Recruitment limitation and competent settlement of sesarmid crab larvae within East African mangrove forests

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ABSTRACT: The functioning of many ecosystems is highly dependent on their faunal communities, dominated by brachyuran crabs. Key to the maintenance of crab populations are the factors that influence their recruitment into the system, particularly the settlement of their megalopae. While spawning is normally highly synchronised, settlement is often not, implying temporal disruption between spawning and settlement. We examined the roles of several key factors (vegetation type, day/night cycle, and lunar phase) on settlement, testing for generality by working in mangroves at 2 sites in Kenya and South Africa. At each site, larval settlement was quantified using artificial collectors placed in 2 vegetation zones for 12 h periods throughout the part of the neap-spring tidal cycle when tide was sufficient to cover the collectors. This continued for 6 mo in Kenya and 11 mo in South Africa, giving the first long-term settlement dataset for east African mangroves. Moon phase proved to have a major role in megalopal settlement, which demonstrated a high degree of spatial specificity. Settlement was correlated across species with tidal amplitude on both short (monthly) and long time scales and focussed on the vegetation zones occupied by conspecific adults. As the distribution of mangrove species is tightly related to local hydrology, this implies a feedback situation with the conservation of mangroves requiring the maintenance of patterns of water flow within the system to support both the vegetation and the associated fauna.

KEY WORDS: Crustacean megalopae · Tidal amplitude · Competent settlement · Community composition

1. INTRODUCTION

The great majority of intertidal invertebrates rely on pelagic larvae to disperse and colonise novel habitats (Morgan & Christy 1995, Becker et al. 2007, Siegel et al. 2008, Cowen & Sponaugle 2009). The proximate and ultimate factors that influence the spatio-temporal patterns of settlement and the recruitment of late-stage larvae are crucial determinants of the composition and dynamics of communities, and understanding these processes has long been a central goal of marine ecology (e.g. Underwood & Fairweather 1989). The drivers of larval production and dispersal include a plethora of biotic and abiotic factors, com-

prising adult reproductive rhythmicity, larval behaviour and mortality, availability of settlement habitats and hydrodynamics (Hughes et al. 2000, Metaxas 2001, Largier 2003, Becker et al. 2007), but we know less about the factors that affect the last phase of the pelagic life and how late stage larvae locate and settle in suitable sites (Moksnes 2002, Pineda et al. 2010).

Worldwide, the reproductive biology of intertidal crabs is highly predictable as it is strongly regulated by cyclical environmental cues, such as tidal ebb and flow, the light–dark cycle, spring and neap tides and seasonal differences in temperature (reviewed by de la Iglesia & Hsu 2010). Synchronous larval release is common in intertidal crabs, ranging from annual to lunar and tidal synchrony (Morgan & Christy 1995, Skov et al. 2005). Tidal synchrony is thought to be advantageous as it reduces the risk of predation (Morgan & Christy 1995) and mitigates the potential effects of lethal salinity and temperature (reviewed by Forward 1987, Christy 2011).

As a consequence of such highly synchronised larval release, megalopal settlement is regular and predictable in some species of crabs and other marine taxa (Morgan et al. 1996, Shanks 2006). In the majority of crab species, however, reproduction and settlement do not show a tight temporal relationship, and settlement patterns are not highly predictable (Paula et al. 2001, 2003, Flores et al. 2002, Olaguer-Feliu et al. 2010). Indeed, cyclic patterns and the factors influencing synchrony, or lack of synchrony, in settlement are not yet clear. Megalopal settlement has been related to the highest spring tides (Paula et al. 2001, Ragionieri et al. 2015), with shoreward transport driven by multiple drivers, such as internal waves over the shallow waters of the shelf (Shanks 1983, Pineda 1991). Predation and physiologically driven selective pressures are also likely to influence the arrival and settlement of megalopae at adult sites, selecting for nocturnal immigration (Papadopoulos et al. 2002, Ragionieri et al. 2015). Potential recruits use chemical cues from the parental habitat and/or conspecifics that are important in triggering larval moult cycles, development and eventually settlement (Gebauer et al. 1998, 2002, O'Connor 2007, Simith & Diele 2008). Some species can independently shift through these life-history events following a genetic and hormonal program, while others rely heavily on environmental and chemical signalling (Gebauer et al. 2005). Late-stage zoeae of brachyurans can rely on stimuli that alter their behaviour so that they position themselves within the water column to take advantage of transportation by depth-dependent currents (Christy

& Morgan 1998, Kingsford et al. 2002, Queiroga & Blanton 2005, Anger 2006). This approach can be used to disperse larvae away from the parent population, return them to the adult habitat, or even to retain larvae in the vicinity of the parents (Epifanio & Cohen 2016).

