The correlation coefficients for all three studies were very low and none were significant (Table 1).

The raw data indicated that the lack of correlation was not due to a lag effect and cross-correlation or lag analysis was clearly unwarranted.

Table 1 Correlation analyses for seasonal, daily 2000 and 2001 data. *r* = correlation coefficient; p = probability value. See text for detailed description of the analyses.

Seasonal correlation		**2000 Daily correlation**		**2001 Daily correlation**	
r	p	***r***	p	***r***	p
-0.08	0.73	0.37	0.20	0.21	0.44

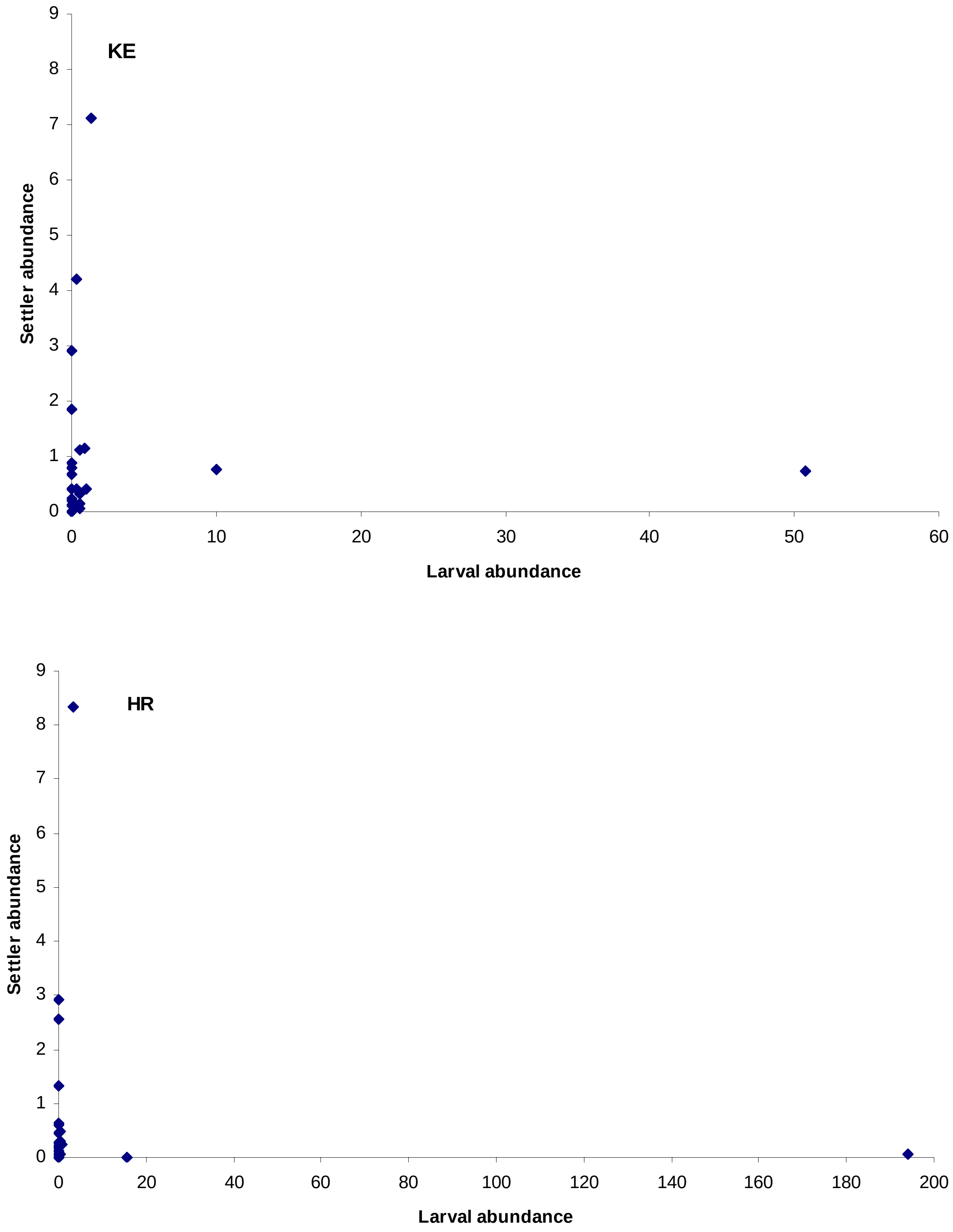


Fig. 1 Seasonal correlation at KE and HR. Larval abundance vs. settler abundance.

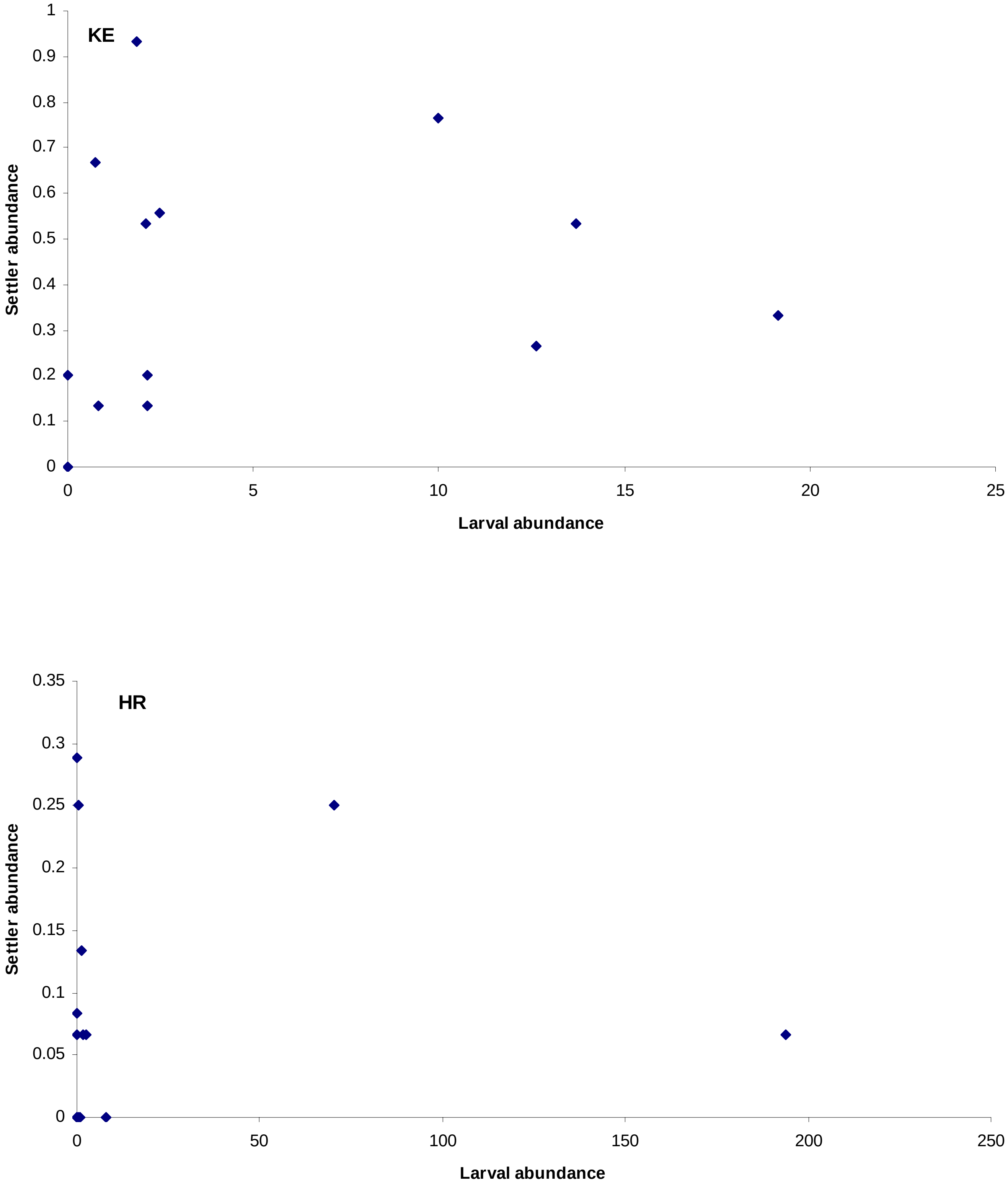


Fig. 2 2000 Daily correlation at KE and HR. Larval abundance vs. settler abundance.

