Recruitment of bivalve molluscs with specific emphasis on *Mytilus galloprovincialis* in the Knysna estuarine embayment, South Africa

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Abstract

Alien invasive species have the ability to transform or alter environments, often causing severe ecological and/or economic impacts. Marine bioinvasions are occurring globally and are most often facilitated (intentially and accidently) through anthropogenic activities including the building of inter-oceanic canals, shipping and commerce. The Mediterranean mussel, *Mytilus galloprovincialis*, is a globally successful marine alien invasive species which was first recorded on the west coast of South Africa in the late 1970s and the south coast in 1988. This species is thought to have reached the Knysna Estuary in the early 2000s and has colonised all man-made hard substrata in the embayment of the estuary. Although there are studies on recruitment of *M. galloprovincialis* on the rocky intertidal coasts of South Africa, there is little information on recruitment of this species in more sheltered estuarine environments. This study aimed to determine recruitment levels of *M. galloprovincialis* and other bivalves within the Knysna estuarine embayment. To determine monthly recruitment, 10 recruit collectors/pads (plastic pot scourers) were placed at three separate locations within the embayment of the estuary for a week on a monthly basis for 20 months. In addition, recruitment of *M. galloprovincialis* over spring and neap tides and different lunar phases was also determined at two sites within the Knysna estuarine embayment during the main reproductive season in 2018. The pads were deployed three days before a neap/spring tide and then collected three days after the respective tide. Finally, to look at how rapidly *M. galloprovincialis* and other macroinvertebrates (when *M. galloprovincialis* was excluded) would re-colonise free space, 18 plots (15x15 cm), consisting of three treatments including a control (A,B and C), were cleared in *M. galloprovincialis* mussel beds and then photographed monthly for 12 months.

Four bivalve taxa (*Mytilus galloprovincialis, Perna perna,* Ostreidae, unidentified mytilid) were recorded during the monthly study. Recruitment levels for all bivalves differed significantly ($P < 0.001$) between months and sites, with peak recruitment occurring from late spring to early autumn (November – March). *Mytilus galloprovincialis* recruitment levels were greater than other bivalves and were up to 4.5x greater than other taxa. Recruitment also varied between years possibly owing to differences in larval supply and/or environmental factors. Spatial variation in bivalve recruitment was observed throughout the study. The greatest recruitment was at the site (Thesen Island Wharf) closer to the entrance of the embayment. By contrast at the site (Railway Bridge) furthest from the entrance lower recruitment was found. This difference is possibly due to differences in hydrodynamics or other biological and/or environmental factors. A preliminary tidal study found that *M. galloprovincialis* had significantly higher ($P < 0.001$) recruitment levels over spring tides than neap tides at Thesen Island Wharf, whereas recruitment at the Railway Bridge on spring and neap tides was not significantly different. In the study undertaken in the reproductive season only, recruitment levels were high over a two week period during both a spring and neap tide, suggesting that factors other than lunar phase and the state of tide are more important in determining the timing and intensity of recruitment. The clearance plots created and photographed over a 12 month period showed that *M. galloprovincialis* rapidly occupied free space (eight months to virtually cover all free space) by encroachment from the adjacent mussel bed. Limpets and barnacles were only able to colonise cleared space when *M. galloprovincialis* was excluded, suggesting that the mussel has the ability to outcompete indigenous macrofauna for space. The high recruitment levels of *M. galloprovincialis* compared to other indigenous bivalves, as well as its ability to occupy space rapidly are traits that must contribute to the success of the invasion of this species within the Knysna estuarine embayment, particulary within Thesen Islands Marina and Thesen Island Wharf.

Keywords: Mollusca, Bivalvia, Mytilidae,Ostreidae, alien invasive species, settlement, re-colonisation

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Chapter 1. Introduction

1.1. Alien Invasive Species

Alien invasive species can be defined as "species that are able to produce self-replicating populations over several generations and that have spread from their point of introduction" (Robinson *et al.,* 2016). Once an alien invasive species has become established in an area it may be extremely difficult to remove it (Molnar *et al*., 2008). Over the past 500 years international trade, shipping and commerce has dramatically increased bringing with them the facilitation and movement of alien invasive species across the globe, some introductions being intentional whilst others accidental (Carlton, 1996; Mooney & Cleland, 2001; Robinson *et al.,* 2005a). The spread of such species have often had a detrimental effect on native species and/or the altering of local habitats may further affect community structure and function (Mooney & Cleland, 2001; Streftaris & Zenetos, 2006; Galil, 2007). The magnitude of the impact of alien invasive species has been so great that they are regarded as one of the major drivers of biodiversity loss, second only to habitat destruction, as well as causing costly socioeconomic impacts, including the management and removal of such species (Carlton, 1996; Streftaris & Zenetos, 2006; Galil, 2007; Rilov & Galil, 2009; Galil *et al.,* 2015; McQuaid *et al.,* 2015). Biological invasions are therefore considered to be a severe wherever they may occur (Carlton, 1996; Streftaris & Zenetos, 2006; Galil, 2007; McQuaid *et al.,* 2015).

There is some support that alien invasive species may have certain traits and mechanisms that prove to be beneficial to their non-native establishment (Kolar & Lodge, 2001). Whilst some traits may be restricted to some taxa, for example in birds their smaller body size may help their invasiveness (Kolar & Lodge, 2001), other traits are more widespread. For sexually reproducing populations having a larger propagule size can be positively correlated with establishment (Colautti *et al.,* 2006; Sinclair & Arnott, 2016). For many plants, reproducing vegetatively or producing small seeds is positively correlated with invasiveness (Kolar & Lodge, 2001). Other factors that may have a positive influence on the invasion success of alien species include the dispersal mechanism of the species (Wilson *et al*., 2009), the availability of an empty niche for the alien invasive to occupy, and the lack of natural predators (enemy release hypothesis) (Keane & Crawley, 2002; Dietz & Edwards, 2006). Furthermore, a common alien invasive characteristic is the ability to establish and colonise environments that are subject to anthropogenic activity and disturbance (Colautti *et al.,* 2006; Erlandsson *et al.,* 2006). The enemy release hypothesis states that non-indigenous species (NIS) may experience a decrease, or absence, of specialist natural enemies and therefore such species may be able to allocate resources usually used for defensive purposes to other important functions, thereby increasing its competitiveness and ultimately the success of its establishment (Keane & Crawley, 2002; Colautti *et al.,* 2004; Dietz & Edwards, 2006). Natural predators of NIS may therefore play a large role in controlling NIS populations (Keane & Crawley, 2002). One of the most influential and studied mechanisms of the establishment of alien invasive species, or NIS, is propagule pressure (i.e. the number/rate of introductions made and the size of the introduction) (Colautti *et al.,* 2006; Sinclair & Arnott, 2016). The study by Colautti *et al.* (2006) has shown that this mechanism is positively associated with the establishment and spread of NIS in invaded areas, and is generally consistent across taxa and the invasive stage. Furthermore, a recent study by Sinclair & Arnott (2016) focusing on propagule pressure of a non-native mysid (*Haemimysis anomala*) in mesocosms suggested that a single large introduction of a NIS had higher chances of surviving and establishing than of smaller, more frequent introductions.

Such characteristics and mechanisms are important in the spread of marine bioinvasions.

Marine bioinvasions are occurring in coastal systems around the world at a rapid rate, and like most invasions they have the ability to transform or alter habitats, displace native species, alter indigenous community interactions, and cause some form of ecological and/or economic impact (such as the collapse of fisheries or aquaculture farms) unless urgent action and management is implemented to remove these species (Carlton, 1996; Naylor *et al., 2*001; Robinson *et al.,* 2005a; Hewitt & Campbell, 2007; Molnar *et al.,* 2008; Rilov & Galil, 2009; Haupt *et al., 2*010; Alexander *et al.,* 2016). Because humanity has relied on ships for transportation (and trade) for hundreds of years we have intentionally and accidently facilitated the movement of marine bioinvasive species across natural barriers of space and time (Carlton, 1996; Molnar *et al.,* 2008; Mead *et al.,* 2011). As global maritime shipping has dramatically increased over the past 500 years due to exploration, globalization and an ever increasing world population so has the spread of marine bioinvasions (Carlton, 1996; Molnar *et al.,* 2008; Rilov & Galil, 2009; Mead *et al.,* 2011; Seebens *et al.,* 2013). A common pathway for transporting alien invasive marine species is by shipping, whereby the fouling of ship hulls, and/or ballast water (water to stabilize the ships) act as vectors that operate within this pathway (Eldredge & Carlton, 2002; Anil *et al.,* 2002; Bax *et al.,*2003; Molnar *et al.,* 2008; Seebens *et al.,* 2013). In a review of literature Bax *et al.* (2003) illustrates that the most common vector of alien marine species introductions in the USA (San Francisco Bay), New Zealand, Australia and the UK is through ship hull fouling. Furthermore, ballast water for ships often contains a multitude of marine species and their propagules, with those able to survive being introduced to novel ranges (Carlton, 1996; Eldredge & Carlton, 2002; Anil *et al.,* 2002; Seebens *et al.,* 2013). It is estimated that over 12 billion tons of ballast water is transported around the world annually, most of which gets released into the vicinity of ports (Anil *et al.,* 2002; Mead *et al.,* 2011; Seebens *et al.,* 2013).

Human engineering projects that have been developed to help facilitate trade and commerce, such as canals and harbours, have often been subject to invasion from alien species (Galil, 2007; Seebens *et al.,* 2013). For example the inter-oceanic canal between the Red Sea and the Mediterranean Sea, the Suez canal, was opened in 1869 and has subsequently led to the rapid invasion of hundreds of taxa within the Mediterranean Sea (Galil, 2007; Edelist *et al.,* 2013; Galil *et al.,* 2015). Approximately half of the 700 non-indigenous species recognised in the Mediterranean Sea today were introduced through the Suez Canal (Galil *et al.,* 2015). Harbours and ports are another anthropogenic engineering example of invasion hotspots due to the large number of ships passing through them and subsequently the large and frequent release of ballast water nearby (Molnar *et al.,* 2008; Mead *et al.,* 2011; Seebens *et al.,* 2013). The aquarium trade has also led to the intentional and unintentional introduction and establishment of alien invasive species, including *Caulerpa taxifolia*, the killer algae, within the Mediterranean Sea (Galil, 2007) and the Pacific lionfish, *Pterois volitans*, introduced into the warm western Atlantic Ocean (Edelist *et al.,* 2013).

The aquaculture industry is considered an important and emerging global source of marine bioinvasions (Molnar *et al.,* 2008; Mead *et al.,* 2011). Numerous alien invasive introductions, both intentional and unintentional, have been made globally through aquaculture. Intentional introductions include the introduction of oysters and/or mussels, as such species may grow faster or be more economically viable to grow in comparison with indigenous bivalves (Robinson *et al.,* 2005a; Streftaris & Zenetos, 2006). Unintentional introductions of alien invasive species through the aquaculture industry include predators, parasites and pathogens hitchhiking upon the introduced bivalve shells to new locations. For example, the predatory whelk, *Urosalpinx cinerea*, has been recently introduced into the Netherlands and was found to prey upon local oyster (Faasse & Lighthart, 2009), whilst the black sea urchin was transported from Chile to South Africa upon oyster shells (Haupt *et al.,* 2010). Furthermore, the parasitic worm, *Terebrasabella heterouncinata,* was introduced into California with abalone from South Africa and shows the ability to deform abalone shells, greatly reducing market prices (Naylor *et al.,* 2001).

The establishment and spread of marine NIS may also be facilitated by the aid of artificial structures such as marinas, concrete walls, pilings and pontoons (Glasby *et al*., 2007; Tyrrell & Byers, 2007; Foster *et al*., 2016). Artificial structures are considered to be equally foreign to the evolutionary history of both native species and NIS and therefore they may level the playing field of the environment in which native species are specifically adapted to (Tyrrell & Byers, 2007). In Sydney Harbour, Australia, native species assemblages were up to 2.5 times greater than NIS on natural rocky reefs, whereas NIS assemblages were 1.5-2.5 times greater than native species on artificial substrata (Glasby *et al*., 2007). Furthermore, in the marina at Wells, USA, Tyrrell & Byers (2007) found that exotic tunicate abundances increased over time on artificial structures at the expense of native species abundances. This suggests that it is common for artificial structures in marine environments to provide a habitat for NIS that may represent entry points for invasion and establishment (Glasby *et al*., 2007; Tyrrell & Byers, 2007; Foster *et al*., 2016).

The introduction of polyvectic species is also important in the spread and establishment of NIS. A polyvectic species is a species that has the ability to be introduced to new locations through more than one type of vector (Ruiz *et al*., 2011). In California, more than half of the 257 non-indigenous species that established were considered to be polyvectic (Ruiz *et al*., 2011). The Mediterranean mussel, *Mytilus galloprovincialis*, has invaded the South African coasts and was introduced along the west coast unintentionally through shipping and subsequently introduced to the south coast intentionally for mariculture (Branch & Steffani, 2004; Robinson *et al.,* 2005a), thus making it a polyvectic species.

1.2. *Mytilus galloprovincialis*

Mytilus galloprovincialis, is a globally successful alien invasive marine species with an anti-tropical distribution and is South Africa's most successful alien invasive marine species (Robinson *et al.,* 2005a; Zardi *et* al., 2006a; Bownes *et al.,* 2008; Nicastro *et al.,* 2010). *Mytilus galloprovincialis* is a filter-feeding mussel native to the Mediterranean and Atlantic coasts of Europe and North Africa, but has subsequently spread due to anthropogenic activities and may now be found along the rocky shores of every continent except Antarctica and is listed among the "100 world's worst invasive species" (Bownes *et al.,* 2008; McQuaid *et al.,* 2015; Zardi *et al.,* 2018). It has been introduced accidentally through the discharge of ballast water, in which their planktonic larvae survive, or by fouling and/or for mariculture (Wonham, 2004; Braby & Somero, 2006; Peteiro *et al.,* 2007; Hanekom, 2008; Zardi *et al.,* 2018). The Mediterranean mussel shows characteristics of an aggressive alien invasive species. It has a high fecundity (van Erkom Shurink & Griffiths, 1991; Hockey & van Erkom

Shurink, 1992), and recruitment rate (Harris *et al.,* 1998), as well as a fast growth rate (Griffiths *et al.,* 1992; Xavier *et al*., 2007), high tolerance and resistance to desiccation (Hockey & van Erkom Shurink, 1992) and sand stress (Zardi *et al.,* 2006b), lack of parasites (Calvo-Ugarteburu & McQuaid, 1998), has fewer predators (Branch & Steffani, 2004), and they are generally a better competitor for space than indigenous species (Hockey & van Erkom Shurink, 1992) – which explains its ability to thrive on coasts around the world (Griffiths *et al.,* 1992; Branch & Steffani, 2004; Robinson *et al*., 2005a). Although this species is generally found on wave-exposed rocky shores it also has the ability to thrive in sheltered environments, such as estuaries, where wave action is low (Molares & Fuentes, 1995; Pollard & Hodgson, 2016; Azpeitia *et al.,* 2017).

One of the Mediterranean mussel's most advantageous characteristics as an alien invasive species within South Africa is its higher fecundity compared with native species (van Erkom Shurink & Griffiths, 1991; Branch & Steffani, 2004; Robinson *et al.,* 2005a; Nicastro *et al.,* 2010; McQuaid *et al*., 2015). International studies have shown that gametogenesis in *M. galloprovincialis* populations occurs during the winter whereas peak reproduction and spawning periods occur from early spring to summer, when water temperatures and chlorophyll-α concentrations increase (e.g. Abada-Boudjema & Dauvin, 1995; Molares & Fuentes, 1995; Atalah *et al.,* 2017; Azpeitia *et al.,* 2017). Reproduction occurs via external fertilization, followed by the dispersal of the planktonic propagules by wind-driven surface currents (McQuaid & Phillips, 2000; McQuaid *et al.,* 2015; Atalah *et al.,* 2017). In South Africa reproductive output of *M. galloprovincialis* can be as high as 20% -200% greater than that of indigenous mussel species and settlement of *M. galloprovincialis* larvae may reach extraordinary densities of up to 2 million recruits.m^{-2} (van Erkom Shurink & Griffiths, 1991; Harris *et* *al.,* 1998; Branch & Steffani, 2004). The mussel larvae can display secondary settlement because the larvae may attach and detach from substrata until a suitable substratum is found (Branch & Steffani, 2004; Atalah *et al*., 2017).