Responses to external stimuli are usually species-specific, and therefore, the relative importance of the physical processes that transport larvae back to the parental habitat is often taxon-dependent (Kingsford et al. 2002, Anger 2006). Brachyuran larvae, however, generally make use of the predictable nature of tides and related current reversals, having frequently evolved convergent behaviours linked to transport mechanisms (Epifanio et al. 1984, Scheltema 1986, Epifanio 1988, Christy & Morgan 1998, Tankersley et al. 2002). In temperate regions, brachyuran megalopae mostly rely on wind-driven currents to aid transportation to estuarine habitats from the nearshore (Botsford 2001, Flores et al. 2002, Domingues et al. 2010, Olaguer-Feliú et al. 2010). Likewise, some tropical species utilise wind-driven currents for transportation to suitable settlement habitats (Paula et al. 2001, 2003).

Here, we examined the proximate drivers and spatial patterns of settlement rates of mangrove crabs across a very wide range of latitudes. We measured temporal and spatial variation in settlement of crabs colonising the equatorial and subtropical regions of the east coast of Africa, allowing us to compare patterns of settlement at the centre and the southernmost limits of mangroves on the continent. In both regions, settlement was monitored to test for correlations with the tidal and day/night cycles. First, we aimed to understand which abiotic factors influence the settlement of megalopae during spring tides and if these factors differ among species and at the 2 different latitudes. Second, we investigated habitat selectivity during settlement to determine if this reflects the clear zonation patterns exhibited by adults in each region (e.g. Emmerson 1994, 2016, Ruwa 1997, Dahdouh-Guebas et al. 2002).

2. MATERIALS AND METHODS

2.1. Study sites

The study sites identified were 2 in the middle and 2 at the southern edge of the distributional range of mangrove ecosystems in eastern Africa. These were Gazi Bay (4° 22′ S, 39° 30′ E) and Shirazi

Creek (4° 31′ S, 39° 25′ E) in Kenya (central) and the Mngazana (31° 42′ S, 29° 25′ E) and Mntafufu (31° 33′ S, 29° 38′ E) estuaries in South Africa (southern limit; Fig. 1). The sites were chosen based on the presence of *Avicennia marina*, *Bruguiera gymnorrhiza*, *Ceriops tagal*, *Heritiera littoralis*, *Lumnitzera racemosa*, *Rhizophora mucronata*, *Sonneratia alba* and *Xylocarpus granatum* in Kenya and of *A. marina*, *B. gymnorrhiza* and *R. mucronata* in South Africa. Within each site, 2 zones with monospecific stands of mangrove species that occur in both countries were identified, one with *A. marina* and one with *R. mucronata*, allowing the comparison of spatial patterns. In both sites, *A. marina* formed the landward limit of the mangrove forest, while *R. mucronata* formed the seaward limit. In South Africa, stands of *R. mucronata* and *B. gymnorrhiza* overlapped, and only those where *R. mucronata* was dominant were chosen.

2.2. Settlement of megalopae

In Kenya, sampling of megalopae occurred between 9 August 2010 and 26 September 2010 at Shirazi and from 9 August 2010 until 24 January 2011 at Gazi Bay. The July–September and November–January periods coincide with the minor and major dry seasons, respectively, and are known to be the 2 peak reproductive periods of mangrove crabs at this latitude (Skov et al. 2005). In South Africa, sampling took place at both sites from 3 November 2010 until 15 October 2011. Sampling was conducted twice a day during spring tides, when the tidal amplitude exceeded 3.00 m for >1 consecutive tide in Kenya and >1.65 m for South Africa (Fusi et al. 2015). This ensured the upper *A. marina* zone was fully submersed during high tide. During each spring tide, the different zones (*A. marina* and *R. mucronata*) were sampled over 5 d in Kenya and 3–8 d in South Africa.