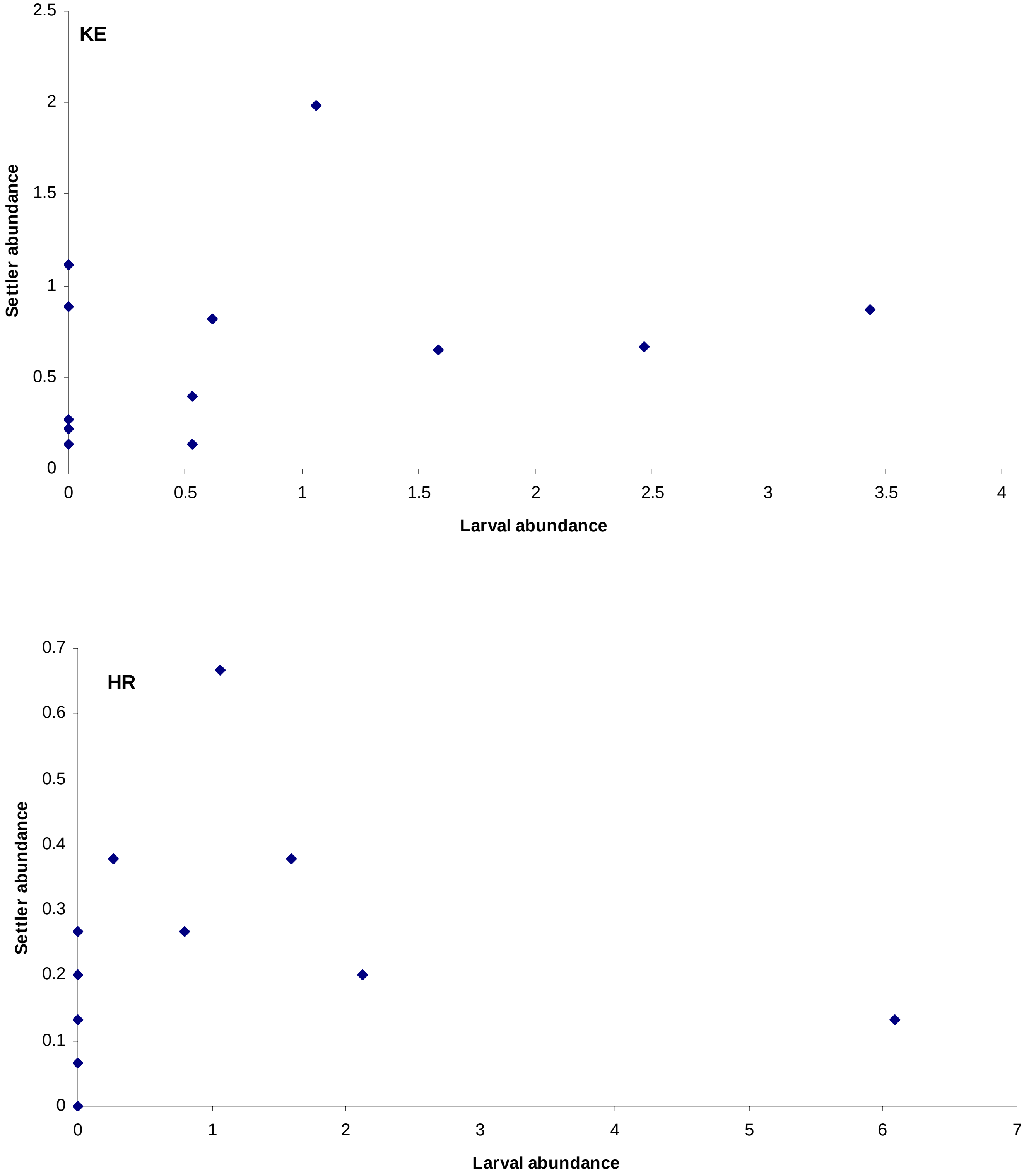


Fig. 3 2001 Daily correlation at KE and HR. Larval abundance vs. settler abundance.

Discussion

The problem arising from external fertilization in open populations, such as sedentary intertidal marine organisms with planktonic larval stages, is that the possible scales of dispersal of larvae may vary widely (Roughgarden *et al*., 1985; Norkko *et al*., 2001; Van Dover *et al*., 2001; Poulin *et al*., 2002). Larvae can potentially disperse over large distances (Widdows, 1991), but it has been demonstrated that longshore dispersal in the majority of mussel larvae on the south coast of South Africa is relatively limited, with most larvae dispersing over scales of less than 10km (McQuaid & Phillips, 2000). Therefore, it could be expected that local populations of larvae in the water column will serve as stocks for local settlement onto local adult mussel beds (Van Dover *et al*., 2001). To investigate this issue, the present study was designed in such a way that intertidal stations for the settlement study were physically opposite offshore stations for the larval availability survey. However, the number of larvae collected at the nearshore stations did not correlate with the abundance of settlers on the adjacent intertidal locations. From these results it emerges that, on scales of hundreds of meters (locations were in fact about 300 meters apart and 500 meters offshore), there is no direct coupling between mussel larval abundance in the water and settlers on the rocks.

Considering only the spatial scales that have been examined in this study, it could be that the settlers collected from a particular location arrive from a source of larvae in the water column not directly opposite the intertidal locations. Dispersal and delivery of larvae would act on scales greater than one kilometer. It appears that the dynamics of water movement and larval transport and delivery are the only explanation of why larval availability and settlement were not coupled, especially if we accept that mussel larvae are transported in the water as passive particles (Bourget, 1988; Harvey *et al*., 1995; McQuaid & Phillips, 2000; Ross, 2001). It seems that water currents have a major role in the transport and delivery of larvae from the water column to the shore, at least on scales of hundreds of meters.

A different explanation for this uncoupling of larval abundance and settlement could be sought in the time scales used in the study. In fact, the abundance of larvae in the water probably could have been correlated with the amount of settlers on the rocks at temporal scales different from the ones investigated in this study. The time scales sampled in the present study could have hidden correlations between dispersal and settlement. Indeed, sometimes the lack of correlation between larval abundance and settlement could derive from sampling problems during quantification of processes. Over or underestimation of patterns could be the real reason for lack of correlation, especially if the distribution of larvae is patchy (Pineda, 2000), as is the case for *Perna perna* larvae (McQuaid & Phillips, 2000).

The examination of correlations was done on a relatively large temporal scale, through fortnightly collections, and on a much finer scale, using daily collections from 2000 and 2001. There is reason to believe that there could be a methodological problem especially for the large time scale in the sampling of larval availability. Perhaps larvae in the water could have arrived to the adjacent intertidal locations at intervals that were not considered in this study. For example, it could be that peaks in abundance of larvae in the water were overlooked due to the timing of the sampling. However, the daily collections should have given more precise information on the possible presence of larvae in the water column, since the samples were collected every day and the chances of missing pools of larvae coming from local sources would have been much reduced. Nevertheless, even the daily scale could have been an insufficiently precise measure of coupling if the abundance of larvae in the water column was determined by short-term effects such as tidal or day/night cycles. Also, a very fine-scale sampling design would not help with the precision of measurements if one considers again the natural patchiness of larvae in the water column (McQuaid & Phillips, 2000).