Settlement and recruitment patterns of the mussel probably reflect local hydrographic conditions of where populations are found (McQuaid & Phillips, 2000). Recent studies suggest that solar irradiance may drive spawning and settling periods, as late winters with high levels of solar irradiance drive earlier spring spawning and settling, while late winters with low levels of solar irradiance delay such processes (Fuentes-Santos *et al.,* 2016; Atalah *et al.,* 2017). Sexual maturity of the mussel is reached within its first year of life and reproductive periods occur at least once per year (Azpeitia *et al*., 2017).

Mytilus galloprovincialis may have severe impacts on local faunal community structures in regions it has invaded. It has displaced native mussel species on the coasts of California, Japan and South Africa (Suchanek *et al.,* 1997; Branch & Steffani, 2004; Wonham, 2004; Braby & Somero, 2006). It has also hybridised with native mussel species in France, California, United Kingdom and Japan - with hybrid offspring of *M. galloprovincialis* often being better competitors than indigenous mussels (Suchanek *et al.,* 1997; Hilbish *et* al., 2002; Wonham, 2004; Braby & Somero, 2006; Lockwood & Somero, 2011; Hilbish *et al.,* 2012; Zardi *et al.,* 2018). Owing to its fast growth rates and its ability to outcompete native species it has also caused negative economic impacts on aquaculture through biofouling in countries such as New Zealand, where it is estimated to cost the Marlborough Sound's region alone US$16 million each year (Atalah *et al.,* 2017). This species, however, is also known to have positive impacts on biodiversity and mussel-culture industries. In South Africa large populations of *M. galloprovincialis* produce an abundance of food for the African Black Oystercatcher, *Haematopus moquini,* and has had the effect of increasing the bird's population size and breeding productivity (Coleman & Hockey, 2008). Furthermore, and in contrast to New Zealand, this alien invasive species is significantly important in the mussel-culture industries of South Africa, as well as its native regions of the Mediterranean, as these industries are entirely dependent on the mussel (Molares & Fuentes, 1995; Robinson *et al.,* 2005a; Azpeitia *et al.,* 2017; Zardi *et al.,* 2018)

The Mediterranean mussel was first recorded in South Africa along the west coast in the late 1970s, where it was most likely introduced through ballast water (Branch & Steffani, 2004; Zardi *et al*., 2018), and by the mid-1980s it had already become the dominant intertidal mussel of west coast rocky shores (Branch & Steffani, 2004; Robinson *et al.,* 2005a; Xavier *et al*., 2007; Pollard & Hodgson, 2016; Zardi *et al*., 2018). The mussel was later introduced to Algoa Bay (Port Elizabeth) on the South African south coast for mariculture and its range has subsequently been extended (McQuaid & Phillips, 2000; Robinson *et al.,* 2005a; Pollard & Hodgson, 2016). *Mytilus galloprovincialis* now occupies about 2800 km of the South African shoreline, although roughly 74% of its biomass occurs along the west coast (Hockey and van Erkom Schurink, 1992; Griffiths et al., 1992), and this is considered to be this species' most rapid invasion ever (Branch & Steffani, 2004; Robinson *et al.,* 2005a; McQuaid *et al.,* 2015; Zardi *et al*., 2018). Recent molecular studies of *M. galloprovincialis* across its entire southern African distribution suggest that its most probable sole source of invasion is from the north-eastern Atlantic shores (Zardi *et al.,* 2018). This mussel is extremely successful along the species-poor west coast of South Africa due to its competitive characteristics and ability to outperform native species such as *Choromytilus meridionalis* and *Aulacomya ater* (Robinson *et al.,* 2005a; McQuaid *et al.,* 2015; Zardi *et al*., 2018). The invasion of *M. galloprovincialis* has also led to a more complex mussel matrix where it dominates primary rock surfaces (Hockey & van Erkom Schurink, 1992; Robisnon *et al*., 2007a). This added complexity has reduced physical stresses of the previously patchier rocky surface habitats and therefore increased invertebrate density and total species richness particularly in the *Granularis* zone (Robisnon *et al*., 2007a). Hockey & van Erkom Schurink (1992) found that the limpet, *Scutellastra granularis*, increased its overall density due to the favourable substratum for juvenile recruits and settlers provided by *M. galloprovincialis* mussel beds*,* although the maximum size of the limpets was limited by the host mussel (Griffiths *et al*., 1992). Furthermore, it was found by Branch *et al.* (2010) that *M. galloprovincialis* outcompeted the limpet *Scutellastra argenvellei* and dominated the rocky substratum on exposed and semi-exposed shores reducing *S. argenvellei* overall population density, however, it also facilitated the recruitment of this limpet. It has been suggested that wave action mediates both the positive and negative interactions between these two species (Branch *et al.,* 2010). Along the southern coast, *M. galloprovincialis* overlaps and co-exists with the indigenous mussel, *Perna perna*, due to partial habitat segregation between the species, with *P. perna* dominating the low shore, *M. galloprovincialis* dominating the high shore and an overlap zone between the two (Robinson *et al.,* 2005a; McQuaid *et al.,* 2015). The invasion of this species has altered native communities and structures, as well as increased the overall mussel biomass along the southern African coastline, providing both positive and negative effects for native fauna as described above (Branch & Steffani, 2004; Robinson *et al.,* 2005a; McQuaid *et al.,* 2015; Pollard & Hodgson, 2016; Zardi *et al*., 2018).

Mytilus galloprovincialis is thought to have reached the Knysna Estuary on the south coast in the early 2000s and has subsequently colonized all man-made hard substrata in the embayment of the estuary (Pollard and Hodgson, 2016). The mussel is restricted to the lower reaches of the estuary and can reach densities of over 1200 per 0.1m² (Pollard & Hodgson, 2016), similar to densities recorded on the nearby coastline of the Tsistikamma National Park (Hanekom, 2008).

1.3. The Knysna Estuary

The Knysna Estuary (34°04'35''S; 23°03'40E) is a permanently open, S-shaped estuary located on the warm-temperate region of the South African southern Cape coast, being the only estuarine bay in the region (Russel, 1996; Maree, 2000; Schumann, 2000; Napier *et al.*, 2009; Allanson *et al.*, 2014). The Knysna River is about 60 km long and emerges from the Outeniqua Mountains entering the estuary at Charlesford (Largier *et al.,* 2000). The estuary channel extends about 19 km in length, has a maximum width of 3.2 km and depth of 12 metres (Day *et al.,* 1981; Napier *et al.*, 2009). Two islands, Thesen Island and Leisure Isle, are present within the lower reaches of the estuary and are accessible to the public via causeways. The estuary has a microtidal range, between 0.4 and 2.0 m, with the tidal prism being estimated to be 19 million cubic meters during spring tides, the influences of the tide being evident for the full 19 km of the estuary (Largier *et al.*, 2000; Allanson, 2000; Napier *et al*., 2009). The middle and lower reaches of the estuary are similar to that of a sheltered marine environment (salinities 30-34 ppt) (Largier *et al*., 2000) owing to the large ebb and flow of seawater that replaces the estuarine water twice daily with low wave action (Maree, 2000; Largier *et al*., 2000; Schumann, 2000; Allanson *et al.*, 2014). The upper reaches of the Knysna Estuary represent a more estuarine environment, as there are lower salinity levels (salinities less than 30 ppt) than the lower and middle reaches of the estuary, owing to the Knysna River freshwater mixing with the more marine waters of the lower reaches (Largier *et al*., 2000; Maree, 2000). The salinity levels of the upper reaches of the estuary depend on the seasonal variability of rainfall received, as the degree of dilution is greater with increased rainfall, while during times of drought the salinity levels can be higher than that of seawater (Largier *et al.*, 2000; Maree, 2000; Allanson, 2000).

The Knysna Estuary is regarded as South Africa's most biodiverse estuary and is ranked highly in terms of conservation importance due to the estuary containing 42.7% of all South African estuarine biodiversity, including the endangered seahorse *Hippocampus capensis* (Day, 1981; Russel, 1996; de Villiers *et al.*, 1999; Allanson, 2000; Marker, 2003; Allanson *et al.*, 2014). High estuarine biodiversity can be attributed to physical factors within the estuary (clear water, permanently open mouth, low wave action and quiet waters) as well the relatively low input of freshwater (de Villiers *et al*., 1999). Due to Knysna's increasing tourist popularity and residential expansion there has been an increase in civil engineering construction (such as roads, bridges, jetties, gabions and walls) that may be found within the estuary. These artificial structures have provided marine invertebrates with a new and change of habitat, increasing their overall contribution to estuarine biodiversity and community structure (Allanson *et al.*, 2014), including the endangered seahorse *Hippocampus capensis* (Claasens *et al.,* 2018). Although the estuary boasts a great estuarine biodiversity it is also Knysna's greatest economic asset as it is picturesque and a popular tourist destination, therefore conservation of the estuary will also be important for economic purposes, especially as development continues and land-use changes occur around the town (Day, 1981; Marker, 2003; Allanson *et al*., 2014).

1.4. Rationale and Aims

Although studies on the recruitment rates of *M. galloprovincialis* have been performed on the rocky intertidal coasts of South Africa (Harris *et al.,* 1998; Hockey & van Erkom Schurink, 1992; Alexander *et al.,* 2016), there is no information on the recruitment rate of this species in sheltered estuarine environments. A recent study by Pollard & Hodgson (2016), however, has shown that gonad index and condition index were greater in *M. galloprovincialis* populations from the Knysna Estuary embayment sites, compared to the open coast sites. This may be advantageous for *M. galloprovincialis,* in terms of reproductive success, and may lead to high recruitment rates in the Knysna embayment that have contributed to its rapid establishment in the estuary. The aims of this study, therefore, were to investigate, with specific focus on the alien invasive mussel *M. galloprovincialis*, (1) monthly recruitment rates of bivalves within the lower reaches of the Knysna estuarine embayment, (2) recruitment rates for *M. galloprovincialis* over neap and spring tides as well over different lunar phases, and lastly (3) settlement of *M. galloprovincialis*, and other space occupiers (molluscs and barnacles), on cleared hard man-made substrata within the estuary. It is hypothesized that one of the reasons for the success of *M. galloprovincialis* in the Knysna embayment is high and continuous recruitment and settlement rates. These studies investigated this aspect of the reproductive biology of this mussel.

Chapter 2. Materials and Methods

2.1. Study Sites

Mytilus galloprovincialis is very abundant in the bay regime (embayment) of the Knysna Estuary with very few mussels occurring above the Railway Bridge (Pollard & Hodgson, 2016). Three locations where mussels were particularly abundant were chosen as study sites. These were a canal wall constructed of gabions (wire baskets filled with rocks) close to the western entrance of Thesen Islands Marina (M), the concrete frame of Thesen Island Wharf (T), and the metal supports of the Railway Bridge (R) (Figs 1, 2a-c). These sites were chosen because all had multi-layered mussel beds, were accessible by boat, but were generally free from human interference.

All three sites were used in a monthly recruitment study, whereas only two sites (T and R) that were about 1.5 km apart were investigated for a neap vs spring tide recruitment study (see section 2.2 below). Finally, only one site (T) was used to investigate colonization of cleared plots within mussel beds. This site was chosen because the concrete frame of the wharf when cleared of mussels offered a flat surface enabling photography to be used to document recruitment of fauna.

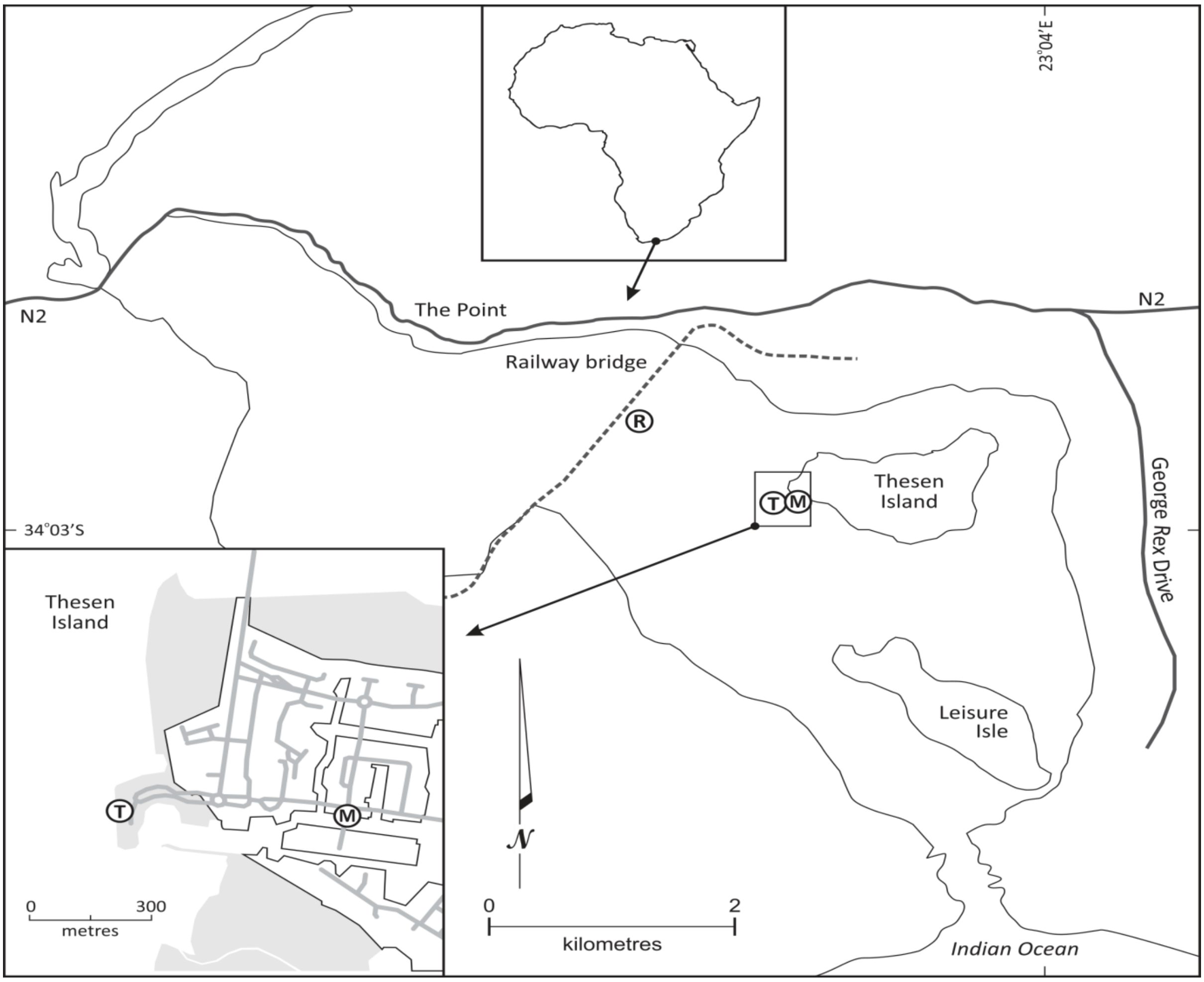

Fig. 1 Map of study sites (Thesen Islands Marina (M), Thesen Island Wharf (T) and the Railway Bridge (R)) within the Knysna Estuary, South Africa.