Megalopae were collected by means of artificial collectors which were made of small mats cut from air-conditioning filters, measuring 33 × 33 × 1 cm to mimic a complex settlement substratum. Artificial substrata to collect megalopae have been extensively and successfully used in various habitats (e.g. Olmi et al. 1990, van Montfrans et al. 1990, Paula et al. 2003, Ragionieri et al. 2015), although recently they proved to be a biased method for some megalopae (Reinsel et al. 2015). For each collection, 4 collectors were pinned haphazardly on the substratum among the trees in the 2 identified stands at each site at the beginning of the low tide. The mats were recovered during the following low tide, after the ebb tide had receded, leaving the traps fully exposed to air. Each mat was placed in a separate transparent pre-labelled Ziplock bag and replaced with a clean mat. The bags and mats were washed out thoroughly using freshwater sprayers to remove any megalopa trapped inside. Only early stage megalopae or recent settlers as defined by Welch et al. (2015) were collected, to avoid the count of later stages, which could be already in the mangroves and secondarily migrate to the collectors. Megalopae were collected and stored in 90% ethanol for later identification. This procedure was carried out at 12 h intervals until the spring tide amplitude was no longer enough to fully submerge all mats in both zones.
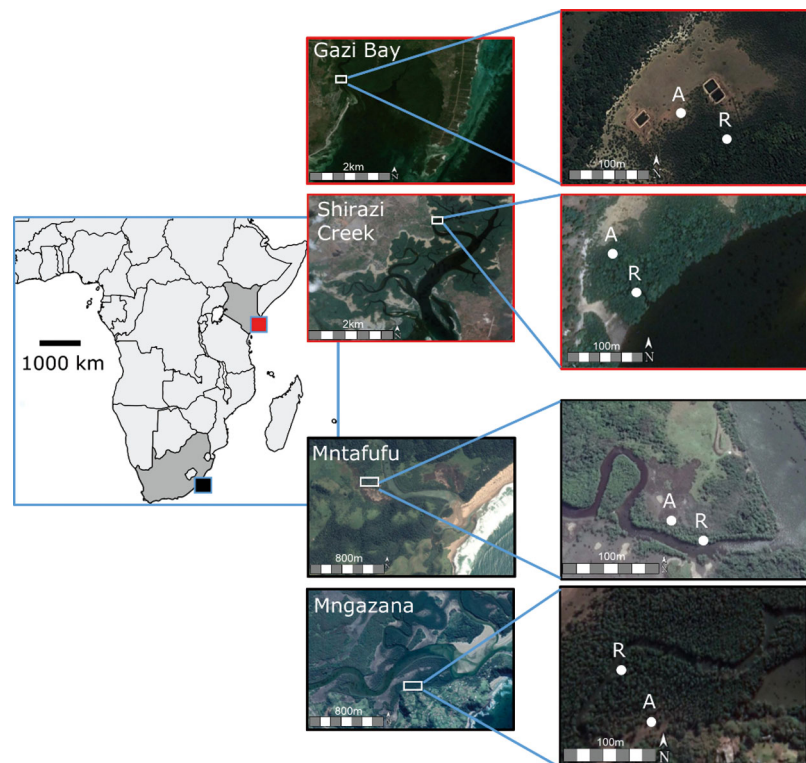

Fig. 1. Aerial view of sampling sites in Kenya (Gazi Bay and Shirazi Creek) and South Africa (Mntafufu Estuary and Mngazana Estuary). A: sampling sites in zones dominated by *Avicennia marina*; R: sampling sites in zones dominated by *Rhizophora mucronata*. All photos courtesy of Google Earth. Africa map shows approximate positions of study areas

2.3. Megalopa identification

Megalopae were identified to species level where possible following Pereyra Lago (1987, 1989, 1993a,b) and Guerao et al. (2011). Individuals that could not be identified were grouped into morphotypes. Then, DNA barcoding was combined to morphological analysis to help identify unknown megalopae or confirm their identification. For this, representative adult specimens of some of the most common crab species of East Africa were collected from Mngazana estuary and sent to the Canadian Centre for DNA Barcoding (CCDB) for DNA sequencing, together with identified megalopae and morphotypes. Sequences were deposited into the BOLD DNA Barcoding Database (www.barcodinglife.org). Specifically, we sequenced the following species: *Epixanthus dentatus* (accession number: SBBM038-13), *Neosarmatium africanum* (accession number: SBBM035-13), *Neosarmatium smithii* (accession number: SBBM034-13), *Ocypode ceratophthalmus* (accession number: SBBM040-13), *Parasesarma catenatum* (accession number: SBBM036-13), *Parasesarma capensis* (accession number: SBBM020-13), *Potamonautes perlatus* (accession number: SBBM042-13), *Scylla serrata* (accession number: SBBM039-13), *Paraleptuca chlorophthalmus* (accession number: SBBM0378-13), *Austruca occidentalis* (accession number: SBBM041-13) and *Tubuca urvillei* (accession number: SBBM019-13).

We then used the software BLAST (National Center for Biotechnology Information) to compare sequences of unknown megalopae to sequence databases and calculate the statistical significance of matches. We also compared the obtained sequences to our own reference sequences. The DNA barcoding allowed the morphological identification of the unknown megalopae with matches >99%.

2.4. Statistical analysis

In total, we sampled through 21 spring tides in South Africa and 12 in Kenya. Because the majority of megalopae collected belonged to different species in the 2 countries and their reproductive period is different, the data for Kenya and South Africa were analysed separately. The number of collected megalopae in each collector was used as a random spatial and temporal replicate, to account for possible non-independence of our data due to our systematic sampling scheme. For both Kenya and South Africa, we investigated differences in megalopal settlement across sites (2 levels, fixed and orthogonal), moon phase (full and new moon, fixed and orthogonal), day and night (2 levels, fixed and orthogonal) mangrove area (*A. marina* and *R. mucronata*, fixed and orthogonal) and across temporal replicates (random, nested within 'moon phase') using a 5-way permutational analysis of variance (PERMANOVA; Anderson 2001). The same model was used in analyses of the number of collected megalopae identified as *Neosarmatium africanum* and *Parasesarma guttatum*, the most abundant larvae found in Kenya, and, in a separate test, those identified as *Parasesarma catenatum* and *P. capensis*, the most abundant larvae found in South Africa. In the case of *N. africanum*, a 4-way PERMANOVA model was applied, excluding Shirazi, where no larvae of this species were ever collected. All datasets were tested for heteroscedasticity using Levene's test, and log transformation was applied when significant differences in variation were found. Post hoc tests were utilised for multiple comparisons among significant interaction factors. All analyses were performed using the PERMANOVA+ routines for PRIMER 7 (Anderson et al. 2008).