The potential methodological problem that arises from the choice of temporal scales in this study seems to be less relevant for the way settlement was investigated. In fact, settlement integrates information on collection of data over time, while larval sampling is instantaneous, even with replicated sampling (Pineda, 2000). In addition, in this study artificial collectors were left on the shore between one tidal cycle and the next, during the fortnightly survey. Therefore, the collection of data was integrated in time. One could argue that some larvae could be lost if the pads were left on the shore for several days (Pineda, 2000). Nevertheless, most of the larvae would actually remain attached and pads should give a good measurement of the settlement that had occurred on a stretch of rocky shore, during a particular time interval (McQuaid, pers. comm.; Lawrie *et al*., in prep.).

Very fine-scale sampling procedures could be the answer to this problem (Pineda, 2000). Collecting samples at fine spatial and especially temporal scales could help in identifying the real patterns of distribution and therefore, give a more realistic and clear indication of coupling of larval availability and settlement. For example, broad sampling time interval seems to be the main reason for uncoupling of larval abundance and settlement in a study of distribution of larvae of *Concholepas* in Chile by Poulin *et al*. (2002).

However, a better approach to sampling in such a variable system, would be an integrated sampling design allowing the recording of larval abundance in the water through a period of time. Integration of larval abundance measurement over time could be achieved by using intertidal traps and/or nets left in the water for a pre-determined period of time. Intertidal traps were tried in the present study, using traps based on models from Setran (1992) and Castilla & Varas (1998). However, the intense sand movement and the wave-exposed nature of shores in the study area made the use of the intertidal traps impossible as they became completely clogged very quickly. Also, plankton nets could not be set and used in nearshore waters because of the absence of calm seas during the present study.

Larvae, especially when transported in the water, are part of a very patchy and extremely variable system (McQuaid & Phillips, 2000; Natunewicz & Epifanio, 2001). One of the main sources of variability for larvae in the water and arrival at the shore is the hydrodynamics of water currents, so that coupling, or its absence, depends strongly on this factor (Pineda, 2000). Therefore, when investigating processes like dispersal and settlement on a particular stretch of coast, preliminary and relatively broad investigations should be carried out to gain an understanding of the main patterns that generally affect these processes. At the same time, scales of investigation should be kept as fine and integrated as possible.

The complexity of this system is so high that studies such as this one are rather uncommon. There are in fact, few investigations that have tried to link different steps in the life cycle of marine invertebrates with their pelagic larval stages (barnacles and ascidians: McGuinness & Davis, 1989; Jenkins *et al*., 2000; ascidians: Hurlbut, 1991; barnacles: Gaines & Bertness, 1992; annelids: Verdier *et al*., 1997; ascidians, barnacles, bryozoans, hydrozoans: Walters & Wethey, 1996; echinoderms: Balch & Scheibling, 2000). In particular, fewer studies have inspected the factors, relationships or variables that regulate the passage of invertebrate larvae from nearshore waters to the intertidal adult habitat (Gaines *et al*., 1985; Pineda, 2000; Satumanatpan & Keough, 2001; Lipcius & Stockhausen, 2002; Pineda *et al*., 2002; Lawrie & McQuaid, in prep.).

Also, those few studies that have looked at possible relationships between larvae in the water and larvae arriving the shore have found that these processes were generally uncoupled. For example, Pulfrich (1996) found that the arrival of mussel larvae on Wadden Sea rocky shores did not match with either the breeding cycle of the adults or with larval abundance in the water column. Also, Miron *et al*. (1995) have looked at possible relationships between the distribution of barnacle larvae at different depths and settlement at three different levels on the shore. Positive correlation was found only between the numbers of larvae in bottom waters and the larvae settling on the low intertidal. When looking at relationships between larval availability and recruitment, rather than settlement, post-settlement mortality may be a major reason for uncoupling of processes (Ross, 2001).

One of the few studies in which positive correlation has been observed between cyprid availability in the water and settlement of barnacles on the rocks was by Minchinton & Scheibling (1991). They also found that settlement was a positive function of recruitment and adult density. In fact, about 80% of variation in recruit and adult density was explained by the numbers of settlers. Lopez *et al*. (1998) found negative relationships between the abundance of echinoid larvae and their recruitment in those years when many larvae were collected, indicating that, in this case, mortality of larvae was density-dependent.

The present study considered temporal scales in such a way that the product of consecutive spawning episodes could be detected, through fortnightly sampling, as mussel larvae are expected to spend up to three weeks in the water column before settling on the rocks (Widdows, 1991; Pulfrich, 1997). Also, the daily 2000 and 2001 samples, lasting three and five weeks respectively, should have allowed the detection of coupling between availability of larvae and settlement, assuming, again, that larval stages in the water become competent after approximately three weeks (Pulfrich, 1997).

In conclusion, considering both relatively large and fine scales, the present study has given a realistic view of the spatial and temporal dynamics that control *Perna perna* larvae in the water and their arrival to the shore, indicating that no correlation existed. The lack of correlation between availability of larvae in the water and settlers on the shore could be due to two main factors. One reason for the lack of correlation could be the mentioned methodological problem regarding the sampling precision and also the lack of integration of larval data. Secondly, the lack of correlation between larval and settler abundances could be due to differential delivery of larvae arriving from nearshore sources. Differential delivery would impose a differential and therefore, uncorrelated distribution of individuals in the water column and on the shore.

Chapter 5

Effect of wind on settlement of *Perna perna*

"Contrariwise- continued Tweedledee- if it was so, it might be,
and if it were so, it would be; but as it isn't, it ain't.
That's logic!"
Lewis Carroll

Introduction

Many studies on the dispersal of invertebrate pelagic larvae and their arrival on the rocky shore have suggested wind as a possible influence (Pineda, 1991; Bertness *et al.*, 1996; Wing *et al.*, 1998; Roman & Boicourt, 1999; Pineda, 2000; Natunewicz *et al.*, 2001; Norkko *et al.*, 2001; Poulin *et al.*, 2002; Barnay *et al.*, 2003). For example, it has been shown that settlement of megalops larvae in estuaries occurs in pulses and that these pulses follow wind events (Natunewicz & Epifanio, 2001). In this particular case, the transport of larvae was suggested to be wind-driven, with northward winds spreading larvae offshore and affecting the size of larval patches (Natunewicz & Epifanio, 2001). Shanks (1998) also suggested that the transport of megalops larvae of *Penaeus* and *Uca* was affected by wind-driven surface currents. Poulin *et al*. (2002) noticed that larvae of the gastropod *Concholepas concholepas,* in Chile, were retained between the shore and the nearshore waters (< 5 km from the coast) due to alternating events of upwelling (when larvae were moved offshore) and relaxation (when larvae were brought back close to the shore). Raimondi (1990) suggested that the highest settlement peaks in barnacles occurred following days of strong winds and before the maximum high tides. In particular, he observed that an increase in wind the day before settlement would move larvae onshore, favouring delivery and settlement.

On the coast of South Africa it has been observed that inshore currents are wind forced (Schumann *et al.*, 1988; Schumann 1989, 1999; Goschen & Schumann, 1994). In particular, the influence of wind on surface currents is direct, while the effect of wind decreases below the surface (Field *et al.*, 1980). Field *et al*. (1980), when investigating the effect of wind on nearshore surface currents in the Benguela system, showed that, at 11m depth, currents were related to the strength and direction of the wind over the previous 36 hours.