2.2. Bivalve Recruitment

2.2.1. Monthly Recruitment.

Bivalve recruitment was investigated at three sites (M, T and R, Figs. 1,2) monthly for 20 months (November 2015 - June 2017) around either full moon or new moon spring tides using plastic kitchen scouring pads as collectors (see King *et al.,* 1990). The woven donut-shaped pads were about 8 cm in diameter and 2 cm thick. Prior to deployment the new pads were placed in embayment water for at least 7 days to leach surface chemicals from them and to develop a biofilm. At each site two ropes (Fig. 2c) about 10 m apart were attached to the structures so that the collectors were positioned in the middle of the mussel beds. The pads, therefore, were placed at similar tidal heights (mid-balanoid region) at all sites. Five pads were attached with cable ties to each rope (Fig. 2c) resulting in a monthly deployment of 10 pads per site. The pads were left for 7 days then collected and each pad placed immediately in a plastic pot containing 70% ethanol and transported back to the laboratory at Rhodes University where they were stored until analysis. In this study we considered recruits to be bivalve individuals that survived on the pads from the moment of settlement until they were sampled (Molares & Fuentes, 1995).

To obtain recruits, each pad was soaked in sodium hypochlorite solution for 5 minutes to detach the bivalve recruits from the pads (Davies, 1974), then rinsed in water filtered through a 75 µm sieve (see Appendix 1 for detailed methodology). The filtrate was preserved in 70% ethanol and decanted into a Bogorov counting chamber. Each sample was then examined under a dissecting microscope, and the bivalve recruits identified to species where possible. Counts of each bivalve taxon were made and in addition the size (shell length) of *M. galloprovincialis* recruits was measured to the nearest 0.04 mm with the aid of an eyepiece graticule. Subsamples of 100 recruits were measured for samples containing greater than 200 recruits.

Salinity and temperature data were also obtained for the duration of the study. These were obtained through the Knysna Estuary Management Platforms, in which permanently Hach sonde devices were deployed and collect data in real time. These data were provided by the Knysna Basin Project.

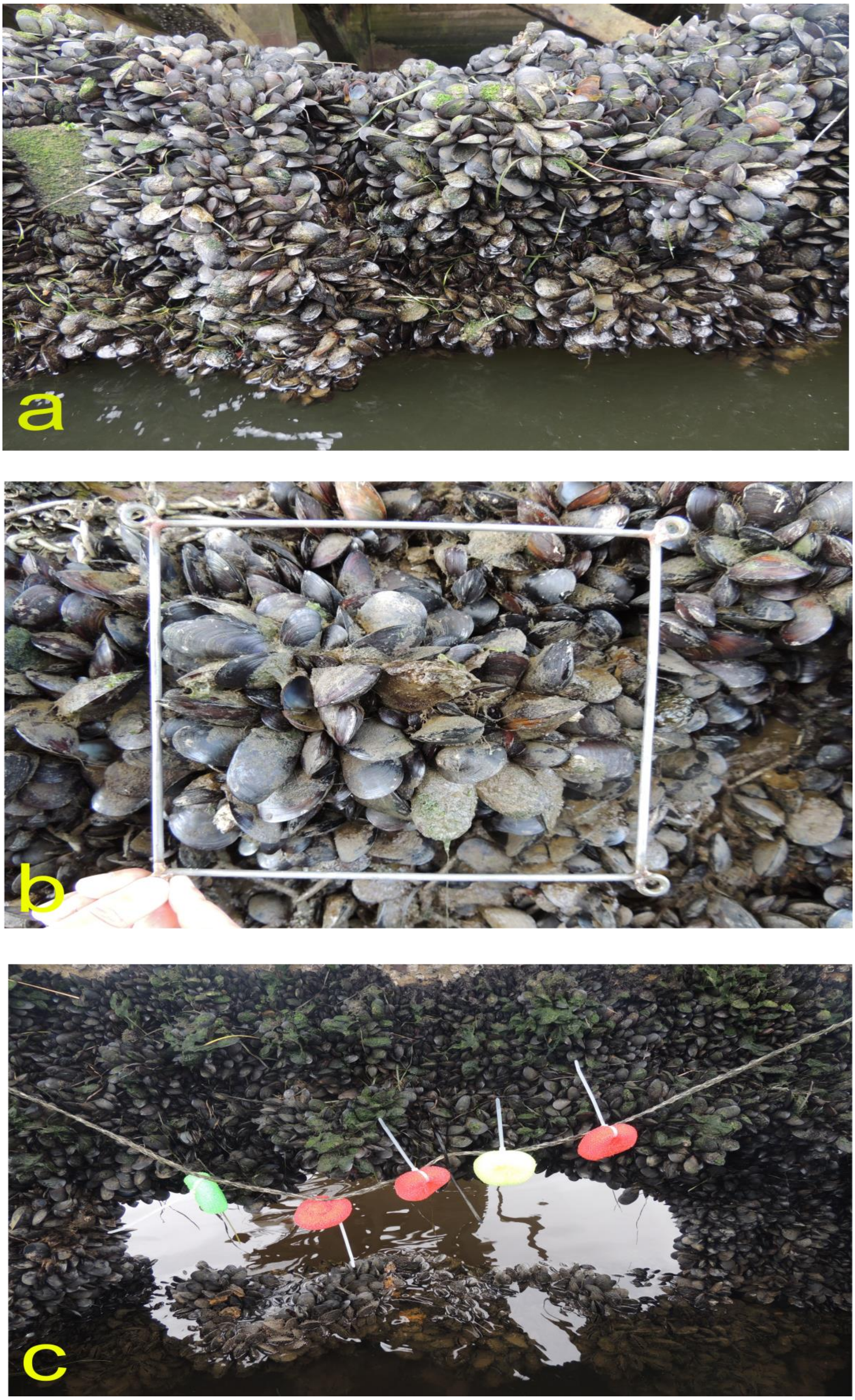

Fig. 2 Photographs of mussel beds within the study sites in the Knysna estuarine embayment: a) Thesen Island Wharf, b) Thesen Islands Marina and c) the Railway Bridge.

2.2.2. Tidal Recruitment – Neap vs. Spring Tides

To investigate whether recruitment of *M. galloprovincialis* varied between spring and neap tides, a preliminary study was undertaken during such tides at three different times of the year. Ten pads (five per rope) were placed on Thesen Island Wharf (T) and ten on the Railway Bridge (R) three days before a neap and a spring tide and then collected three days after the respective tide in March 2017, August 2017 and October 2017. These months were chosen as they fell within three different seasons. After collection the pads were processed as explained in 2.2.1 and the number of *M. galloprovincialis* recruits were counted.

2.2.3. Lunar Phase Recruitment

To investigate in more detail whether recruitment of *M. galloprovincialis* was affected by phase of the moon (First Quarter, New Moon, Last Quarter and Full Moon), and therefore spring and neap tide, a more intensive study was undertaken over the summer months (February and March) of 2018, as it is the summer months when bivalve recruitment was greatest (see Results section 3.1.2). The sampling method was identical to that explained previously (section 2.2.2) except that it took place over a six-week period. After collection the pads were processed as explained in 2.2.1 and the number of *M. galloprovincialis* recruits were counted. The preliminary study suggested that there may be an effect of tide on recruitment (see Results section 3.3).

2.2.4. Recruitment and Re-Colonisation of Clearance Plots

To further investigate recruitment and re-colonisation of mussels and other space occupiers, 18 plots (15 cm x 15 cm) were cleared of mussels within the mussel beds on Thesen Island Wharf in February 2017. Every month six plots were cleared of all mussels (treatment A) that settled or encroached on the plots (to investigate which fauna would occupy these plots when mussels were absent), six plots were left completely undisturbed (treatment B) and six plots had all fauna removed except mussels (treatment C). Plots were photographed and then photographed monthly for 11 further months i.e. until January 2018.

2.3. Statistical Analysis

All statistical analyses were done using the program R Studio v.1.0.136 (R Core Team, 2018). The effect of location and date on the abundance of settlers for all bivalves were investigated by performing negative binomial regressions (using the model: Recruits (counts) ~ Date*Site) (function: ‘MASS::glm.nb’). The negative binomial regressions were chosen to accommodate for the over-dispersion of the means in the observed data. Tests for patterns of residuals against predicted values suggested a negative binomial regression. These models were used to predict recruitment values for bivalves which were then compared with the observed data (function: ‘stats::predict’). Likely-hood ratio tests (Chi-squared) were also performed on the models to test for significance between date, sites and for an interaction between the two. Additionally the effect of the location on the distribution of *M. galloprovincialis* recruit sizes was tested by a Kruskal-Wallis test (function: ’stats::kruskal.test’), followed by a Dunn’s post-hoc test to identify where shell lengths differed significantly (function: ‘FSA::dunnTest’).

To investigate the effect of recruitment rates of *M. galloprovincialis* at two sites (T and R) during different tides during three time periods in 2017 involved creating separate negative binomial regressions (model: Recruits ~ Time Period + Tide). These models were used to create predictions (where applicable) that were then compared with the observed data. The lunar phase study looking at the differences between lunar phases and recruitment levels over summer 2018 (February and March) was analysed by using Kruskal-Wallis test (function: ’stats::kruskal.test’) to test for a significant difference between recruitment levels of *M. galloprovinvialis* over the different lunar phases for both sites T and R, followed by a Dunn’s test (function: ‘FSA::dunnTest’) to identify where significant differences occurred.

When analysing the photographed plots it was difficult to calculate densities of *M. galloprovincialis* in treatments B and C due to the 3-D structure of the mussel beds. Therefore, the average percent coverage of the various invertebrates of each treatment (A-C) was calculated to determine how fast colonisation of free space occurred among *M. galloprovincialis* and other macro-invertebrates. Each treatment was then analysed by one-way analysis of variance (ANOVA) followed by Tukey HSD post-hoc test to identify where differences occurred for each treatment.

Chapter 3. Results

3.1. Monthly Recruitment of Bivalves

3.1.1. Temperature and Salinity of the Embayment Water During Study

Water temperature of the Knysna estuarine embayment showed seasonal fluctuations. Mean embayment water temperatures were highest during typical summer months (November – March) and was highest at up to 25°C; mean = 23.1±1.6°C (December), however, it was also when water temperatures varied the most (Fig. 3). Summer water temperatures varied between 15 to 25°C (January 2016) a result of upwelling that occurs regularly at this time of year (Schumann, 2000). Lower water temperatures predominated winter months (May - September) and declined to just below 15°C (July) but were less variable when compared to summer. With the exception of the first month of the study (November 2015) water salinity was relatively constant (about 35 ppt.) (Fig. 3).

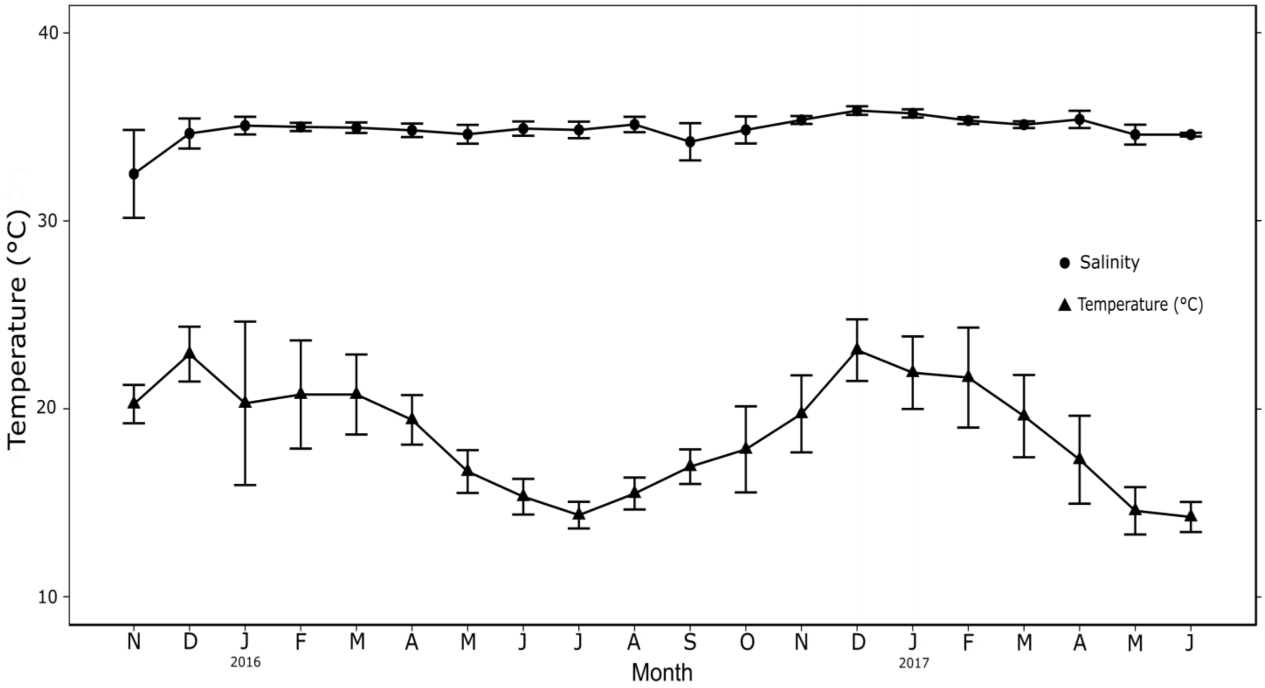

Fig. 3 Mean (±SD) water temperature (°C) and salinity (ppt) of the Knysna Estuarine embayment. Data were obtained from the Knysna Estuary Monitoring Platforms, the Knysna Basin Project.

3.1.2. Monthly Recruitment of Bivalves

The recruits of four bivalve taxa were identified at all sites. *Mytilus galloprovincialis* (Fig. 4a) and *Perna perna* (Fig. 4b) were identified based on shell morphology (Bownes *et al*., 2008), whereas it was not possible to identify the other Mytilidae (Fig. 4c) and Ostreidae (Fig. 4d) recruits to the species level. It is possible that the unidentified bivalve species may be the estuarine mussel *Arcuatula capensis* (Fig. 4c) as this species is common to the lower and middle reaches of the estuary (Allanson *et al.,* 2014).

Recruitment occurred on all dates for all bivalve taxa, and the temporal pattern of recruitment was similar (Fig. 5). The majority of recruits were *M. galloprovincialis*, with peak recruitment (March 2016) levels up to 4.5x greater (mean max = 340±66 per pad) than the bivalve taxon with the next highest peak recruitment (Ostreidae, March 2016) (mean max = 76±22 per pad), 9x greater than *Perna perna* (February 2017) and up to 17x greater than the unidentified bivalve species (March 2016).

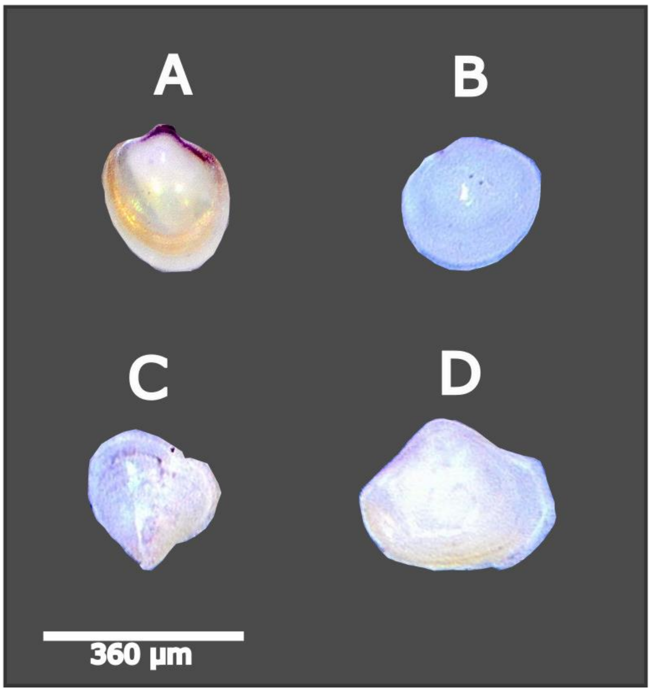

Fig. 4 Images of bivalve taxa larvae found in the Knysna estuarine embayment. A) *Mytilus galloprovincialis*, B) *Perna perna*, C) Ostreidae, D) unidentified bivalve spp.