3. RESULTS

In total, 94 megalopae were captured in Kenya, of which 68 and 24 were attributed to *Parasesarma guttatum* and *Neosarmatium africanum*, respectively. In South Africa, 506 megalopae were collected, of which 449 and 57 were identified as *P. catenatum* and *P. capensis*, respectively. The highest number of megalopae collected per trap was 5 (*N. africanum*) and 3 (*P. guttatum*) in Kenya, corresponding to a megalopal density of 45.9 and 27.5 ind. m^{-2}, respectively. In South Africa, the highest number of megalopae collected per trap was 70 (*P. catenatum*) and 5 (*P. capensis*), corresponding to 643 and 45.9 ind. m^{-2} respectively. In particular, the high density of *P. catenatum* was due to the exceptionally high number (267) collected during one single occasion (25–30 September 2011). During the other sampled tides, the highest density of *P. catenatum* megalopae was 82.6 ind. m^{-2}.

In Kenya, *P. guttatum* megalopae settled throughout the sampling period, while *N. africanum* larvae were mainly collected in December to January, with very few recorded in September (Fig. 2). The number of collected megalopae varied significantly according to lunar phase and the mangrove zone (Table 1, Figs. 2 & 3). Overall, settlement was significantly higher at full moon, across all factors, and in the *Rhizophora mucronata* dominated area (Fig. 3A).
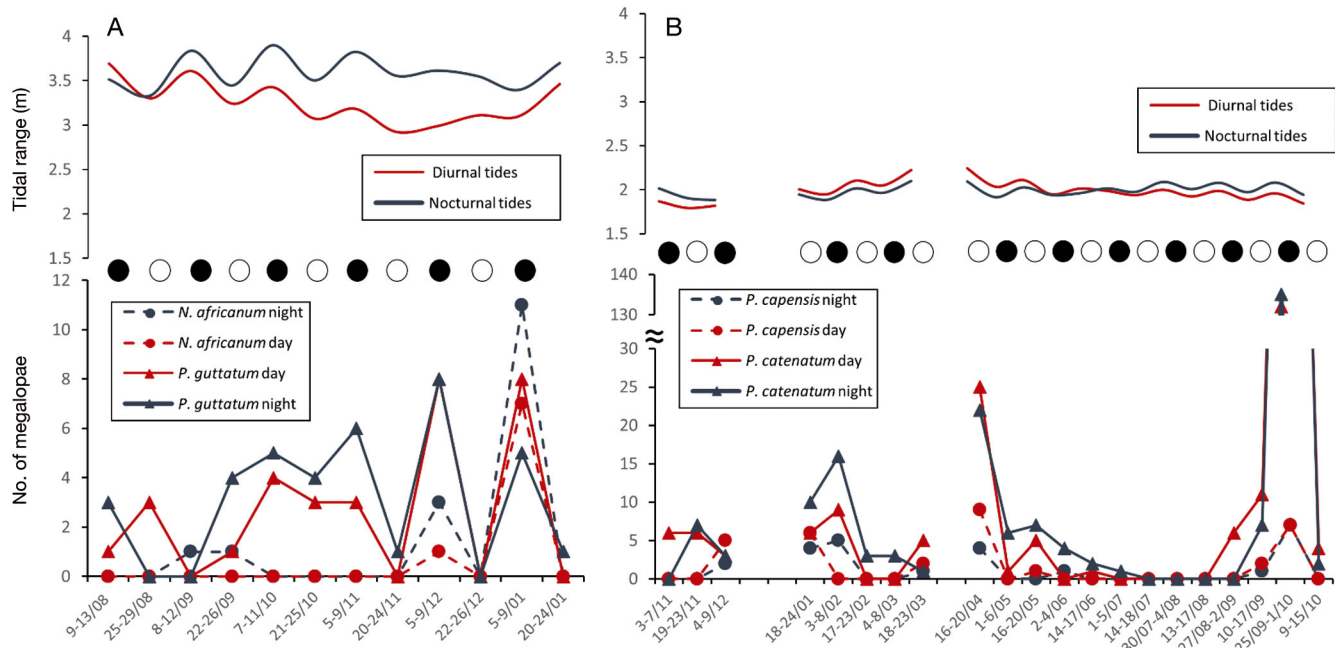
Fig. 2. Tidal amplitude and total numbers of settlers in the collectors deployed at the 2 sites in (A) Kenya and (B) South Africa. The moon phases are also shown (open circles: full moons; black circles: new moons)

P. guttatum megalopae were mostly captured in the *R. mucronata* zone (settlement in *R. mucronata* zone at full moon vs. settlement in *Avicennia marina* zone at full moon: $t = 3.36$, p = 0.002, post hoc test; Fig. 3B) and at full moon (settlement at full moon in *R. mucronata* zone vs. settlement at new moon in *R. mucronata*: $t = 3.39$, p = 0.002, post hoc test; Fig. 3B). *N. africanum* megalopae in Gazi Bay traps were significantly more abundant at full moon and were mostly collected in the *A. marina* zone (Table 2, Fig. 3C).