However, most investigations on coastal current circulation on the south coast of South Africa have been limited to Algoa Bay (see Fig.1, chapter 2). Current patterns and the influence of the wind may change on very fine spatial scales, especially in the nearshore waters where local variability of the coastal and bottom topography are important (Field *et al*., 1980; Goschen & Schumann, 1988). In addition to this, the action of wind, internal waves and tides and, on this coast, the Agulhas current, can have different effects in bays and on the open coastline (Goschen & Schumann, 1988; Pineda, 2000). For example, Goschen & Schumann (1988) found that the influence of wind is reduced on the open coast just outside the eastern end of Algoa Bay, compared to within the Bay itself. Conversely, the effect of the Agulhas current is greater outside Algoa Bay, than in the Bay (Goschen & Schumann, 1988).

Despite the importance of wind effects on pelagic larval transport and delivery, the number of studies on the relations between local wind patterns and larval transport and delivery is very limited in South Africa. Moreover, most of these studies have been restricted to the examination of dispersal, transport and retention and settlement mechanisms of the ichthyoplankton, especially of commercially important fish species like sardines, mackerel, anchovies, pilchards and soles (Beckley & van Ballegooyen, 1992; Boyd *et al*., 1992; Mann, 1992; Olivar *et al*., 1992; Roy et al., 1992; Villacastin-Herrero et al., 1992; Tinley *et al*., 1997; Hutchings *et al*., 1998). Such larvae are less likely to behave as passive particles than those of invertebrates. Hutchings *et al*. (2002)

have considered the strong currents around the Southern African coastline as a potential risk for offshore dispersing planktonic eggs and larvae from broadcast spawners, especially pelagic, demersal and inshore-dwelling fishes. Boland (1997) has suggested that the geographic differences in adult barnacle abundance between the south and west coasts of South Africa could be due to pre-settlement processes. Larval transport would be regulated by onshore winds on the south coast and offshore wind-driven currents on the west coast, leading to a differential distribution of larvae along the two coasts (Boland, 1997).

In general, very few studies have examined the distribution of invertebrates in nearshore waters (Barange & Boyd, 1992). Even fewer have explored the relations between the processes that influence the population dynamics of benthic species and wind-forced currents (Commito *et al*., 1995; McQuaid & Phillips, 2000).

Providing evidence of the relationships between physical and biological processes becomes very important especially when trying to estimate, predict or simply to understand the final size of benthic populations. For example, mathematical models have been applied to predict the recruitment of the annelid *Owenia fusiformis* in the Gulf of Lyon, France. In this case, larval dispersal and recruitment were dominated by wind-driven currents. In particular, the simulations indicated that different proportions of larvae would be lost depending on the wind climate; 60% of the larvae reached adults habitats during maximum downwelling events as opposed to only 15% during upwelling events (Verdier-Bonnet *et al*., 1997). Therefore, clarification of the relationships between wind patterns and settlement rates of a species such as *Perna perna*, is very important in order to understand and predict population dynamics and distribution.

Materials & Methods

In this study possible relationships between wind direction and settlement rates were considered. During this study, the data analysed for settlement were taken from the fortnightly seasonal pad collections completed during the settlement study (chapter three). The mean numbers of settlers collected at each location during the fortnight full spring tides were considered. The average of all locations was then calculated and used for correlation analysis in association with the wind data.

Hourly records of wind direction and speed were taken from the Port Alfred weather station (about 30 km from the study sites), for March 2000 to June 2001.

The wind records were initially divided into four main directions, NE, SE, SW and NW, the predominant winds in this area. However, since the rocky shores of both Kenton-on-Sea and High Rocks are south east oriented, only offshore and onshore winds were considered in the analysis for purposes of plotting. Southeasterly winds were considered onshore while the northwesterly winds were regarded as offshore. Also, the other two directions of wind (NE and SW) made a low contribution to the annual and seasonal wind patterns.

At first, the hours for which the wind had blown in one of these two main directions were summed up for each month. The total hours of southeasterly winds in each month and the total hours of northwesterly wind were plotted against the fortnightly means of settlers.

Because surface water currents, and the particles carried with them, can change direction quickly and the maximum influence of the wind on surface water currents is given by the wind during the previous 36, 24 and 12 hours (Field *et al*., 1980), the total hours of wind blown in each of the considered directions were calculated for 12 and 24 hours previous to the collection of pads for the settlement counts. Settlement data, for each fortnightly collection, were plotted against the corresponding 12-hour totals of southeasterly and northwesterly winds. The correlation analysis was performed using the Statistica 6 software package.

Results

Preliminarily, correlation was run to examine the effect of wind on daily 2000 and 2001 settler abundance. However, since this analysis did not show even the most tenuous relationship, these results are not going to be discussed here.

The relations between northwesterly, southeasterly winds, time of the year as a circular variable and fortnightly means of settler abundance were initially determined by using partial correlation. Partial correlation considers the relation between two parameters holding others constant. Vectors calculated from the wind direction and speed were also considered and used for partial correlation analysis. However, no significant results were observed from any of these analyses and, therefore, the results of the partial correlation are not shown here.

The examination of possible relationships between the abundance of settlers in the scouring pads and the direction of the main offshore and onshore winds was particularly difficult, and probably biased, for one main reason: the abundance of settlers arriving at the shore was very low throughout almost the entire sampling period. Therefore, possible negative or positive correlations between settler abundances and wind direction would not have been easy to show. Nevertheless, a possible relationship between wind and settlers can be observed when plotting the mean of seasonal settler abundances against, respectively, the sum of southeasterly and northwesterly winds blown during the month previous to the pad sampling (Fig.1).

Settlers seemed to be most abundant when northwesterly winds dropped and southeasterly onshore winds intensified (Fig. 1). This coincided with the start of spring and continued for the whole summer. Westerly and easterly winds follow seasonal fluctuations, with west winds prevailing throughout the year, but dropping during summer, from October/November to March (Hunter, 1981; Schumann *et al.*, 1991; Schumann, 1999; Fig. 1). On the other hand, the maximum duration of easterly winds is recorded during the summer months (Schumann *et al.*, 1982; Phillips, 1994).

When examining the relationship between the fortnightly means of settler abundance and the sum of southeasterly winds in the 12 hours previous to sampling, no significant correlation could be detected (Table 1). However, plotting of the data suggested a positive relationship between the two variables. Whenever southeasterly winds were minimal, or had just started blowing, settler abundance increased. It seems that the onset of southeasterly winds may enhance the number of settlers (Fig.1). Therefore, although the number of settlers was generally low, there was a slight (non-significant) positive relation between these winds and the abundance of settlers (Fig. 2).

Figure 1 suggests that, as the duration of offshore northwesterly winds increased, there was a decrease in the number of settlers, while an increase in settler abundance followed periods during which this wind was not as dominant. A weak negative relation between northwesterly winds and settler abundance can be detected, although this is biased by a single point (Fig.2). On a seasonal basis, it appeared that when the northwesterly winds prevailed (winter months), the abundance of settlers was very low, while maximum numbers of settlers were collected in the pads when these winds almost reached their minimum (27 December 2000; see Fig.3, chapter three). Nevertheless, these possible patterns were extremely weak and correlation analysis showed no significant relationship in either case (Table 1).

Table 1 Correlation between wind direction and settler abundance. *r* = correlation coefficient; p = probability value.