The negative binomial models created for each bivalve taxon fitted the data well and explained more than 80% ($r^2 > 0.80$) of the recruitment variability in each case (Figs. 6-9). The Chi-squared likelihood ratio tests for each model show highly significant differences in recruitment between date, sites and for an interaction between date and sites for all species ($P < 0.001$ in all cases) (see Appendix 2, Tables 3-6). All models showed that recruitment occurred throughout the study, however there was a clear seasonal pattern in recruitment within the Knysna estuarine embayment. Recruitment primarily occurred from late spring to early autumn (November-March) with considerably fewer recruits during the rest of the year. At all three sites (Thesen Island Wharf (T), Thesen Islands Marina (M), and the Railway Bridge (R)) there were particularly high levels of all bivalve taxon recruitment during December 2015, March 2016 and February 2017 (Figs. 6-9). Recruitment of Ostreidae, however, was slightly different with high recruitment levels occurring at all sites during March 2016 and February 2017, while during March 2017 higher recruitment levels were only found at sites T and R (Fig. 7). Furthermore, the Ostreidae at site T displayed recruitment further into autumn and winter (March – June 2017) in contrast with sites M and R and the other bivalve taxa (Figs. 5-9). All negative binomial models showed that sites T and M had similar recruitment patterns throughout the study and both sites had greater recruitment levels than site R. Yearly differences were also noticeable as all bivalves (except the oyster species) showed greater and more prolonged recruitment over the summer and early autumn months (December - March) of 2015/2016 compared to the summer months of 2016/2017. Bivalve recruitment levels in 2017 were only high during February.

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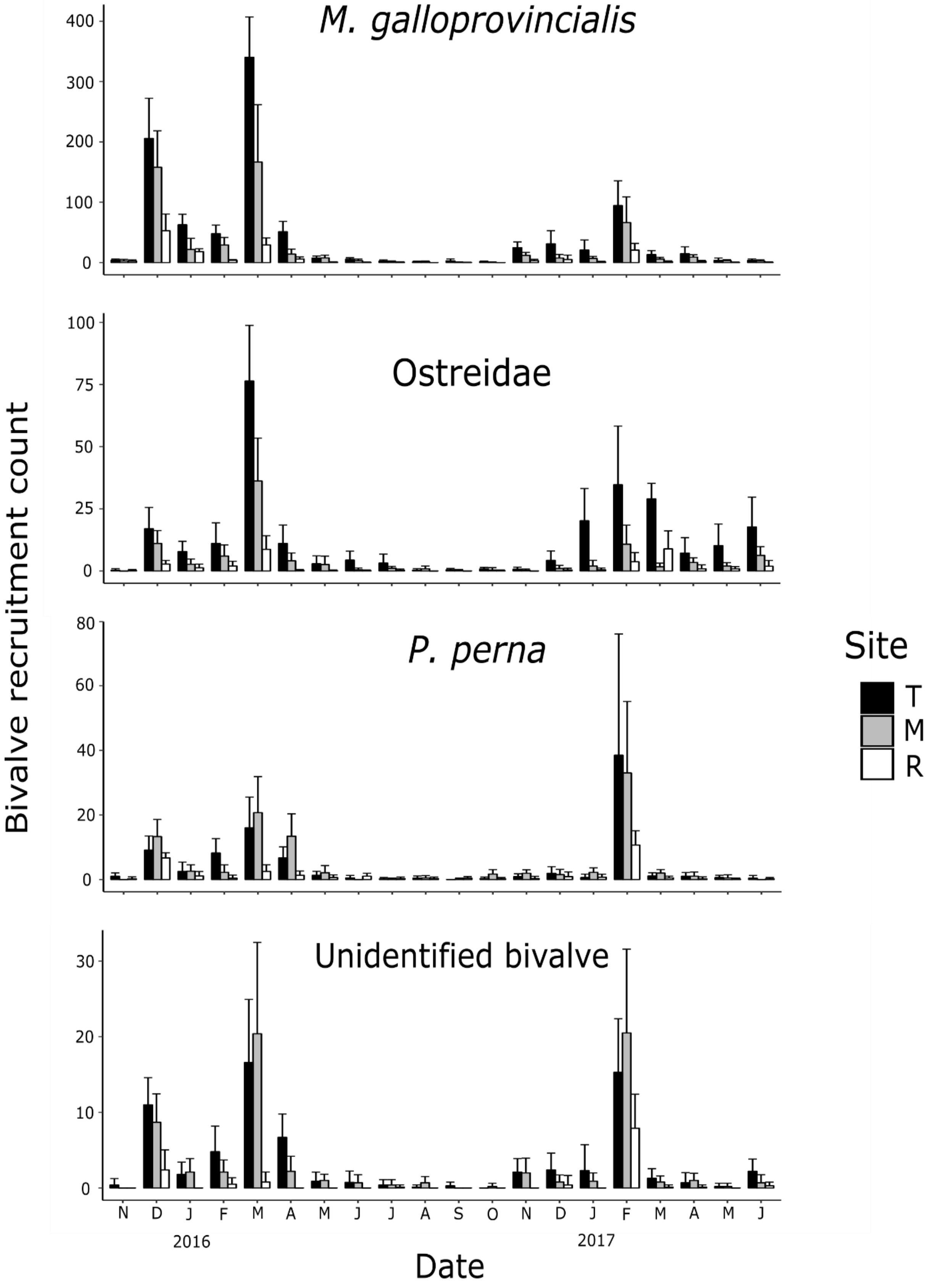

Fig. 5 Mean (±SD) recruitment per pad of four bivalve taxa at Thesen Island Wharf (T), Thesen Islands Marina (M), and the Railway Bridge (R) within the Knysna estuarine embayment.

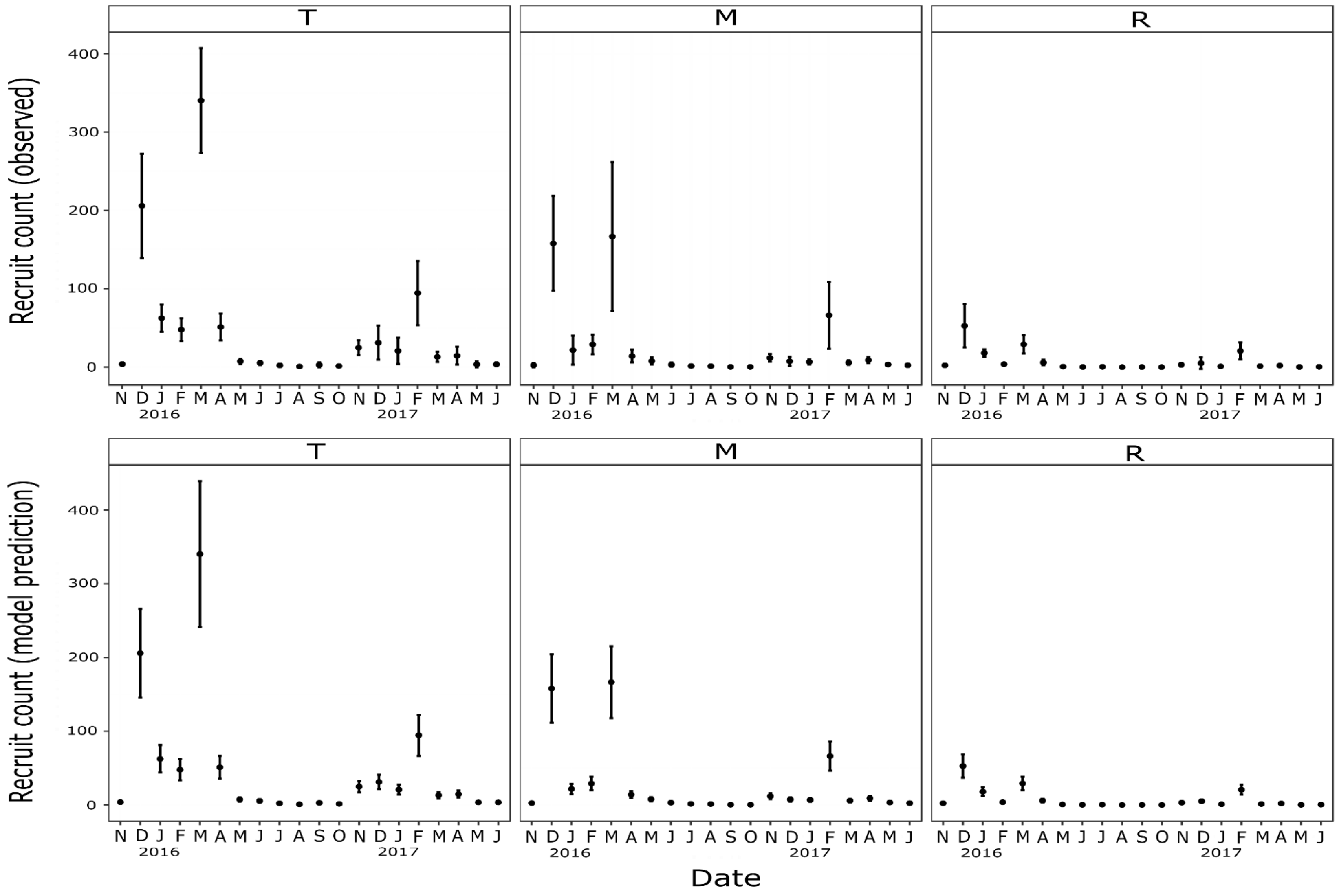

Fig. 6 Mean (±SD) recruitment of *M. galloprovincialis* per pad at Thesen Island Wharf (T), Thesen Islands Marina (M) and the Railway Bridge (R) in the Knysna estuarine embayment.

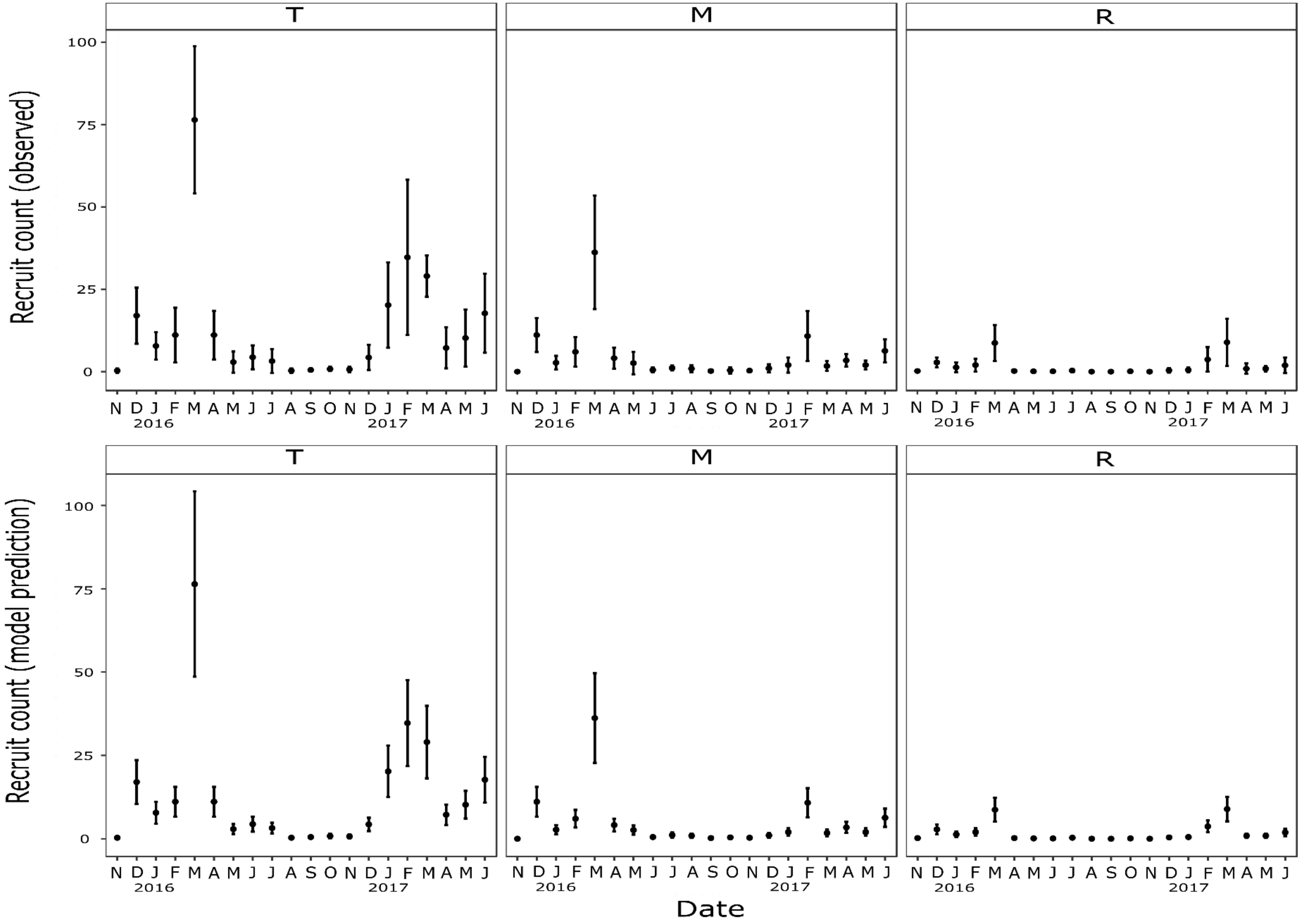

Fig. 7 Mean (±SD) recruitment of Ostreidae per pad at Thesen Island Wharf (T), Thesen Islands Marina (M) and the Railway Bridge (R) in the Knysna estuarine embayment.

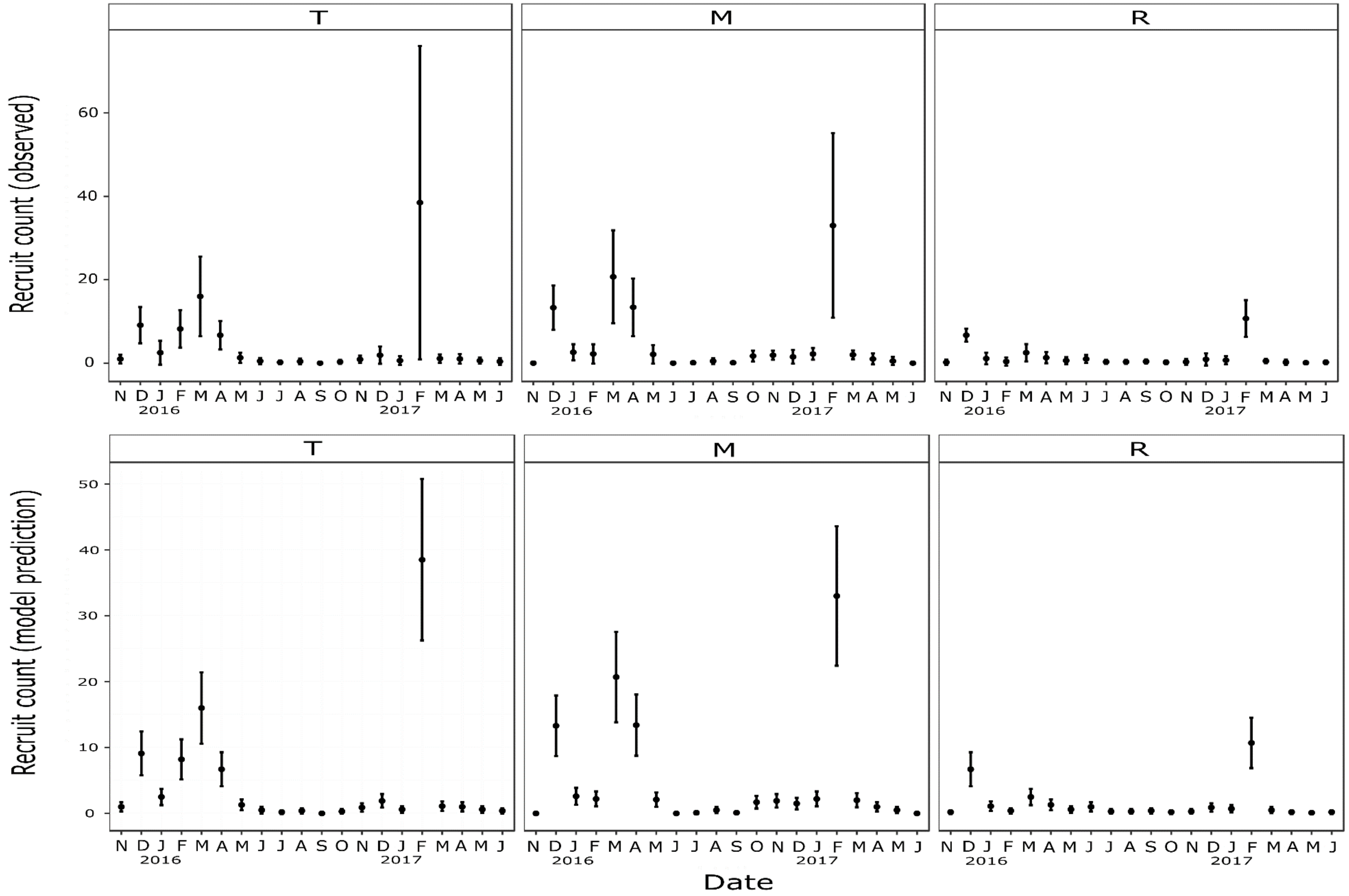

Fig. 8 Mean (±SD) recruitment of *Perna perna* per pad at Thesen Island Wharf (T), Thesen Islands Marina (M) and the Railway Bridge (R) in the Knysna estuarine embayment.