In South Africa, megalopal settlement was also recorded throughout the sampling period, with a notable break in July to August. Neither *P. catenatum* nor *P. capensis* settlement was influenced by lunar phase or the day/night cycle (Fig. 4, Table 3), although the exceptional settlement event occurred during a new moon night. The *A. marina* dominated zone proved to be significantly preferred across species (Fig. 4, Table 3). This result appears to have been driven mainly by the preference exerted by *P. catenatum* larvae,

Table 1. Results of the 5-way PERMANOVA models applied to test for differences in total numbers of megalopae and *Parasesarma guttatum* megalopae collected at the 2 investigated sites (Si) in Kenya across moon phase (Mo), mangrove zones (Zo) and day and night (D/n)

| Source	df	Total			*P. guttatum*		
		MS	Pseudo-*F*	p	MS	Pseudo-*F*	p
Moon	1	4.453	7.782	0.017	2.144	9.225	0.009
Zone	1	0.279	1.652	0.211	1.051	18.591	<0.001
Site	1	0.015	0.569	0.530	0.015	0.569	0.537
Day/night	1	0.005	0.043	0.835	0.060	0.560	0.460
Rep(Mo)	10	0.942	8.573	<0.001	0.345	3.750	0.001
Mo × Zo	1	0.094	0.559	0.465	0.521	9.228	0.003
Mo × Si	1	0.038	1.431	0.354	0.038	1.431	0.350
Mo × D/n	1	0.005	0.048	0.827	0.092	0.855	0.364
Zo × Si	1	–	–		–	–	
Zo × D/n	1	0.173	2.397	0.125	0.111	1.576	0.212
Si × D/n	1	0.193	1.021	0.424	0.193	1.021	0.424
Zo × Rep(Mo)	10	0.217	1.971	0.036	0.028	0.306	0.980
Si × Rep(Mo)	2	0.026	0.240	0.787	0.026	0.287	0.753
D/n × Rep(Mo)	10	0.107	0.969	0.469	0.120	1.300	0.227
Mo × Zo × Si	1	0.005	0.158	0.738	0.005	0.158	0.731
Mo × Zo × D/n	1	0.021	0.298	0.582	0.021	0.303	0.586
Mo × Si × D/n	1	0.015	0.079	0.804	0.015	0.079	0.808
Zo × Si × D/n	1	0.316	9.683	0.094	0.316	9.683	0.088
Zo × Si × Rep(Mo)	2	0.033	0.297	0.746	0.033	0.355	0.701
Zo × D/n × Rep(Mo)	10	0.042	0.379	0.957	0.054	0.584	0.828
Si × D/n × Rep(Mo)	2	0.189	1.720	0.184	0.189	2.055	0.137
Mo × Zo × Si × D/n	1	–	–		–	–	
Res	192	0.110			0.033	0.355	0.694
Total	255				0.092		
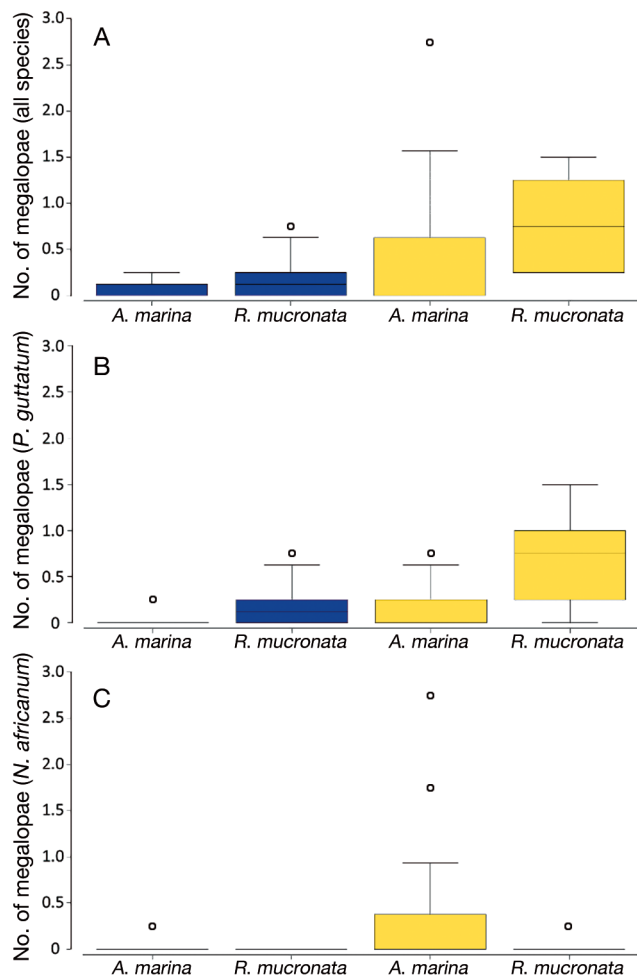
Fig. 3. Number of megalopae, averaged across traps, of (A) all the collected species, (B) *Parasesarma guttatum* megalopae and (C) *Neosarmatium africanum* megalopae collected in the *Rhizophora mucronata* and *Avicennia marina* dominated zones of 2 sampling sites in Kenya (A and B) and in Gazi Bay only (C). The new moon and full moon samplings are shown in blue and yellow, respectively. Bar: median; box: interquartile range; whiskers: 5th–95th percentiles; dots: outliners