Correlation SE winds vs. settlers		Correlation NW winds vs. settlers	
r	p	*r*	p
0.18	0.36	-0.13	0.49

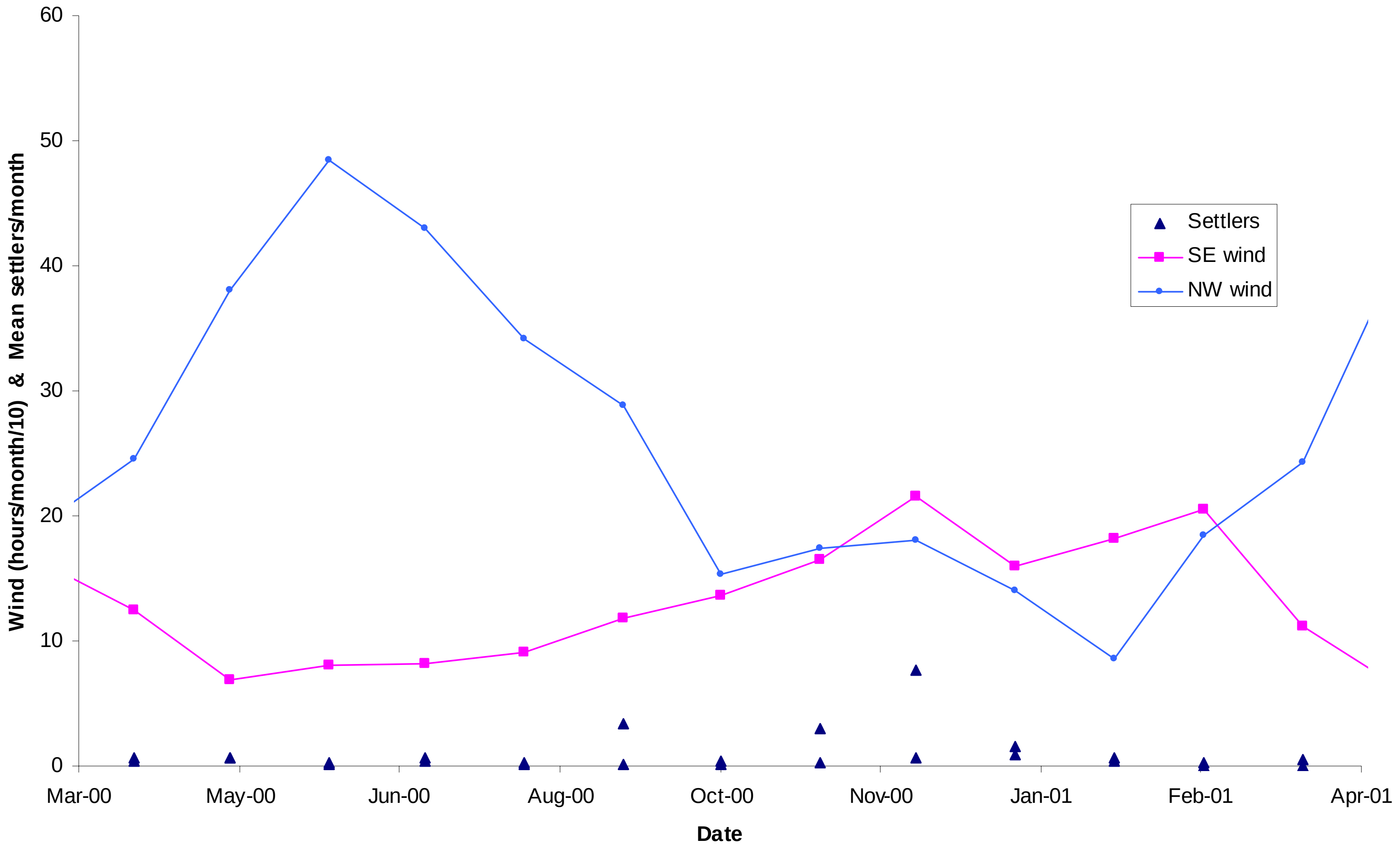
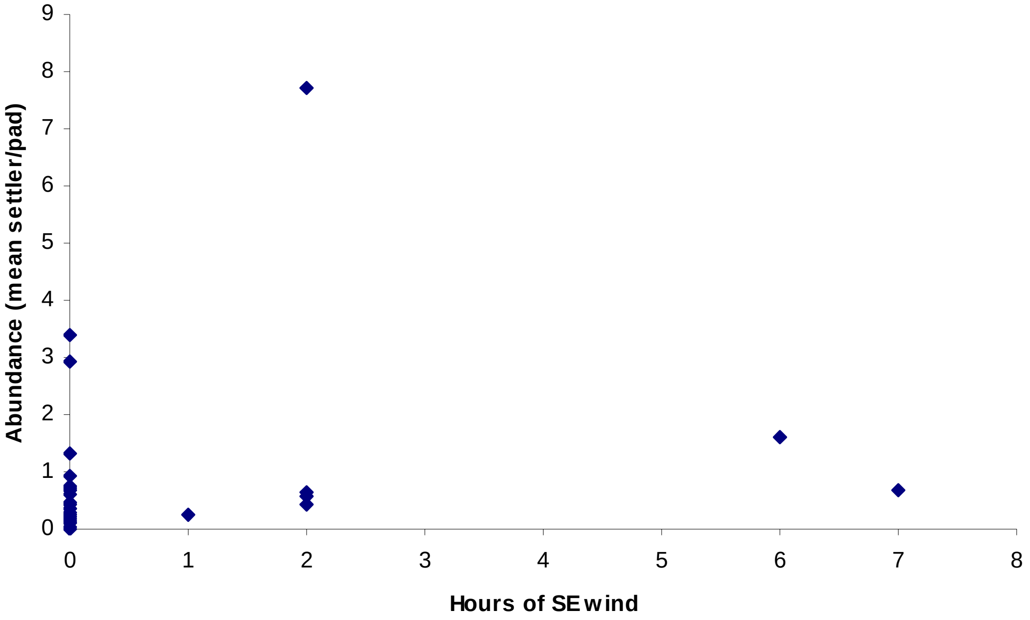


Fig. 1 Northwesterly (NW) and southeasterly (SE) winds and settler abundance.



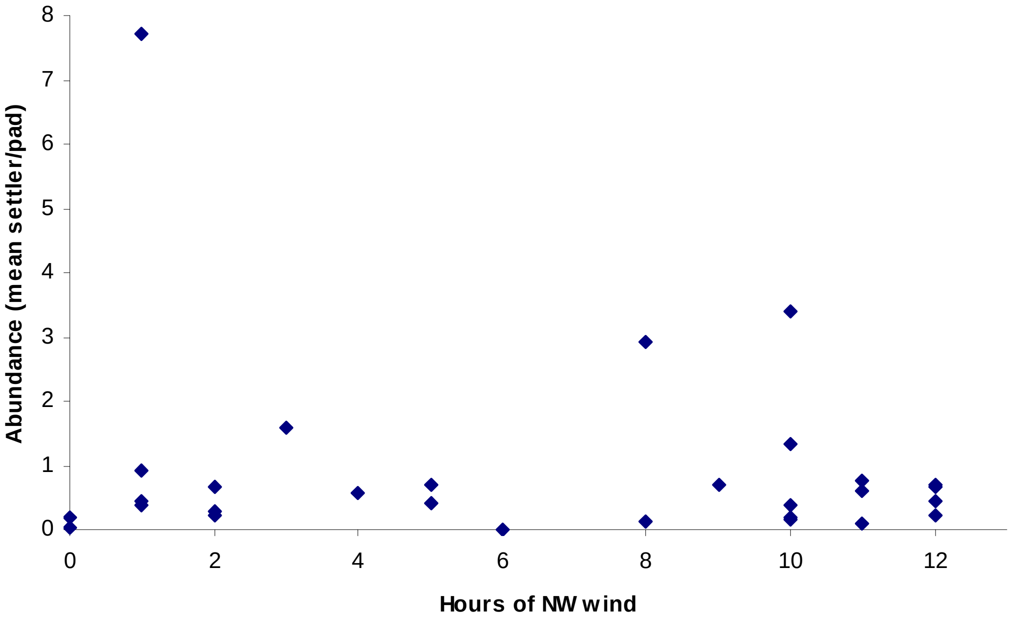


Fig. 2 Scatter diagram of wind (SE top; NW bottom) and settler abundance.