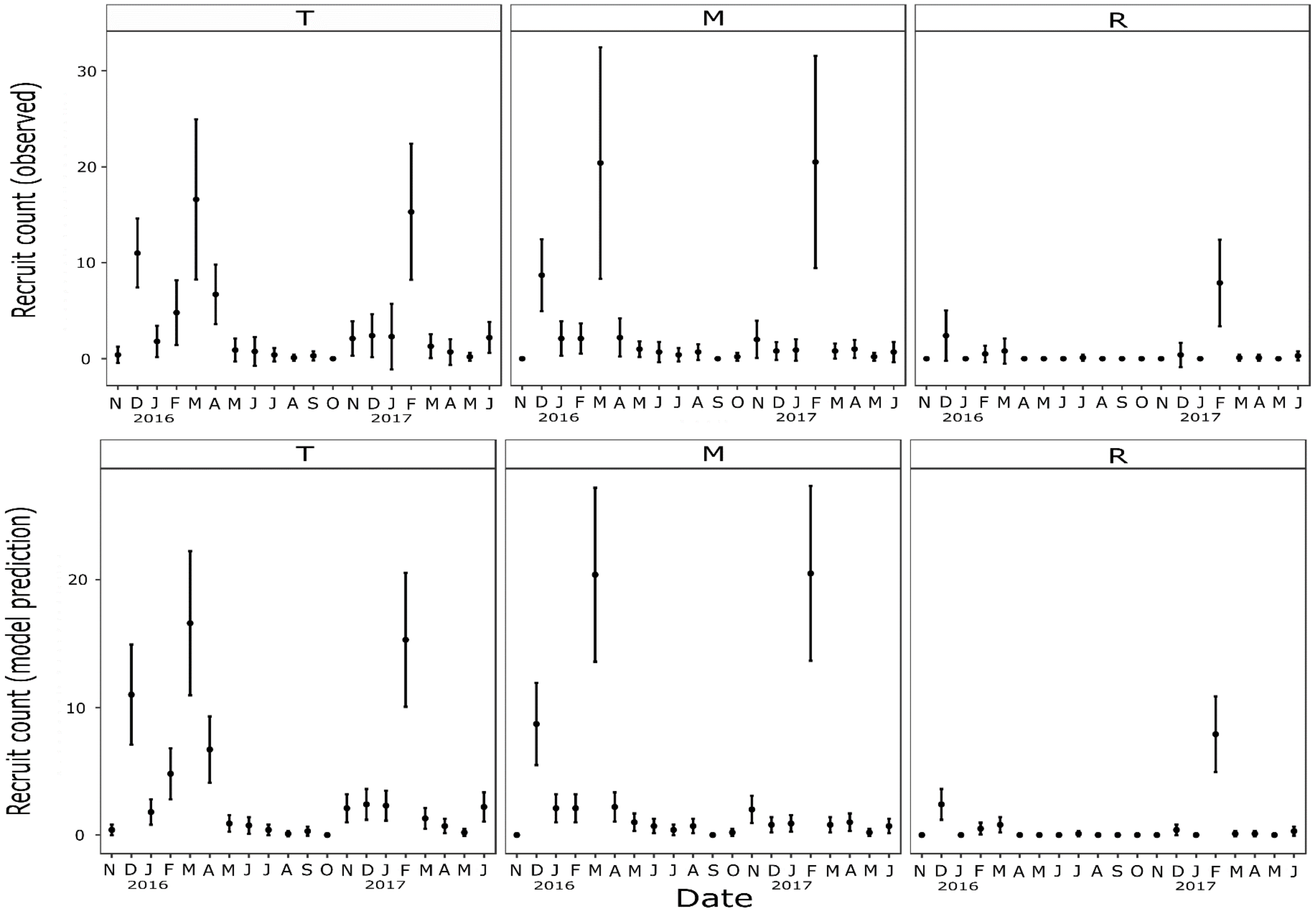

Fig. 9 Mean (±SD) recruitment of the unidentified bivalve per pad at Thesen Island Wharf (T), Thesen Islands Marina (M) and the Railway Bridge (R) in the Knysna estuarine embayment.

3.2. Size at Recruitment of *M. galloprovincialis*

Because *Mytilus galloprovincialis* recruits were the most abundant and the focus of this study, their size was measured. Although the size range of *M. galloprovincialis* recruits at all three sites during the 20 months of sampling was variable, a mode length of 320 µm was found across all three sites (Table 1). Furthermore, at each site the majority (> 70%) of recruits had a shell length between 320 and 400 µm (Fig. 10). Even though most recruit sizes tended to be similar at each site the Kruskal-Wallis (Chi-squared = 63.29, P < 0.001, df = 2) and Dunn's post-hoc analysis (see Appendix 2, Table 7) showed that there was a highly statistically significant difference between all sites (Table 1). This may be due to different numbers of *M. galloprovincialis* recruit shell lengths being measured at all sites. Furthermore, *M. galloprovincialis* recruits at site T had greater variability in shell lengths, as slightly more than 10% of recruit shell lengths at site T were equal to, or greater than, 800 µm whereas less than 2% of recruits in sites M and R were equal to, or greater than, 800 µm (Fig. 10).

Table 1. Central tendencies of *M. galloprovincialis* recruit lengths per pad at Thesen Island Wharf (T), Thesen Islands Marina (M), and Railway Bridge (R) in the Knysna estuarine embayment. Identical letters indicate no significant differences between sites.

Length (µm)	**Site**		
	T	**M**	**R**
Mean ± SD	436.23 ± 198.29	374.03 ± 94.58	359 ± 82.98
Max	1520	1200	1200
Min	280	240	280
Mode	320	320	320
Dunn's test	a	b	c
n	1343	1190	636

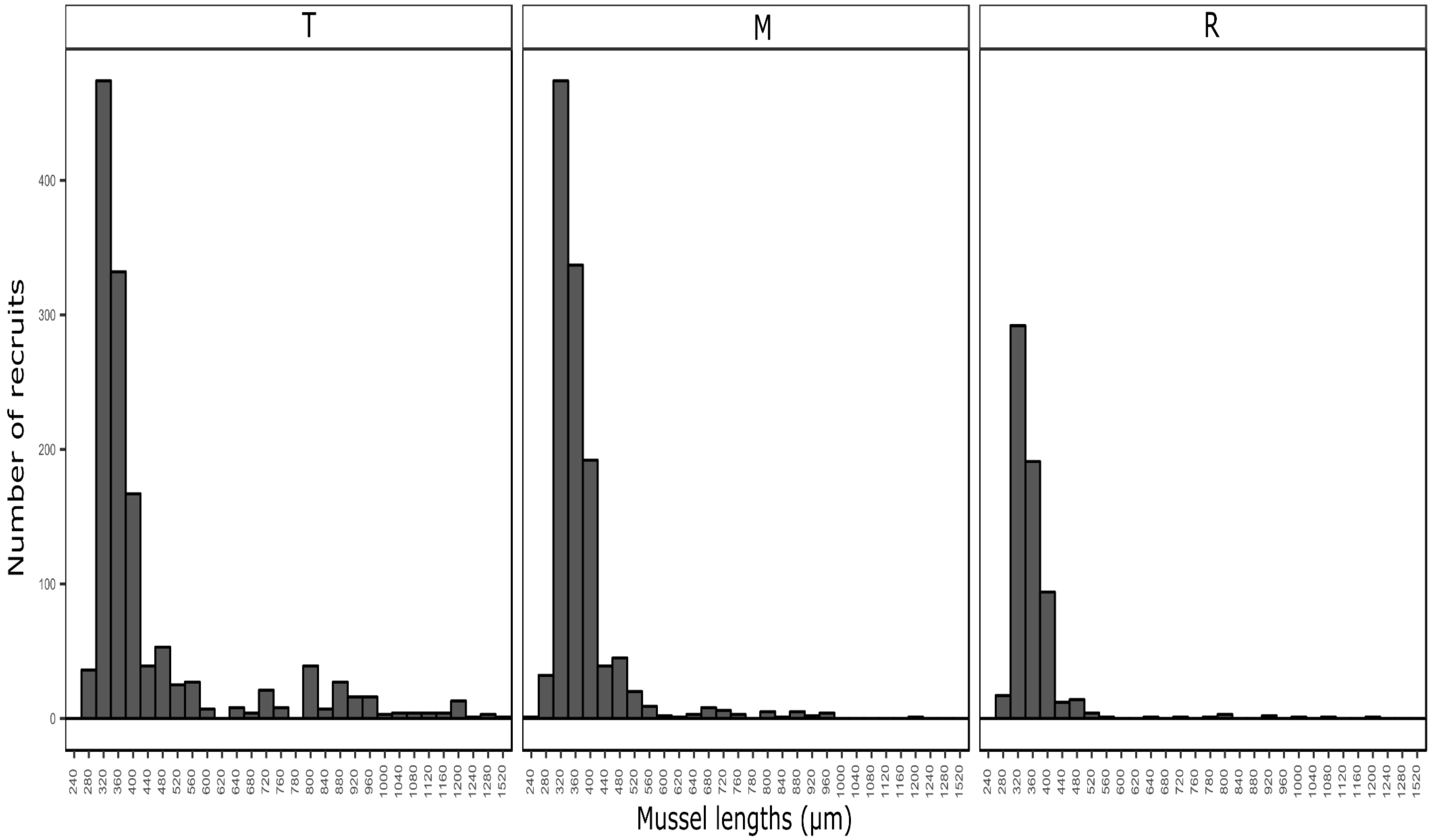

Fig. 10 Frequencies of *M. galloprovincialis* recruit lengths (µm) per pad at Thesen Island Wharf (T), Thesen Islands Marina (M), and the Railway Bridge (R) in the Knysna estuarine embayment.

3.3. Tidal Recruitment of *Mytilus galloprovinvialis* – Neap vs. Spring Tides

There appeared to be an effect of tidal phase and time of year on recruitment levels of *M. galloprovinciali*s at Thesen Island Wharf (site T) (Fig. 11). The Chi-squared likelihood ratio tests for the negative binomial model showed highly significant differences (P < 0.001) between tides and time of year, however, no significance was found for an interaction between tides and time of year (P = 0.42) (see Appendix 2, Table 8). Recruitment in August 2017 was low regardless of the tide. The negative binomial model created indicated a significant difference in recruitment between August and March as well as between August and October, whereas no difference was found between March and October. Recruitment also differed significantly tidally with greater recruitment on spring tides especially in March and October (Fig. 11).

At the Railway Bridge (R) recruitment was low during all three months investigated and for both spring and neap tides (Fig. 12). The highest mean number of recruits found at the Railway Bridge during the spring tide study was four per pad and a maximum of two recruits per pad for the neap tide study. Although it appeared that spring tides have higher average recruitment levels than that of neap tides the negative binomial regression suggested that there was no significant difference between time of year and between tides (Fig. 12). Due to the lack of significance between time of year and tides no predicted values were created for this negative binomial model. It was also noticeable that both sites displayed similar patterns of recruitment despite the actual number of recruits differing.

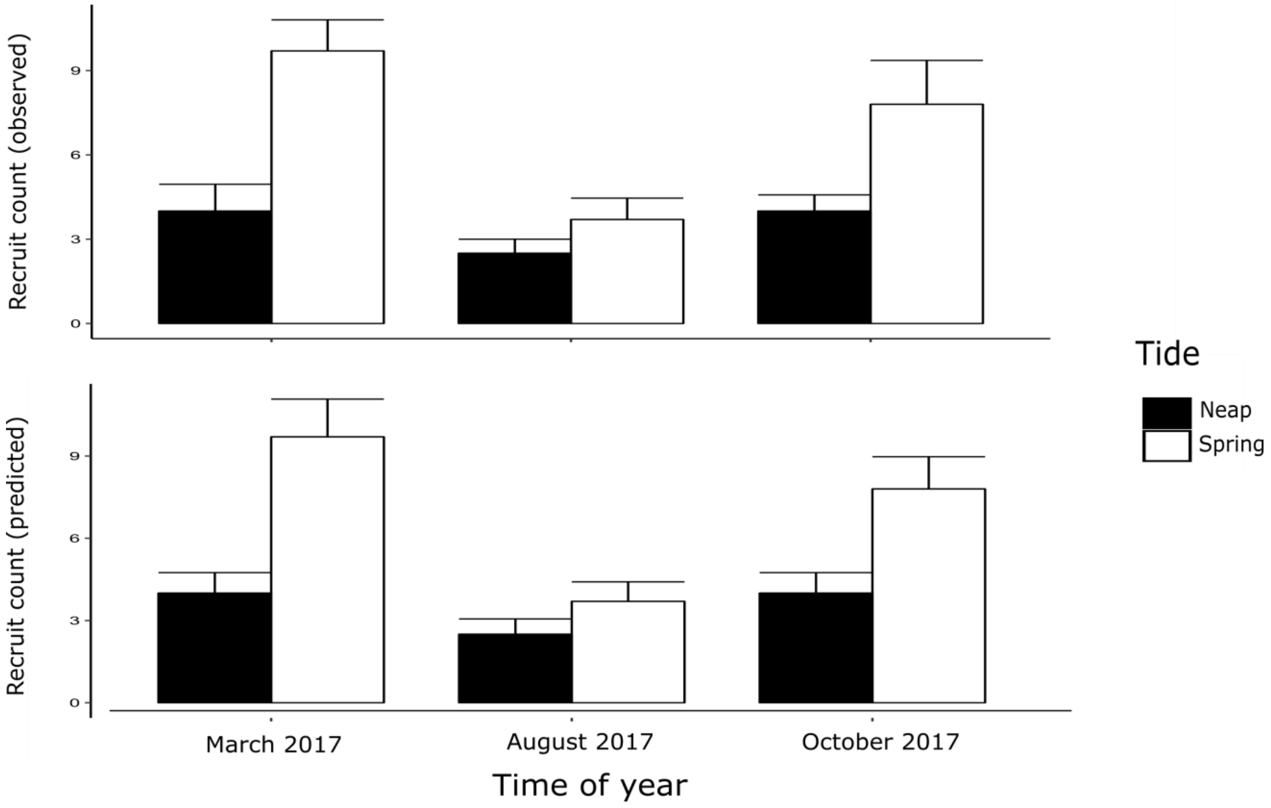

Fig. 11 Mean (±SD) recruitment of *M. galloprovincialis* per pad over neap and spring tides during different months at Thesen Island Wharf (T).