Table 2. Results of the 4-way PERMANOVA model applied to test for differences in the total numbers of *Neosarmatium africanum* megalopae collected at Gazi, Kenya, across moon-phases, mangrove zones and day and night (abbreviations as in Table 1)

Source	df	MS	Pseudo-*F*	p
Moon	1	0.647	1.872	0.202
Zone	1	0.647	1.893	0.196
Day/night	1	0.060	4.880	0.053
Rep(Mo)	10	0.346	11.818	**<0.001**
Mo × Zo	1	0.496	1.451	0.259
Mo × D/n	1	0.021	1.713	0.217
Zo × D/n	1	0.021	3.435	0.095
Zo × Rep(Mo)	10	0.342	11.687	**<0.001**
D/n × Rep(Mo)	10	0.012	0.422	0.937
Mo × Zo × D/n	1	0.002	0.334	0.573
Zo × D/n × Rep(Mo)	10	0.006	0.211	0.995
Res	144	0.029		
Total	191			

which were collected in higher numbers in this zone, though only in Mngazana (settlement at *A. marina* zone in Mngazana vs. settlement at *A. marina* zone in Mntafufu: $t = 2.39$, p = 0.01, post hoc test; Fig. 4B).

4. DISCUSSION

This study sheds light on the factors that regulate the settlement of the late-stage larvae of intertidal animals, a long-standing and critical debate in marine ecology. Although we confirmed that settlement of megalopae of populations of crabs inhabiting tropical and sub-tropical mangroves are not as predictable
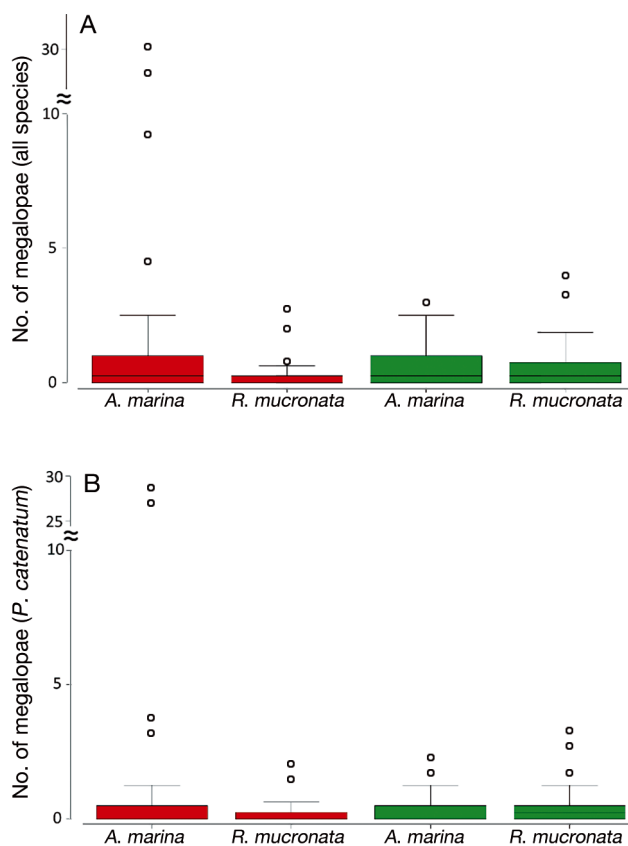

Fig. 4. (A) Total amount of megalopae and (B) *Parasesarma catenatum* megalopae collected in the *Rhizophora mucronata* and *Avicennia marina* dominated zones of 2 sites in South Africa, Mngazana (red) and Mntafufu (green). Boxplot features as in Fig. 3

Table 3. Results of the 5-way PERMANOVA models applied to test for differences in total numbers of megalopae in *Parasesarma catenatum* and *P. capensis* collected at the 2 investigated sites in South Africa across moon phase, mangrove zones and day and night (abbreviations as in Table 1)