Discussion

The influence of wind on surface currents, larval transport and settlement of invertebrates is likely to be very important. In fact, unless larvae are deep in the water, hydrodynamics are amongst the main factors affecting the arrival of larvae on the shore, determining settlement spatial variability and, therefore, giving shape to the final adult population size and distribution (Gaines *et al*., 1985; Raimondi, 1990; Gaines & Bertness, 1992; Bertness, *et. al*. 1996).

In the case of mussels, the effect of wind on larval delivery should be relatively easy to detect because larvae, initially dispersed in the water column, are transported like passive particles. Therefore, the direct action of wind on surface currents can limit larval dispersal and influence the delivery of larvae to the rocks (Phillips, 1994).

Since the sites used for the present study were southeasterly oriented, the dominant winds considered here were onshore and offshore winds, on the sea-land axis, rather than longshore winds. It has been suggested that the biggest changes in wind direction and speed occur on the sea-land axis, while the local coastline and topography also add variation to the wind patterns (Jury & Guastella, 1987; Schumann *et al*., 1991).

In this study, relationships between wind patterns and settlement were difficult to detect mainly because of the low numbers of settlers collected from the scouring pads throughout the whole survey. In fact, no clear evidence of any real relationships could be established in this case. Nevertheless, the results do suggest a possible trend of *Perna perna* settlement on the shore mainly when offshore northwesterly winds drop and onshore southeasterly winds increase.

Many investigations have examined the relationship between wind patterns and settlement, mostly of barnacles. These studies have often produced different results regarding the influence of onshore or offshore winds on larval settlement. For example, Shanks (1986) found no obvious relationship between settlement of barnacles and wind-generated currents, while Hawkins & Hartnoll (1982) observed that the maximum settlement of barnacles occurred during onshore winds, or better, during onshore flow. Bertness *et al.* (1996) also confirmed the positive influence of onshore winds, when examining wind-driven patterns in barnacle settlement. In contrast, Bennell (1981) found a positive relation between settlement and offshore winds. Indirect evidence of the influence of wind on settlement and recruitment was also given by the onshore accumulation and delivery of larvae on a Californian shore, during periods of calm weather (Roughgarden *et al*., 1991). Blanton *et al*. (1995) also found that the maximum settlement of crab megalops larvae was reached during periods of calm winds. Lambert & Harris (2000) suggest that sea urchin larvae from the Gulf of Maine concentrate onshore during persistent onshore wind periods, when larval settlement reaches its maximum.

In this study, the wind patterns observed followed the normal pattern of seasonal wind fluctuations in this area (Schumann *et al*., 1988; Schumann *et al*., 1991; Phillips, 1994).

Phillips (1994) confirmed that on the south coast of South Africa the predominantly northwesterly winds of winter favour gametogenesis and spawning of mussels, while recruitment is enhanced by south- and northeasterly winds, which are more frequent from spring to autumn. Seasonal fluctuations in mussel spawning have also been observed in two other studies carried on this coast. Both studies confirmed that the main spawning of gametes occurred during the austral winter months, from May to September (Ndzipa, 2002; Lawrie & McQuaid, in prep.), when there are strong offshore winds and maximum localised upwelling (Schumann *et al.*, 1982; Lasiak, 1986; Phillips, 1994).

Obviously spawning seasonality is important. In fact, during the winter months, when spawning occurs, the probability of mussel larvae settling on the rocks is minimal. The problem is that, during the winter months, the absence of settlement could be due to two variables: wind and, or spawning. The low numbers of larvae collected during this study, from May to August, could be due either to the timing of spawning (Ndzipa, 2002; Lawrie & McQuaid, in prep.) or to the offshore winds that prevail during this time of the year (Schumann *et al.*, 1988; Phillips, 1994). The prevalence of offshore winds in winter would transport larvae away from settlement sites. Alternatively, settlers could be missing from the shore in winter because spawning had just occurred, since after external fertilization, mussel larvae spend 3-4 weeks in the water column before becoming competent to settle. However, at the temporal scales that have been investigated in this study, no larvae were collected from the water column during winter. Therefore, it is more likely that offshore wind could have transported larvae over distances greater than 500m. Alternatively, a possibility could exist that winter spawning was minimal or absent during the year of the study, but this aspect was not measured in this study.

During the rest of the year, in particular during spring and summer, the predominance of onshore southeasterly winds increases, possibly resulting in larvae being transported ashore. The peak of settlement observed at the end of December 2000 could have been favoured by the slight increase of onshore winds. However, this interpretation, although suggested from the plotting of settler abundances and wind data, is highly speculative as no significant correlation was recorded.

In conclusion, it seems that the low numbers of settlers collected in the scouring pads during this study have probably masked the possibility of finding significant relationships between wind and settlement.

In order to establish the possible effects of wind direction on the settlement of *Perna* larvae, further investigations would be necessary. These studies should probably be carried out in bays, to maximise the abundance of settlers, as settlement rates are usually higher in bays or harbours than on the open coast (Phillips, 1994; Archambault & Bourget, 1999; pers.obs; Bownes, pers. comm.). However, when examining the effects of wind on inshore larval transport and delivery, we should also consider the importance of the shoreline topography and the extreme variability of wind on very fine spatial scales (Xie & Eggleston, 1999). Wind does influence the surface and deeper water currents, but its direct effect is controlled and modified by the local topography, the sea-land interface, boundary layer structure and finally by the choice of sites where wind measurements are performed (Goschen & Schumann, 1988; Schumann, 1989; Archambault & Bourget, 1999).

Chapter 6

General Discussion

> “ *If we knew what we were doing, it would not be called research*”
> **Albert Einstein**

Sexual reproduction through external fertilization is a very common strategy found in organisms living on land and sea, from plants, to many marine invertebrates, especially sessile and sedentary forms (Crisp, 1976). External fertilization often leads to high dispersal of gametes and zygotes or larvae. In the case of sedentary organisms, external fertilization also leads to settlement and recruitment of juveniles to the adult substrata (Bhaud, 2000).

External fertilization, with the associated potentials for high levels of dispersal and the necessity for larval return to suitable substrata via settlement, leads to many evolutionary trade-offs. External fertilization involves both advantages and disadvantages. For example, in the case of the rocky shores and mussel populations, there is high mortality of gametes during spawning. Gametes can disperse rapidly in such a high wave energy environment and prezygotic selection is almost absent in mussels (Thorson, 1950; Underwood & Keough, 2001). Only few sedentary forms, like barnacles, copulate; many organisms do not show courtship behaviour and there is no barrier to gene flow due to incompatibility of genital organs, since no copulation occurs (Knox, 1963). Moreover, the eggs spawned by one female can mix with sperm released by different males. Therefore, the great disadvantage of external fertilization is that there is no individual selection for the best partner. This removes several potential barriers to hybridisation and also leads to potential loss of energy caused by the generally high rates of hybridisation failure.

On the other hand, there are advantages to external fertilization. The most important and obvious are the greater chances of gene exchange and recombination in external fertilizers than in species with asexual or internal fertilization strategies (Stearns, 1993). To ensure fertilization success, especially in a variable environment like water, many species have adopted synchronization of spawning, to increase egg and sperm encounters (Knox, 1963; Morgan, 1995; Morgan, 2001; Underwood & Keough, 2001). Among external fertilizers, including mussels, the only pre-zygotic barrier available to prevent hybridisation is incompatibility of the gametes. However, this type of barrier is not entirely effective since there are successful cases of mussel hybridisation. For example, *Mytilus edulis* and *M. galloprovincialis*, on the southwestern British coast, do hybridise successfully (Gilg & Hilbish, 2003). Nevertheless, Bierne *et al.* (2002) suggest that assortative selection and reduced hybrid fitness maintain and limit the mussel hybrid zone in Europe.