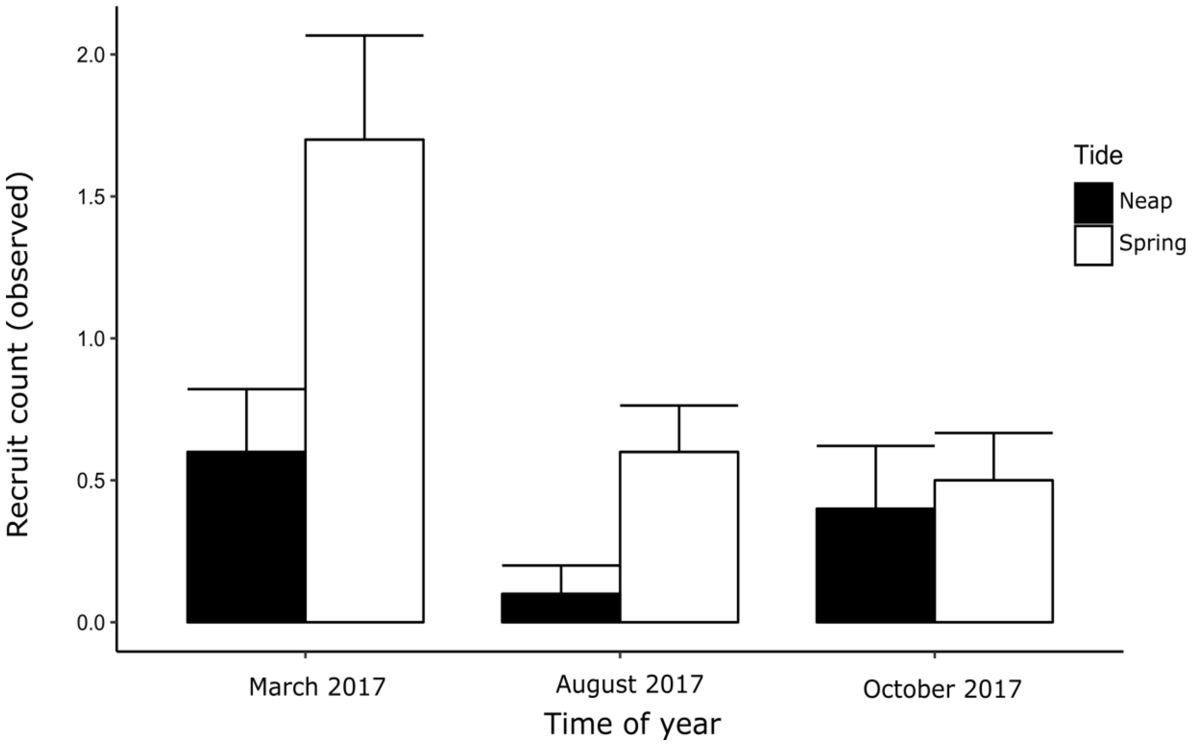

Fig. 12 Mean (±SD) recruitment of *M. galloprovincialis* per pad over neap and spring tides during different months at the Railway Bridge (R).

3.4. Lunar Recruitment of *M. galloprovincialis*

Highly significant differences ($P < 0.001$) between the phases of the moon at sites T (Chi-squared = 33.94, $P < 0.001$, df = 5) and R (Chi-squared = 36.44, $P < 0.001$, df = 5) (Fig. 13) were found by a Kruskal-Wallis test followed by a post-hoc Dunn's test (see Appendix 2, Tables 9 and 10). The greatest *M. galloprovincialis* recruitment occurred at both sites between the 20th February and 6th March 2018 during the last quarter and full moon, although variation of recruit numbers was also greatest during these phases of the moon. About 50% fewer recruits were found during other phases of the moon. Recruitment was also always greater at Thesen Island Wharf (T) than the Railway Bridge (R).

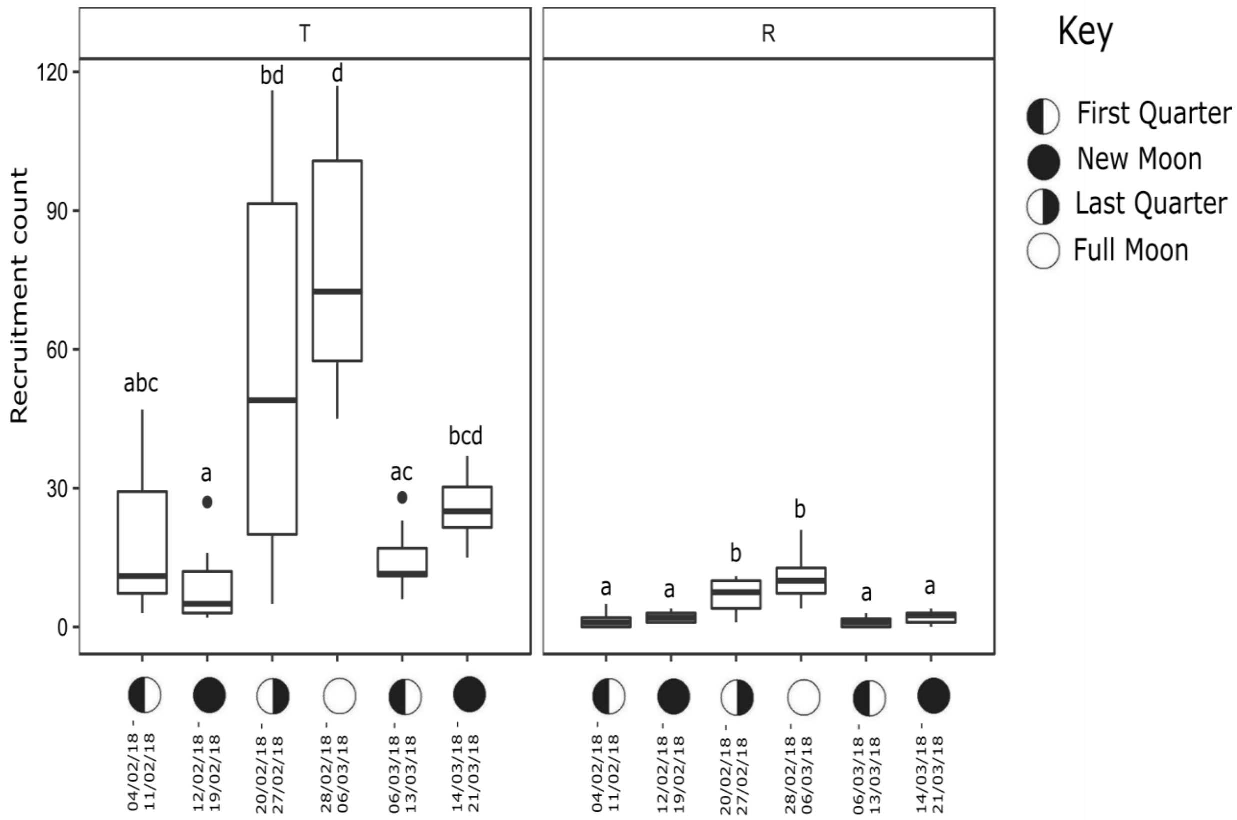

Fig. 13 Recruitment count distribution of *M. galloprovincialis* per pad during different moon phases at Thesen Island Wharf (T) and the Railway Bridge (R). Identical letters indicate no significant differences between phases of the moon.

3.5. Settlement and Re-Colonisation of Clearance Plots

In treatment A, in which plots were kept free of mussels only, the substratum was not colonised by macroinvertebrates for five months (Fig. 14). In August 2017 the first signs of re-colonisation had occurred with a few barnacles (*Amphibalanus amphitrite*) present in the plots. By October 2017 some limpets (*Siphonaria* spp.) had arrived. It was only by January 2018 that the Tukey HSD post hoc indicated significant differences ($P < 0.05$) of percentage coverage in treatment A (Fig. 14) whereby *A. amphitrite* and *Siphonaria* species occupied about 4% of the space (Figs. 14, 17). By contrast in treatment B (plots left untouched) and treatment C (all macroinvertebrates except mussels removed) the space in the cleared plots was very rapidly occupied by the encroachment of *M. galloprovincialis*. One month post-clearance the mussels occupied about 20% of the plots, increasing to 50% four months post clearance. Significant differences in percentage coverage could be seen for treatments B and C after two to three months post-clearance for each respective treatment (Figs. 15,16). Within eight months the alien invasive mussels had virtually occupied all the space (Figs. 15-17). It should also be noted that in treatment B, where the cleared plots were undisturbed throughout the study, all cleared space was occupied by *M. galloprovincialis* (Fig.17). Furthermore, no *M. galloprovincialis* recruits were found in treatments B and C, and free space was occupied through encroachment only.

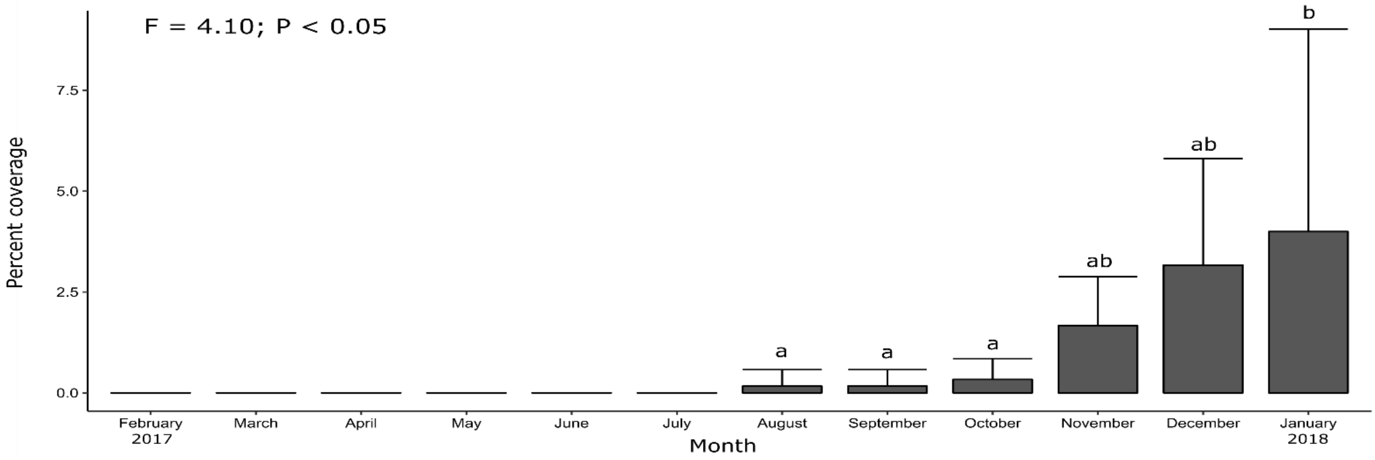

Fig. 14 Mean (±SD) percentage coverage of macro-invertebrates (treatment A) per cleared plot over a 12 month period (February 2017 – January 2018) at Thesen Island Wharf (T). Identical letters indicate no significant differences between months.

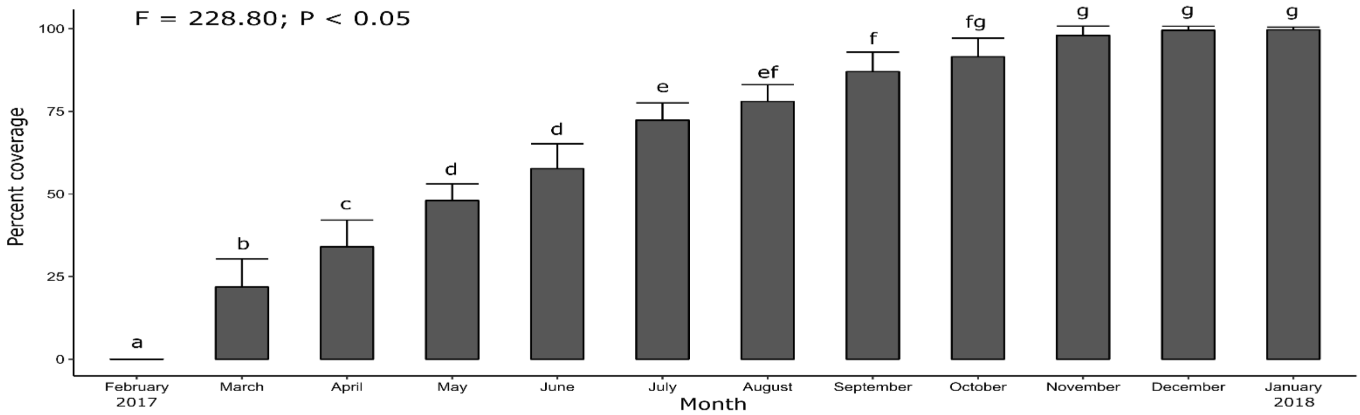

Fig. 15 Mean (±SD) percentage coverage of *M. galloprovincialis* (treatment B) per cleared plot over a 12 month period (February 2017 – January 2018) at Thesen Island Wharf (T). Identical letters indicate no significant differences between months.

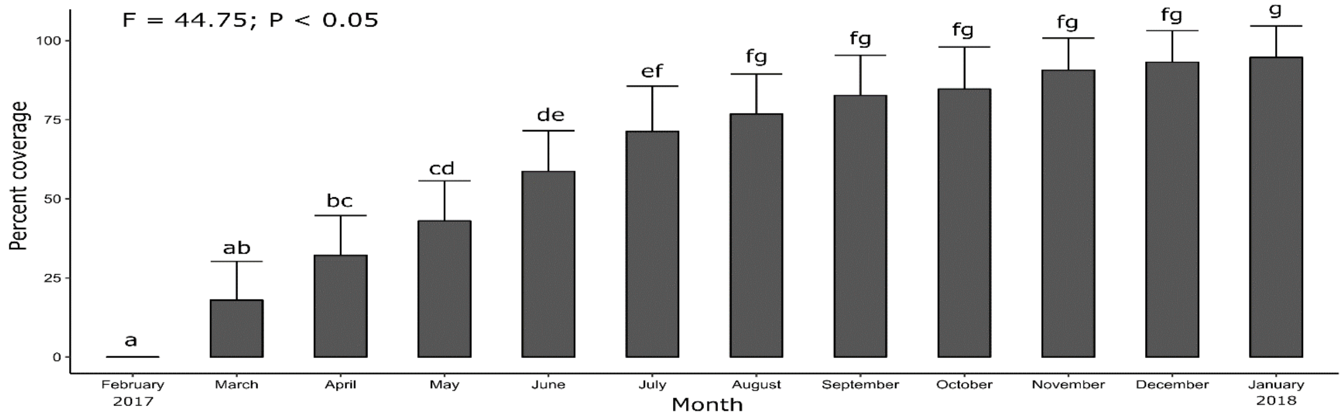

Fig. 16 Mean (±SD) percentage coverage of *M. galloprovincialis* (treatment C) per cleared plot over a 12 month period (February 2017 – January 2018) at Thesen Island Wharf (T). Identical letters indicate no significant differences between months.