Source	df	Total			P. catenatum			P. capensis		
		MS	Pseudo-*F*	p	MS	Pseudo-*F*	p	MS	Pseudo-*F*	p
Moon	1	0.297	0.065	0.799	0.399	0.125	0.735	0.001	0.004	0.949
Zone	1	3.870	5.421	0.033	2.237	3.419	0.078	0.033	0.916	0.354
Site	1	0.072	0.084	0.775	0.036	0.061	0.805	0.000	0.000	0.997
Day/night	1	0.019	0.091	0.779	0.007	0.051	0.827	0.016	0.505	0.488
Rep(Mo)	19	4.603	32.966	<0.001	3.197	25.349	<0.001	0.183	5.041	<0.001
Mo × Zo	1	0.035	0.050	0.831	0.183	0.280	0.599	0.066	1.816	0.203
Mo × Si	1	0.076	0.088	0.770	0.048	0.081	0.786	0.005	0.051	0.826
Mo × D/n	1	0.000	0.000	0.996	0.001	0.006	0.936	0.052	1.695	0.211
Zo × Si	1	3.372	5.420	0.033	3.469	5.395	0.032	0.212	3.899	0.064
Zo × D/n	1	0.348	1.542	0.232	0.031	0.346	0.561	0.196	2.365	0.137
Si × D/n	1	0.234	1.211	0.287	0.308	1.858	0.187	0.039	0.647	0.432
Zo × Rep(Mo)	19	0.714	5.113	0.000	0.654	5.189	0.000	0.036	0.993	0.462
Si × Rep(Mo)	19	0.858	6.143	0.000	0.597	4.737	0.000	0.104	2.866	0.000
Si × Rep(Mo)	19	0.858	6.143	0.000	0.597	4.737	0.000	0.104	2.866	0.000
D/n × Rep(Mo)	19	0.205	1.466	0.091	0.132	1.045	0.414	0.031	0.847	0.648
Mo × Zo × Si	1	1.071	1.722	0.206	1.120	1.742	0.194	0.007	0.124	0.732
Mo × Zo × D/n	1	0.025	0.113	0.744	0.019	0.219	0.640	0.137	1.656	0.207
Mo × Si × D/n	1	0.840	4.348	0.054	0.519	3.126	0.086	0.028	0.466	0.511
Zo × Si × D/n	1	0.099	0.618	0.439	0.063	0.458	0.504	0.053	1.445	0.246
Zo × Si × Rep(Mo)	19	0.622	4.456	0.000	0.643	5.098	0.000	0.054	1.494	0.086
Zo × D/n × Rep(Mo)	19	0.226	1.616	0.046	0.088	0.701	0.820	0.083	2.279	0.002
Si × D/n × Rep(Mo)	19	0.193	1.384	0.129	0.166	1.315	0.163	0.060	1.651	0.043
Mo × Zo × Si × D/n	1	0.334	2.079	0.163	0.164	1.188	0.288	0.010	0.263	0.614
Res	504	0.140			0.126			0.036		
Total	671									

as their related spawning events, the use of megalopal traps across a wide geographical range and across prolonged sampling periods provides novel information on the proximate causes of spatio-temporal patterns in settlement.

From a quantitative point of view, the main result of this study is the very low abundance of megalopae that settled on the deployed traps in both Kenya and South Africa. Across the whole sampling period, <10 megalopae were collected per lunar temporal replicate in Kenya and <20 per lunar replicate in South Africa. Moreover, over half of the total megalopae collected in South Africa were collected during a single settlement event. This low abundance of settlers could be related to our sampling methodology, which proved, in one case, to be inefficient in trapping megalopae of some fiddler crabs (but was successful for *Minuca pugnax*; see Reinsel et al. 2015). We, however, successfully trapped the settlers of the most abundant sesarmid species at the 4 sites, and our data are in accord with a number of published results showing how populations of a number of intertidal animals dwelling on east and southern African coasts appear to be recruitment-limited. For example, this limitation has been advocated for various east African populations of mangrove crabs (Paula et al. 2001, 2003, Ragionieri et al. 2015) and southern African rocky shore populations of the mussel *Perna perna* (Harris et al. 1998, Reaugh-Flower et al. 2010), which all show very limited, variable and apparently random settlement patterns of their final stage larvae. This pattern might be linked to very dynamic alongshore transport (Reaugh-Flower et al. 2010) once exported outside the estuaries.

Temporally, our results show that, regardless of the differences in average tidal range between Kenya and South Africa, tidal amplitude affects settlement more than any of the other physical parameters measured. In particular, in Kenya, where the tidal range is larger than in South Africa, settlement peaks were recorded at full moons, characterised by the highest tides during the sampling period. This tidal trend was statistically significant for both the medium-sized sesarmid *Parasesarma guttatum*, which spawns all year round (Skov et al. 2005), and the large-sized species *Neosarmatium africanum*, which has a much shorter reproductive season (Skov et al. 2005). In South Africa, where the tidal range is narrower than

in Kenya, the 2 most abundant settlement events were recorded just after the March and September equinoxes, when the amplitude of the tides was at its maximum.