Dispersal and settlement of larvae involve trade-offs too. Again, using mussels as an example, the biggest disadvantage is the high, unavoidable larval waste due to the mortality of the larvae while still in the water, or during their transport away from the shore (Thorson, 1950; Todd, 1985; Dame, 1996; Underwood & Keough, 2001). Because larval wastage cannot be avoided; it seems that the concept of "spreading the risk" proposed by Reddingius & den Boer (1970) applies. If larvae are retained, there is a risk of rapid disappearance of local populations whenever patches of adult populations are removed, due to physical or biological disturbance (Underwood & Keough, 2001).

Yet, the largest evolutionary advantage is the gene flow that is guaranteed by larval transport and delivery (Scheltema, 1986; Strathmann *et al.*, 2002). Since one of the main causes of adult mussel mortality is competition for space on the shore (Griffiths & Hockey, 1987), dispersal of larvae ensures the establishment and success of open populations. The adults will be far from their offspring and offspring will settle far from their siblings (Strathmann, 1974; Todd, 1985). Because of the pressures of intra- and interspecific competition for space, sedentary forms like mussels also need to maximise their occupation of space and to exploit any empty areas available (Todd, 1985; Dame, 1996; Seed *et al*., 2000). Dispersal guarantees effective occupation of space at different spatial scales (Crisp, 1976), while re-colonisation of empty patches after physical or biotic removal of adult population will also be faster than in closed populations (Underwood & Keough, 2001).

The last major advantage of the reproductive strategy exhibited by mussels is the production of a long planktotrophic larval stage, able to feed while being transported in the water column (Bayne, 1976; Todd, 1985; Scheltema, 1986). This could be seen as a negative point for the development and survival of the individual itself, since the newly hatched larva, requiring a large amount of food, depends totally on extrinsic phytoplankton resources, and larval mortality rates in the water are very high (Thorson, 1950; Todd, 1985; Lutz & Kennish 1992). However, it appears that within species, competition for food in the water column is not a restricting factor for planktonic marine larvae (Strathmann, 1996). In addition, planktotrophic larvae are energetically cheap to produce since the eggs contain a small quantity of yolk (Thorson, 1950).

It seems rather that the main risk for mussel larvae is to be transported over large distances before returning to the settlement sites. Therefore, being able to provide food for itself and especially being able to delay metamorphosis (Thorson, 1950; Bayne, 1976), if necessary, are effective adaptations in an environment where the unpredictable direction and strength of currents are amongst the main determinants of transport and delivery of larvae (Underwood & Keough, 2001).

In addition to the evolutionary trade-offs associated with the reproductive and life strategies of mussels, there are also ecological trade-offs. For example, since there is little or no behavioural influence on larval transport in the water column, or on larval delivery to the shore, it appears that dispersal and settlement are powerfully affected by abiotic factors, in particular, currents (McQuaid & Phillips, 2000). In fact, currents can control the scales of dispersal of larvae and individual survival rates. Balanced against this are the positive evolutionary effects of currents in terms of external fertilization, dispersal and settlement. In particular, the action and influence of abiotic parameters like currents on larval transport and delivery vary greatly at different spatial and temporal scales. The variability of currents can make dispersal and settlement of larvae even more unpredictable processes making the prediction of final population structure even more difficult.

This study has confirmed the high levels of variability in time and space when examining the abundance of larvae in the water and of settlers on the shore. Temporal variability was particularly obvious in this study, both on large (seasonal, lunar phase and tidal) and finer (daily) temporal scales. Since the density of larvae varies so much in time, reliable and precise predictions of the presence or arrival of larvae cannot be made on this coast.

Nevertheless, the summer season proved to be the period with the highest abundances of settlers. Different studies, performed on this stretch of coast, have demonstrated that the main mussel spawning time is during the winter months. However, the different studies have not agreed on specific months during winter (Lawrie & McQuaid, in prep; Ndzipa & McQuaid, in prep). The same seems to apply to the seasonality of larval settlement. The summer months have proven to be the preferred months for mussel settlement, but again, different studies show peaks of settlement during different months in this season (Lawrie *et al*., in prep; present study).

Summer appears to be an appropriate season for settlement, as the weather and water conditions are more benign than during the stormy and turbulent conditions typically encountered during the winter months. However, even in summer, hydrodynamic conditions in the southern hemisphere are probably less predictable than in the northern hemisphere (Dame, 1996).

Many studies have shown the influence of the tidal and lunar phases on spawning, transport and delivery of pelagic invertebrate larvae (Eggleston *et al*., 1998; Jeffery & Underwood, 2000; Paula *et al*., 2001; Flores *et al*., 2002; Tankersely *et al*., 2002). This effect could be an additional mechanism for ensuring synchronisation of spawning, dispersal and settlement (Knox, 1963). Being transported simultaneously and, especially, being delivered together, could help larvae to reduce losses caused by predation, although it could also increase intraspecific competition.

The present study does not show a clear positive relation between tidal or lunar phases and settlement. However, although the statistical analyses are equivocal, the data suggest that there is a certain synchronisation of settlement during the new moon phase.

This could also be interpreted as a tidal effect, especially of tidal amplitude, on larval delivery. In fact, the settlement peaks for this study corresponded with new moon phases during the months of September, November, December 2000 and January, February 2001. In almost all these cases, the tidal amplitude during these spring tides was relatively small, with maximum tidal height being rather low. This could appear to be problematic for colonisation of substrata, as small tidal amplitude would give larvae less chance to settle on the shore during maximum spring tides. On the other hand, smaller tidal amplitude could also mean relatively small turbulence, less wave impact at the delivery sites and, thus, less risk of the newly settled larvae being removed by currents. However, this interpretation of the new moon influence on larval delivery is speculative since no statistically significant pattern was observed. Also, more work should be done, focusing not only on the study of the tidal influence on settlement and delivery, but also on the hydrodynamics of waves at very fine spatial scales that act directly on the delivery of larvae on the shore.

Another factor that could have a strong impact on the delivery of larvae to the shore is the wind (Gaines & Bertness, 1992; Bertness *et al*., 1996; Forward *et al*., 1997; Natunewicz & Epifanio, 2001; Underwood & Keough, 2001; Flores *et al*., 2002; Tankersely *et al*., 2002). The wind can show seasonal patterns in speed and direction and certainly this is the case for the south east coast of South Africa. Offshore winds dominate during winter, while onshore winds prevail in summer (Phillips, 1994; pers obs). Previous studies, carried out in this area, have shown that spawning of mussels occurs mainly during the winter months (Ndzipa, 2002; Lawrie & McQuaid, in prep.) when the prevailing winds are offshore. Therefore, gametes and larvae are likely to be transported offshore during this season (Phillips, 1994; pers obs.).

In this study, wind did not seem to be strongly linked to the arrival of larvae onto the shore, though there was a tendency for settlement to occur during the onshore winds, characteristic of the summer (Fig. 1, chapter 5) when larvae are likely to be passively transported back to the shore.

The measurement of dispersal of invertebrate larvae concerns the possible spatial scales over which larvae are transported in the water column. If the spatial scales of transport and delivery are known, predictions on the inshore larval distribution can be made and, population structure could also be estimated indirectly (Underwood & Denley, 1984; Raimondi, 1990; Minchinton & Scheibling, 1991; Minchinton & Scheibling, 1993; Connolly *et al*., 2001; Morgan, 2001).

This study shows clear spatial patterns of distribution, which differ for larvae in the water and settlers on the rocks. Larval distribution in the water column was not significantly affected by scales of a few kilometers (site scale), although differences in larval abundances were found between the two sites. Also, the availability of larvae did not show a significant location effect. However, location (scales of hundreds of meters) consistently showed significant effects on settler abundances. For example, the location KE B showed persistent maximum numbers of settlers during both daily settlement studies and the neap tide recruitment study.