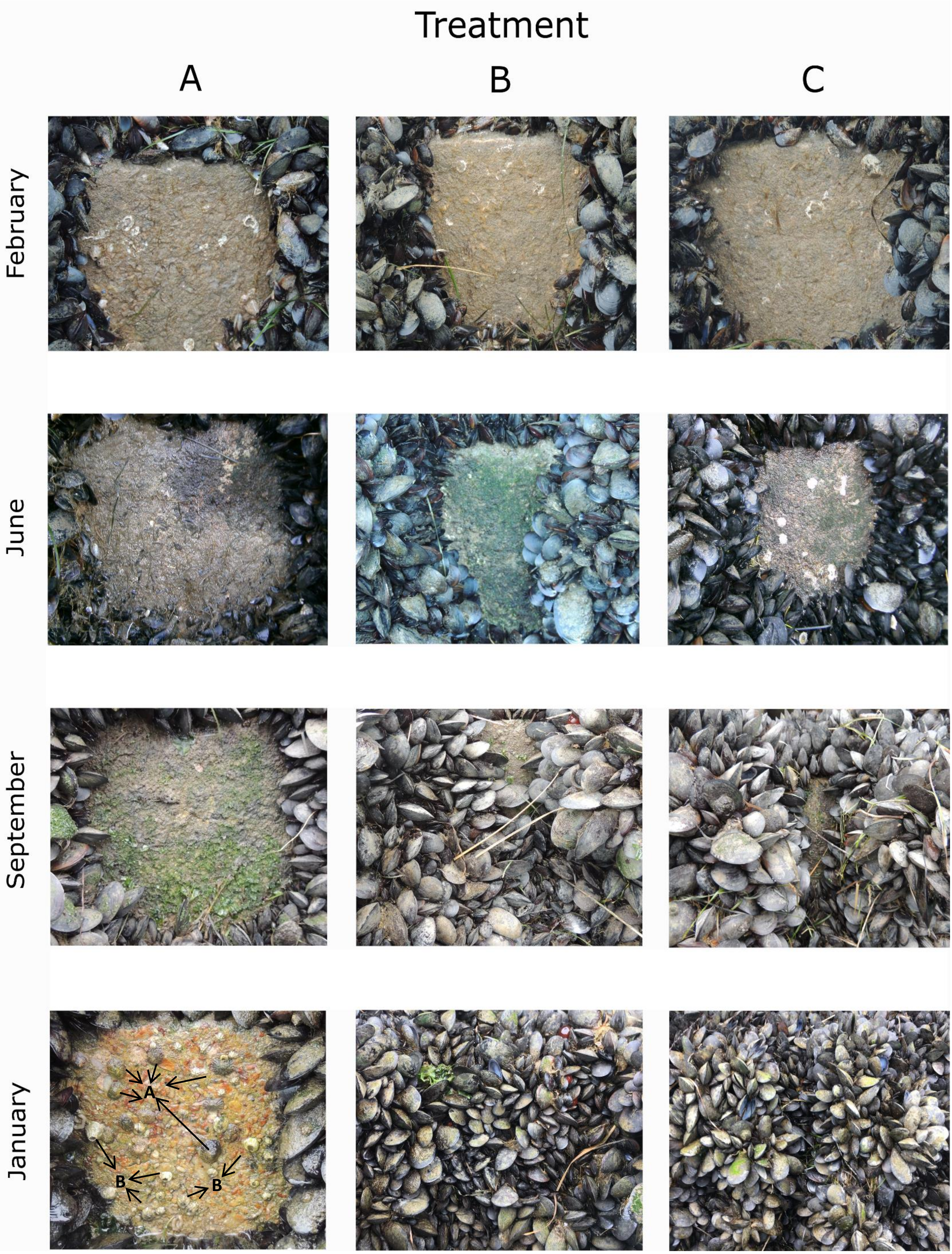

Fig. 17 Examples of cleared plots (treatments A-C) over a 12 month period (February 2017-January 2018). Arrows pointing to letter A indicate *Siphonaria* species whereas arrows pointing to letter B indicate the barnacle *Amphibalanus amphitrite.*

Chapter 4. Discussion

4.1. Monthly Bivalve Recruitment Patterns

The recruits of four bivalve taxa, *Mytilus galloprovincialis, Perna perna,* Ostreidae and an unidentified mytilid (possibly the estuarine mussel *Arcuatula capensis*) were recorded from the pads deployed in the Knysna estuarine embayment. The temporal pattern of recruitment at all sites was similar, with some recruitment throughout the year but with a peak in summer months.

The majority of recruitment studies on *M. galloprovincialis* from other regions of the world have found that in both the southern and northern hemisphere recruitment primarily occurs from late spring until early autumn, with little to no recruitment during winter (Table 2). Recruitment of *M. galloprovincialis* in the Knysna estuarine embayment, therefore, did not differ from these findings. Spring and summer reproduction is also typical of most marine invertebrates from the southern coasts of South Africa (Hodgson, 2010). An exception to this trend is populations on the west coast of southern Africa (Namibia and South Africa) where some studies have indicated more protracted recruitment of *M. galloprovincialis* throughout autumn and winter (Harris *et al*., 1998; Bownes & McQuaid, 2009; Reaugh-Flower *et al.,* 2011). Furthermore, Hodgson (2010) found that along the west coast of South Africa several species of invertebrates, including the white mussel *Donax serra*, reproduce during winter.

Table 2. Examples of recruitment studies of *M. galloprovincialis* and *P. perna* from different areas of the world.

Country	Hemisphere	Season of peak recruitment	Month(s) of peak recruitment	Reference
M. galloprovincialis				
Turkey	N	Spring - summer	March - August	Çelik *et al*. (2016)
Italy	N	Spring - summer	May - June	Ceccherelii & Rossi (1984)
Spain	N	Spring - autumn	March - September	Molares & Fuentes (1995)
Spain	N	Spring - autumn	March - October	Fuentes-Santos *et al*. (2016)
Spain	N	Spring - autumn	March - September	Caceres-Martinez *et al.* (1993)
Algeria	N	Spring - summer	June - August	Abada-Boujema & Dauvin (1995)
Morocco	N	Spring - autumn	May - September	Halla *et al*. (2018)
USA (California)	N	Spring - autumn	May - November	Johnson & Geller (2006)
Australia	S	Spring - summer	October - January	Sievers *et al*. (2014)
New Zealand	S	Spring - summer	October - December	Atalah *et al*. (2017)
Namibia	S	Winter - spring	NA	Deelie (2015)
Namibia	S	Protracted	December	Harris *et al*. (1998)
South Africa	S	Protracted	March - May	Bownes & McQuaid (2009)
South Africa	S	Spring - summer	October - February	Pfaff *et a*l. (2011)
South Africa	S	Protracted	NA	Harris *et al*. (1998)
South Africa	S	Spring - autumn	December - March	*Current study*
P. perna				
Algeria	N	Spring - summer	June - August	Abada-Boujema & Dauvin (1995)
Morocco	N	Spring - summer	April - August	Halla *et al.* (2018)
South Africa	S	Summer - autumn	January - April	McQuaid & Lawrie (2005)
South Africa	S	Spring - summer	October - January	Lasiak & Dye (1989)
South Africa	S	Spring - summer	October - March	Bownes McQuaid (2009)
South Africa	S	Summer - autumn	January - April	McQuaid & Lindsay (2007)
South Africa	S	Winter	June - September	Lasiak & Barnard (1995)
South Africa	S	Spring - autumn	December - March	*Current study*

Peak recruitment periods of the indigenous mussel, *Perna perna,* found in the current study also showed spring to early autumn recruitment and similar patterns have been found from open south coast sites in South Africa, as well as in north Africa in Algeria and Morocco (Table 2). By contrast on the southeast coast of South Africa recruitment of *P. perna* peaks during winter months (Lasiak & Barnard, 1995). It should, however, be noted that there are limited global studies on *Perna perna* recruitment patterns (Table 2), and most studies investigating this topic have been restricted to South Africa. Peak recruitment periods of *Mytilus galloprovincialis* and *Perna perna* are a result of spring and summer spawning, which is associated with increases in water temperature, food availability and generally more favourable environmental conditions (Abada-Boujema, 1995; Knights *et al.,* 2006; Çelik *et al.,* 2016; Azpeitia *et al*., 2017; Halla *et al*., 2018). Which of these environmental factors are the main exogenous drivers of the timing of reproduction has yet to be determined experimentally in these two species.

Upwelling may also be an important factor involved in seasonal reproduction and recruitment of bivalves in southern Africa. Reaugh-Flower *et al.* (2011) demonstrated that recruitment levels of four inter-tidal mussels (*M. galloprovincialis, P. perna, Semimytilus algosus and Aulacomya atra*) were highest and recruitment protracted along the western coasts of Southern Africa, which have year-round, wind-induced upwelling. By contrast the southern coast of South Africa experiences seasonal wind-induced upwelling and mussel recruitment during summer (Reaugh-Flower *et al.,* 2011). Furthermore a study by Pfaff *et al*. (2011) showed that along ~400km of the Atlantic coastline of South Africa, mussel recruitment (of which the majority of recruits were thought to be *M. galloprovincialis*) was greatest during austral summer which coincides with equatorward winds that drive coastal upwelling. Upwelling events may control the delivery and supply of particulate matter and nutrients, which are important food sources for filter-feeding larvae, as well as concentrate mussel larvae and therefore increase propagule pressure during upwelling events (Menge *et al*., 2003, Reaugh-Flower *et al*., 2011). This study illustrated that recruitment levels of all bivalve taxa in the Knysna estuarine embayment were greatest during spring and summer, which is when upwelling events occur (Schumann, 2000) and higher productivity (high phytoplanktonic Chl-α levels) is found within the region (Allanson *et al.,* 2000). The results of this study are similar to those of Menge *et al*. (2003), Reaugh-Flower *et al*.

(2011) and Pfaff *et al*. (2011), and support the predictions of Reaugh-Flower *et al*. (2011), who proposed that recruitment levels of mussels on the southern coast of South Africa would have a strong seasonal pattern in relation to upwelling. However, it must be noted that data on upwelling events was not collected in the present study and that the timing of bivalve recruitment and upwelling events within the Knysna estuarine embayment may be coincidental.

It is typical for newly settled marine invertebrate larvae to be subject to varying levels of mortality caused by different biological and environmental factors. These factors may include competition among larvae for food and space, the level of food supplied to larvae (Olson & Olson, 1989), local hydrodynamic processes (Hunt & Scheibling, 1997; Pineda *et al.*, 2010), physical and biological stressors (Hunt & Scheibling, 1997) and most noticeably predation on larvae - including cannibalism (Porri *et al.,* 2008; Pineda *et al.*, 2010). Such factors may have played an important role in the inter-annual variations of bivalve recruitment levels found in the Knysna estuarine embayment. Low levels of food and/or productivity may increase marine invertebrate larvae mortality as the affected larvae would starve to death or face longer periods of metamorphosis which may expose the larvae to prolonged periods of predation (Olson & Olson, 1989). Varying levels of food throughout the current study may have attributed to the inter-annual differences among bivalve recruitment levels within the Knysna estuarine embayment. Future studies should address the temporal availability of food for bivalves within the Knysna estuarine embayment to consider whether this may be contributing to bivalve larval survival.

Predation has been widely considered one of the most important factors regarding mortality levels of marine invertebrate larvae (Olson & Olson, 1989; Hunt & Scheibling, 1997; Porri *et al.,* 2008b; Pineda *et al.*, 2010). In Alaska, USA, the exclusion of certain starfish and whelks led to an increase in the recruitment of barnacles in a year that experienced low settlement levels (Caroll, 1996). In Sweden the greatest cause of mortality among juvenile shore crab (*Carcinus maenas*) settlers was predation (Moksnes, 2002). Similarly, a major cause of mussel and mussel larvae mortality has been considered to be the predation of fish (Crooks, 2002; Morrisey *et al*., 2006). In Spain, 2005, Peteiro *et al.* (2007) could not assess *M. galloprovincialis* recruitment densities as fish predators (*Spondyliosoma cantharus* L.) eradicated the settled populations. Furthermore, work by Lehan & Davenport (2004) in Ireland found that *Mytilus edulis* ingested around 90% of bivalve larvae (*Ruditapes phillipinarium*) delivered to it, including a large range of size classes, and work by Porri *et al.* (2008b) in South Africa found that adult mussel ingestion of *Perna perna* and *Mytilus galloprovincialis* larvae through opportunistic filter-feeding may remove up to 77% of potential settlers. It is possible that during the present study various forms of predation (including cannibalism through filter-feeding) on bivalve larvae varied throughout the months, leading to the inter-annual recruitment levels found. Future work should identify the potential predators of bivalve larvae within the Knysna estuarine embayment and as well as their potential impact on bivalve mortality.

Of the four bivalve taxa, the recruitment levels of *M. galloprovinvialis* were by far the greatest. There are a number of possible reasons for this. Within the Knysna estuarine embayment *M. galloprovincialis* is the most abundant mussel while other bivalves are not as abundant (Hodgson, personal observation), which might explain why its recruitment levels were higher than the other bivalves. However, it is possible that *M. galloprovincialis* recruits are attracted to the adult mussel beds, which is where the pads were placed. A study by Robinson *et al.* (2007b) showed that along the west coast of South Africa *M. galloprovincialis* preferentially recruited onto adult mussel beds, and juvenile *M. galloprovincialis* were able to colonise shingle that was trapped in adult mussel beds. Furthermore, recruitment densities of *Perna perna*, and other mussels, along the coast of South Africa have shown significant positive relationships with adult mussel beds (Erlandsson & McQuaid, 2004; McQuaid & Lindsay, 2007; Reaugh-Flower *et al.,* 2011; McQuaid *et al.,* 2015) although such relationships were often weak. Along the coast of California, however, *M. galloprovincialis* and *Mytilus trossulus* settled in greater densities where adults of these species were rare (Johnson & Geller, 2006). Further studies within the Knysna estuarine embayment should identify whether recruitment differs significantly between sites with large densities of *M. galloprovincialis* adults, and sites where the adults are rare or absent.

Fecundity might also be a factor in explaining the high recruitment of *M. galloprovincialis*. This species on South African rocky shores has reproductive outputs 20 - 200% times greater than those of indigenous mussels (van Erkom Schurink & Griffiths, 1991; Branch & Steffani, 2004). In addition, Hockey & van Erkom Schurink (1992) illustrated that annual gamete release by the indigenous Brown mussel, *Perna perna*, was only 42% of that of *M. galloprovincialis*, demonstrating the alien invasive mussel's superior fecundity. Within the sheltered Knysna estuarine embayment *M. galloprovincialis* may have greater reproductive potential. Pollard & Hodgson (2016) showed that these populations of *M. galloprovincialis* had greater gonad indices and lower attachment strength compared to populations at the Knysna estuary mouth where wave action was greater. It is therefore likely that within sheltered environments the alien invasive mussel directs energy that would otherwise have been used for attachment strength into higher reproductive output (Zardi *et al*., 2006a; Pollard & Hodgson, 2016). In contrast, the Brown mussel *P. perna* invests more energy into attachment strength than *M. galloprovincialis*, leading to less energy invested in its reproductive output (Zardi *et al*., 2006a). This energy invested into reproductive output of *M. galloprovincialis* may in part be account for the large recruitment levels found within the Knysna estuarine embayment. Finally, larval retention of *M. galloprovincialis* may contribute to high recruitment, as this has been found to be greater in bays (McQuaid & Phillips, 2006; Bownes & McQuaid, 2009) which promotes successful establishment, recruitment and expansion of the mussel (McQuaid & Phillips, 2006). It is possible that in the sheltered Knysna estuarine embayment there is a relatively high degree of larval retention, therefore leading to the high recruitment levels recorded.

The Ostreidae recruits found may be those of the alien invasive Japanese oyster, *Magallana gigas*, upon which the South African oyster industry is largely based (Robinson *et al.,* 2005b). This species is restricted to estuaries along the southern coast of South Africa, including the Knysna Estuary, with no populations being recorded along the open coast (Robinson *et al.,* 2005b). A more recent study by Keightley *et al.* (2015), however, found that *M. gigas* had since disappeared from the Knysna Estuary. It is possible that they had missed this species or that a new invasion has been detected in which this study may provide a baseline for the recruitment levels of *M. gigas* within the Knysna estuarine embayment. Further work should aim to raise the Ostreidae spat within the Knysna estuarine embayment or perform genetic tests to identify which species has been found. The Ostreidae had the second highest recruitment levels of the bivalve taxa recorded, and had more protracted recruitment over autumn and winter months (April - June), particularly at Thesen Island Wharf (T). These higher recruitment rates and ability for protracted recruitment over indigenous bivalves may be due to alien invasive characteristics similarly seen in *M. galloprovincialis*.