Although we have identified that tidal amplitude was the strongest proximate factor for settlement, the ultimate factors driving this process are still difficult to ascertain. Indeed, reproduction of mangrove crabs is also strongly related to peaks in tidal amplitude (Morgan & Christy 1995, Skov et al. 2005) such that the observed peaks in settlement could be an obvious consequence of highly synchronous mass larval releases happening during those periods. The recorded settlement patterns, however, may not necessarily be coupled to spawning (Flores et al. 2002, Skov et al. 2005, Fratini et al. 2016). It is worth noting that 52.7 % of all megalopae collected during the year were caught during a single sampling event closely following the September equinox; such an extraordinary settlement event is unlikely to be explained by reproductive synchrony only.

As previously highlighted by other studies carried out on megalopal settlement in East African mangrove crab populations (Paula et al. 2003, Ragionieri et al. 2015), no strong relationship between settlement and moon phase as well as day/light cycle was observed. None of the identified species at either site showed any preference for settlement during new moon or at night; thus, the hypothesis of behavioural avoidance of visual predators proposed by Christy (2003) was not supported.

Alternatively, the onshore/estuarine transport could be enhanced behaviourally during spring tides, through increased active response by megalopae due to increased salinity during flooding (Tankersley et al. 2002). The 2 systems considered during this study, however, are not exposed to large salinity alterations as the Kenyan mangroves occur in a marine creek and the South African swamps are present in the lower reaches of an estuary, with very limited saline excursions.

Physical mechanisms, such as tidally forced internal waves and tidal bores have also been proposed for the shoreward transport of megalopae during spring tides in shallow water systems (Shanks 1983, Pineda 1991). Although direct measurements of the hydrodynamics adjacent to the mangrove sites could not be made, the explanation of the transport of larvae driven by internal waves cannot be entirely dismissed, especially for South Africa, where the sampling locations are adjacent to a relatively narrow shelf (Schumann & Brink 1990, Hutchings 1994). Linked to the mechanic drivers of transport, the effect

of tidal flux is also a plausible explanation especially for the largest settlement peak during the September equinox, when an increased number of competent megalopae would have been carried within the mangroves during flooding (Epifanio 1995). The spatial pattern of megalopal settlement in the studied populations is clear and novel. All the identified species except *P. capensis* were highly competent at finding the 'correct' habitat, i.e. the area of the forest colonised by conspecific adults. This habitat selectivity was particularly clear in Kenya, where both *P. guttatum* and *N. africanum* significantly preferred the areas dominated by their respective adults, while in South Africa, only the very abundant and dominant *P. catenatum* showed a spatial preference for the *Avicennia marina* dominated area where adults are most abundant. The lack of a strong effect of mangrove area for *P. capensis* is not surprising because the sampling site in South Africa occurs at the extreme edge of the geographical range of this species, and *P. capensis* is sparsely distributed across the whole forest, although somewhat more abundant in areas characterised by *Rhizophora mucronata* (Fratini et al. 2019).

Importantly, megalopae of different species enter the mangroves using the same medium of transport, high tides, but they do not settle by chance onto any substratum. If this was the case, they would probably mostly settle in areas dominated by *R. mucronata*, the habitat closest to the low water mark, which is inundated for longest, being the first to be covered by the incoming tide and the last to be exposed on the ebbing tide (Paula et al. 2001). In contrast, we have shown for the first time that megalopae of these crab species select specific settlement habitats, regardless of their inundation time or accessibility.

We could not disentangle the proximate causes of such spatial selectivity or the mechanisms, which remain obscure. Olfactory, visual and sound stimuli coming from the adult populations or from the substratum are likely to play a role in initiating settlement, as shown for other brachyuran species (Gebauer et al. 1998, 2002, 2005, O'Connor 2007, Simith & Diele 2008), but an alternative and novel experimental approach has to be implemented to answer this question. Generally, active choice of settlement and post-settlement habitats has benefits for survival and feeding (Fernandez et al. 1993, Thiel & Dernedde 1994, Orth & van Montfrans 2002), while ontogenetic shifts in habitat occupation are also common (Moksnes 2002).

Because crabs are among the most important constituents of both natural and restored mangrove ecosystems (Cannicci et al. 2008, Kristensen et al. 2008),

this study has important ecological and conservation implications. From an ecological point of view, we confirm that tidal amplitude plays a major role in megalopal settlement of mangrove crabs and demonstrate, for the first time, the spatial specificity of the event. Our long-term sampling allowed us to show robustly that settlement is directly correlated with tidal amplitude on a monthly basis as well as on a yearly basis. This latter and novel evidence is critical for understanding the impact of such events on population dynamics and community composition of mangrove crab assemblages (Fratini et al. 2016). Moreover, we showed that the settlement of larvae conserves the spatial distribution and zonation of the different adult crab populations within mangrove forests, and because this settlement is related to the overall hydrological conditions of the mangroves, this functional hydrology must be preserved. From a conservation point of view, our results clearly show that the maintenance, or the restoration, of the natural hydrology of mangrove forests is critical not just for the floral component of the ecosystem (Lewis 2005), but also for the protection of the spatial distribution of the macrofauna, which has implications for the functionality of the system as a whole.

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