The effect of location on settler abundance highlighted differential delivery of larvae on the shore at scales of hundreds of meters. It may even be that differential delivery could act at smaller scales, with settler distribution being regular even on scales of meters, but this was not tested here. Adult mussel distribution on the shore is very irregular, varying between isolated patches of a few animals to large areas entirely covered with mussels. Wahl (2001) observed a very patchy distribution of organisms when examining variability of settlers of algae and blue mussels among replicates 50 meters apart. The patchiness, often encountered in adult populations, could reflect what happens on the shore during settlement. If larvae are delivered in a uniform way, without discrimination among settlement areas, post-settlement mortality would determine the final coverage of juveniles and adults on the shore (Knox, 1963; Underwood & Denley, 1984; Erlandsson & McQuaid, unpubl. data). Alternatively, larvae could show differential settlement with settlement itself being highly variable at very fine scales (Underwood & Denley, 1984). de Vooys (2003) showed that chemical communication stimulates aggregation behaviour in adults of *Mytilus edulis*, but he also suggests that the same chemical cues used by the adults could also influence settlement. The possibility of settlement cues provided by juvenile or adult conspecifics was not considered during this survey, but it makes a very interesting topic for future studies on mussel settlement and its influence on final population structure.

In order to understand better the scales of transport and delivery of larvae and to make possible predictions on the future of larvae and the structure of adult populations (Seed, 1976; Underwood & Keough, 2001), dispersal and settlement should be assessed simultaneously (Suchanek, 1985; Underwood & Keough, 2001). Local studies on mussel larval dispersal suggest that the scales of transport of the majority of larvae are relatively limited (of the order of 10's of kilometers; McQuaid & Phillips, 2000). However, the influence of factors like currents and wind can enhance these scales considerably. Large or increased scales of dispersal may not be advantageous for planktotrophic larvae and may lead to a great loss of larvae, because of advection away from suitable settlement areas (Palmer & Strathmann, 1981). Many studies of fish and invertebrates suggest larval retention, with various kinds of control retaining larvae relatively close to the sites of reproduction (Keough, 1988; Gaines & Bertness, 1992; Underwood & Keough, 2001). Sale (1991) suggests that it would be "safer" for coral reef fish larvae to settle "close to home" rather than far away, although variation in settlement rates, at fine spatial scales, is still observed due to the patchy distribution of adults on the reef. In general, we could, therefore, speak of "ideal intermediate" scales of dispersal, where larvae are transported over distances that still guarantee successful settlement on the shore after a few weeks.

This study did not show any coupling between the abundance of larvae in the water and of settlers on the rocks. This observation reinforces the idea of the importance of the scales of larval transport and delivery as regulated by physical factors, especially by hydrodynamics. Although the results of this study do not provide direct insight on the scales of dispersal, we can see that larvae are transported over spatial scales that do not couple with the scales at which they are delivered to the shore. Being transported far, but not too far from the parental sites could be an advantage for the survival of the individual and the population as a whole. Mussels are still guaranteed good gene flow and are still able to re-occupy territories after disturbance or colonise new areas. They are also able to ensure the settlement of offspring far from their parents to avoid competition for space amongst related individuals. However, mussel larvae have no control over their transport in the water. If hydrodynamics are so important, this could explain the contrasting results from different studies.

To conclude, this study, by examining the variation in larval availability and settlement of *Perna perna* larvae on a range of spatial and temporal scales, has highlighted the importance of those ecological components, especially hydrodynamics, operating at different steps of the life history and influencing the survival of individuals and the structure of the population. Larval availability and settlement are among those parameters that crucially regulate the distribution and abundance of marine mussel populations (Morgan, 2001; Underwood & Keough, 2001). The combined investigation of both larval availability and settlement has allowed us to understand the different spatial scales of distribution of mussel larvae and settlers on this coast. In particular, through this design, it has been possible to determine the spatial scales of settlers arriving to the shore (hundreds of meters). It has also been possible to show that differential delivery of larvae, rather than differential distribution of larvae in water, determines the final distribution of settlers, and possibly of adults, on the shore. However, in addition to differential delivery, post-settlement mortality should also be taken into account when trying to understand the distribution of adult and the final size of the adult populations. The main findings of this study could be summarised in two conceptual models represented in figure 1. These models show two possible ways of structuring adult mussel populations from the point of view of differential spatial distribution of larvae in the water column or differential delivery of settlers on the shore. Adults on the shore are characterised by different population sizes (A or B in figure 1). The final size could be determined either by a different amount of larvae, distributed differentially in the water or by differential delivery, acting at settlement level. Both models should include a certain degree of post-settlement mortality, which was not taken into account in this study. The model that best suits the results of this study is differential delivery, model number 2 (Fig. 1).

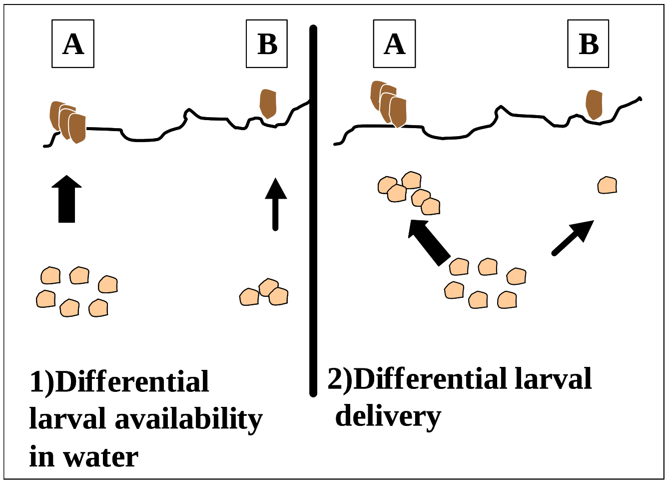


Fig. 1 Conceptual models for mussel distribution on the shore. 1) Final population size determined by differential larval availability. 2) Final population size determined by differential settlement. A and B represent two possible adult population on the shore (scales hundreds of meters); Black lines represent the shoreline; circular bodies represent mussel larvae in the water; long bodies represent adult mussels on the shore.

Final Summary and ideas for future research

The spatio-temporal approach of this study has been fundamental to a deeper understanding of the variability of *Perna perna* larval abundance in the nearshore waters and their settlement on the rocks.

First, the analysis of the spatial variability of larval and settler abundance has highlighted the importance of differential delivery of larvae from the water to the shore. This process seems to provide good support for what could be identified as spatial synchronisation, meaning the accumulation and assemblage of larvae towards the settlement substrata.

The temporal aspect of the study has allowed an understanding of synchronisation of arrival on the shore, despite the strong variability in timing of larval abundance in nearshore waters. This suggests the importance of wind and lunar/tidal action on the delivery of *Perna perna* larvae. However, this aspect should be carefully tested using a specific experimental design focused on the effects of tidal and lunar phases and on the precise action of the wind on the surface water currents. This last feature would require an understanding of local hydrodynamics and coastal water circulation patterns.

Finally the lack of coupling between the abundance of larvae in the water column and settlers on the shore emphasises the fact that dispersal, under the influence of currents, determines the transport of larvae from nearshore waters to the shore. However, this process operates at different scales, depending on how far offshore the larvae are carried. In addition, even if past studies have proved dispersal to be limited to local scales, it still operates at scales that are at least larger than a kilometer. Nevertheless, future studies on the scales of dispersal and on the spatio-temporal relationships between dispersal and settlement should seriously consider a much finer temporal scale sampling design. The presence and abundance of larvae in the water is highly variable, but an intense and frequent sampling design could facilitate the interpretation of the coupling phenomenon and determine in a more accurate way the real scales of distribution of larvae while still in the water. To conclude, all these studies, dealing with the abundance and distribution patterns of invertebrate pelagic larvae in the water and their arrival onshore, should include knowledge of local hydrodynamic conditions, which have been claimed to be one among the most important causes of variability in terms of both time and space.

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“Probable impossibilities are to be preferred to improbable possibilities”
Aristotle

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