Bivalve recruitment within the Knysna estuarine embayment varied among sites with the more seaward locations, Thesen Islands Marina (M) and the Thesen Islands Wharf (T), having significantly higher recruitment levels than the inner estuarine location (Railway Bridge (R)). Similar recruitment patterns of *Mytilus* mussels in estuarine environments have been documented before in England (McGrorty & Goss-Custard*.,* 1991) and Spain (Molares & Fuentes, 1995; Peteiro *et al*., 2007) where recruitment was greater towards seaward regions of the estuaries. Within the Knysna estuarine embayment the Railway Bridge is characterized by a transition region between the bay regime and lagoon regime, where salinity levels can be lower (less than 30) more often (Largier *et al.,* 2000). Tidal currents and water exchange may be weaker in this region compared to Thesen Island Wharf (Largier *et al.,* 2000) and presumably Thesen Islands Marina. These changes in tidal currents may restrict bivalve recruitment in that region of the estuary. Porri *et al.* (2008a) found that at scales of 100s of metres hydrodynamics can play a crucial role in the delivery of *Perna perna* larvae among sites. Furthermore, in a review of the literature Pineda *et al.* (2010) discussed that settlement of larvae is greater in waters with a higher turbulence and a faster flow, compared to more still waters. In the Ria de Ares-Betanzos, Spain, recruitment of *M. galloprovincialis* was greatest in regions where high current velocities were present (Peteiro *et al.,* 2007). Furthermore, Knights *et al.* (2012) found that within estuarine sites along the coastline of South Carolina, USA, recruitment of the eastern oyster, *Crassostrea virginica*, was approximately 58% higher in sites with higher water flow. It may also be possible that reduction of water flow and tidal currents near the Railway Bridge limits the dispersal of bivalve larvae and therefore an increase in predation of larvae may occur, contributing to the spatial differences of bivalve recruits within the Knysna estuarine embayment. It is unlikely that particulate food levels are influencing low levels of recruitment at the Railway Bridge as Allanson *et al.* (2000) showed that there was no significant spatial variation in Chl-α levels between the Railway Bridge region and more seaward locations.

In conclusion it is likely that the spatial recruitment patterns of *M. galloprovicialis* and other bivalves within the Knysna estuarine embayment are not due to a single factor but likely a combination of environmental factors, greater gonad indices (Pollard & Hodgson, 2016), local hydrodynamic conditions (Porri *et al.,* 2006; Porri *et al.,* 2008a; Pineda *et al.,* 2010) oceanographic processes (Menge *et al.,* 2003; Pfaff *et al*., 2011; Reaugh-Flower *et al.,* 2011) and predation levels (Porri *et al.,* 2008b; Pineda *et al.,* 2010).

4.2. Size Classes of *M. galloprovincialis*

Bayne (1964) proposed the primary and secondary settlement hypothesis suggesting that primary settlers (mussel larvae <0.5 mm) attach to filamentous substrata, whereby they undergo a period of growth before detaching themselves and reattaching as secondary settlers onto mussel beds (Bayne, 1964). *Mytilus* spp. first settle at about 220 µm in shell length after four weeks (Knights *et al.,* 2006), and then their shell lengths grow at about 25 µm per day (Bayne, 1964). Based on this growth rate it can be assumed that all *M. galloprovincialis* primary settlers within the Knysna estuarine embayment were no older than 40 days. The sizes of *M. galloprovincialis* recruits found in this study were similar at all sites within the Knysna estuarine embayment, with more than 80% of recruits at each site being primary settlers (sizes <0.5 mm), and the remaining 20% being secondary settlers (sizes >0.5 mm). The results of this study may indirectly support the primary and secondary hypothesis as the majority of recruits found on the filamentous pads were primary settlers, and it very probable that secondary settlers found on the pads came from the adjacent mussel beds. Similarly, McQuaid & Lindsay (2005) and Porri *et al*. (2006) found mussel recruits greater than 0.5 mm on the filamentous substrata (pads) in their studies which was likely due to the collection pads being placed near mussel beds and hence the secondary settlers actively, or passively, dislodge and reattach at local scales (Porri *et al.,* 2006). The significant differences in *M. galloprovincialis* recruit lengths between sites is probably due to the fact that different amounts of shell lengths were recorded for each site. Furthermore, there were fewer than 10% of secondary settlers present at the Thesen Islands Marina (M) and the Railway Bridge (R) and almost double the percentage of secondary settlers found at Thesen Island Wharf (T). Porri *et al.* (2008a) found that levels of late plantigrades of *Perna perna* differed among sites at small scales, implying that hydrodynamics had an effect of the distribution of the size classes of mussel recruits at various sites. It is possible that differences in local hydrodynamics near the Railway Bridge (Largier *et al.,* 2000) and heavy siltation (sand stress is correlated to mortality levels of *M. galloprovincialis* (Nicastro *et al.,* 2010)) within the Thesen Islands Marina and the Railway Bridge (personal observation) may account for lack of secondary settlers as environmental conditions may not be as favourable.

4.3. Tidal and Lunar Phase Recruitment of *M. galloprovincialis*

Lunar cycles may play an important role as a cue for reproduction and settlement of marine coastal invertebrates. For example lunar periodicity in settlement has been documented in barnacles in Portugal (Jacinto & Cruz, 2008) and crabs along Caribbean coastal reefs (Reyns & Sponaugle, 1999). Little information, however, is available for mussels. Variability of marine invertebrate recruitment within coastal lagoons may be due to differences in larval transport over different tides. Because greater water mass is transported during spring tides it would be expected that a greater number of recruits would be found on these tides (Pineda *et al*., 2010). The preliminary tidal study indicated that recruitment levels of *M. galloprovincialis* were greater over the spring tides than neap tides at three different times of the year. In contrast to this study Porri *et al.* (2006) investigated whether spring tides*,* along the south coast of South Africa, would influence settlement of the indigenous brown mussel, *Perna perna,* due to increases of tidal amplitude and found no clear effect. A further study by Porri *et al.* (2008a), however, found that *P. perna* had peaks in settler abundance following spring tides although the relationship between settlement and tide was not significant. Recruitment of *Mytilus* spp. larvae along the Irish Sea similarly showed no effect of tidal phase (i.e. spring and neap tides) on recruitment as recruitment levels were similar for both tidal phases (Knights *et al.,* 2006). However, Knights *et al.* (2006) found a significant effect of tidal state (i.e. flood or ebb state during spring/neap tide) on larval distribution that was always greatest during flood tide, regardless of the tidal phase. It possible that the results obtained in the present study were misleading because (1) sampling was confined to single spring and neap tides from 3 months only and (2) sampling was not concentrated during the peak reproductive season. For this reason the investigation was expanded with the lunar study during the summer of 2018. When sampling took place over three consecutive neap and spring tides. When looking at the lunar study results and interpreting them into the tidal study, recruitment was found to be highly variable among tides and no clear relationship between tidal phase and recruitment was evident. The two main pulses of recruitment occurred over a two week period in which both a spring tide (full moon) and neap tide (last quarter moon) occurred (Fig. 13).

The lunar study indicated no clear relationship between recruitment levels of *M. galloprovincialis* and phase of the moon. Although there was generally more recruitment on a full moon and new moon compared to the first and last quarter phases of the moon, this was not always the case and therefore differences in recruitment over different lunar phases were not found. It was also found that high numbers of recruits could occur on both a spring and a neap tide in the summer of 2018. Porri *et al*. (2006) found that *Perna perna* generally had greater, but not significantly different, recruitment levels over a new moon than a full moon. It should be noted, however, that only full moon and new moon phases were investigated by Porri *et al*. (2006) and that last and first quarter phases of the moon were unaccounted for. As mentioned above the main recruitment of *M. galloprovinvialis* was over a two week period in which a full moon and last quarter moon were experienced. This suggests that other factors may be more important on the levels and timing of recruitment or spawning within *M. galloprovincialis* populations, and that tidal and lunar phase may not have a direct effect. It is probable that recruitment of *Mytilus galloprovincialis* within the Knysna estuarine embayment is influenced by a magnitude of temporal and spatial factors such as mentioned above within the monthly bivalve recruitment study. A shortfall of the lunar study, however, should be mentioned. During this study recruitment levels were recorded over two first quarter phases of the moon and two new moon phases, whereas recruitment levels for full moon and last quarter phases were recorded once. Further studies should try to look at recruitment levels over consecutive tides as well as having the same number of lunar phases to address the shortfall of this study and to establish unequivocally whether there is no relationship between tides and lunar phases with recruitment of *M. galloprovincialis* within the Knysna estuarine embayment.

4.4. Settlement and Re-Colonisation of Cleared Plots

The study examining the re-colonisation of clearance plots illustrated that where *M. galloprovincialis* is adjacent to unoccupied substrata, it rapidly encroaches the free space. This suggests that within the Knysna estuarine embayment, at the tidal height studied, *M. galloprovincialis* is a successful competitor for space. In this study cleared plots that were left untouched throughout the study (treatment B) and cleared plots in which all macroinvertebrates except mussels were removed (treatment C) were virtually overgrown by *M. galloprovincialis* within eight months. Erlandsson *et al.* (2006) studied the re-colonisation rates of *M. galloprovincialis* and *P. perna* following major disturbances along the southern coast of South Africa and found that *M. galloprovincialis* can re-colonise cleared space up three times more rapidly than *P. perna*. *Mytilus galloprovincialis* has also shown competitive dominance over limpet species by outcompeting them for space on primary rock surfaces along the west coast of South Africa (Hockey & van Erkom Schurink, 1992). In the present study other invertebrates (limpets and barnacles) arrived to the plots only when *M. galloprovincialis* was prevented from occupying space (treatment A). Percentage coverage of other invertebrates was relatively low (roughly 1%) and it took about nine months before their coverage became notable. The increase of percentage coverage found between treatments during this study demonstrates the alien invasive mussel’s superior ability to colonise a disturbed habitat in comparison to the local estuarine fauna.

It is interesting to note that the re-colonisation of the treatments B and C occurred through encroachment from the sides of the cleared plots rather than recruitment of the mussels onto the plots. An absence of physical and/or chemical cues may result in the recruits actively choosing not to settle on the substrata (Lutz & Kennish, 1992; Seed & Suchanek, 1992; Littorin & Gilek, 1999), although Bracewell *et al.* (2013) found that an absence chemical cues did not affect the recruitment levels of the invasive barnacle *Austrominius modestus* at relatively small spatial scales. As noted before, primary settlers of *Mytilus* species actively choose to settle on filamentous structures (Bayne, 1964). A similar study by Littorin & Gilek (1999) who cleared 15 x 15 cm plots of *Mytilus edulis* in Sweden found that the mussels generally re-colonised free space by invading from the sides, and a general trend of mussels encroaching from the periphery leaving the centre of the cleared plots with less mussel cover. This general trend was observed in the photographs of the present study. Similarly, Paine & Levin (1981) showed that within mussel beds of *Mytilus californianus* the size of disturbance plots could have a critical effect on the re-colonisation process of those mussels, whereas in small disturbance plots re-colonisation occurs from mussels encroaching from the sides, especially where adjacent mussel beds were thick. Littorin & Gilek (1999) suggested that this encroachment may occur as an effect of individual growth of the mussels, and new recruits, in the adjacent mussel beds with the growth of mussels pushing each other into the cleared space. It is thought that if larger plots were cleared this would result in a longer period of recovery, and therefore this longer recovery period may have sufficient time to allow for recruitment enhancing structures to develop onto the cleared plots which would in turn allow for more primary and secondary settlement of recruits in the cleared plots (Littorin & Gilek, 1999), although it should be noted that Bracewell *et al.* (2013) found that the size of clearance plots did not affect the rate of recruitment with the barnacle *A. modestus*. Furthermore, work by Hodgson *et al.* (2018) found that populations of *M. galloprovincialis* at Thesen Island Wharf and Thesen Islands Marina within the Knysna estuarine embayment showed rapid growth rates and mussel bed thickness at Thesen Island Wharf was about 100 mm. This growth rate and thickness of the mussel beds may be influencing the rapid encroachment seen in the present study.

Suchanek (1985) illustrated that tidal height may play an important role on the re-colonisation process of *M. californianus*. Plots that were cleared of *M. californianus* on the low-shore and mid-shore were re-colonised at rapid rates (1-2 years) whereas plots that were cleared on the high-shore had extremely low rates of re-colonisation (up to 10 years), although re-colonisation rates were also highly correlated with mussel bed thickness. This may have had an important effect on the re-colonisation rates of *M. galloprovincialis* within this study as the cleared plots were performed on the mid-balanoid zone of the Knysna estuarine embayment and therefore this may have resulted in the rapid re-colonisation rates seen.

Although other explanations, such as the enemy release hypothesis, illustrate why alien species become successful invaders in non-indigenous environments this was unlikely the case in this study. Predation of mussels by starfish (*Marthasterias africana*) was observed within the Knysna estuarine embayment (Fig. 18) although the starfish were primarily observed at the littoral/sublittoral interface. Whelk predation is also unlikely as there are few whelks in *M. galloprovincialis* beds (Hodgson, personal observation).

Fig. 18 Starfish (*Marthasterias africana*) preying on *Mytilus galloprovincialis* mussel bed within the Knysna estuarine embayment.

4.5. Conclusion

This study has illustrated important alien invasive traits of the Mediterranean mussel *M. galloprovincialis* within the Knysna estuarine embayment. *Mytilus galloprovincialis* has superior recruitment and re-colonisation of disturbed space in comparison to indigenous bivalves within the Knysna estuarine embayment. In terms of the aims of the current study it was found that: (1) recruitment levels of the alien invasive mussel were higher than those of the indigenous bivalves, with all bivalve taxa recruiting throughout the year although strong seasonal (spring - autumn) recruitment occurred with inter-annual differences; (2) *M. galloprovincialis* recruitment was not unequivocal over spring and neap tides, and it is unlikely that lunar phases influence recruitment; and (3) re-colonisation of disturbed plots was dominated by *M. galloprovinvialis* encroachment, which excluded the re-colonisation barnacles and limpets. This work therefore partially agrees with the initial hypothesis (one of the reasons for the success of *M. galloprovincialis* in the Knysna embayment is due to high and continuous recruitment and settlement rates) because high recruitment levels were evident in *M. galloprovincialis* beds within the Knysna estuarine embayment, however, recruitment mainly occurred during spring until autumn and high recruitment levels were not recorded throughout the study. These characteristics of *M. galloprovincialis*, along with the environmental conditions (i.e. marine-like conditions, sheltered, large tidal inflow etc.) and artificial substratum of the Knysna estuarine embayment have enabled the success and establishment of the mussel within such an environment. It may therefore be assumed that the greatest biological factors contributing to the success and establishment of *M. galloprovincialis* within the Knysna estuarine embayment are its high fecundity and recruitment levels, as well as its ability to re-colonise disturbed environments at fast rates and outcompete indigenous fauna for space. Future work should address the factors that influence the temporal and spatial factors that influence bivalve larval mortality and recruitment levels within the Knysna estuarine embayment. Such work should address the relationships between upwelling and food availability with bivalve recruitment; temporal and spatial impacts of predators on bivalve larvae and as well as the temporal and spatial differences in local hydrodynamics in relation to bivalve recruitment.

Appendix 1

Washing Scouring Pads

1. Add 9/10ml of bleach per 250ml to sample in 250ml sample bottle. Shake well and leave for 5 minutes to soak.
2. After 5 min pour contents of bottle over 75µm sieve.
3. Remove the scouring pad from the bottle and place in a 500ml bucket.
4. Carefully rinsed the sample bottle out over 75µm sieve until clean.
5. Cut the scouring pad an unravel it leaving it in the bucket.
6. Using a hose pipe rinse down the scouring pad. Holding your thumb over the opening of the pipe will increase water pressure and help to remove the debris from the pad.
7. Take care not to splash ANY water out of the bucket.
8. When the bucket is full pour the water out over the same sieve used above.
9. If there is a lot of debris stuck to the pad swirl it quite vigorously around within the bucket in a little water taking care not to splash.
10. Repeat steps 6 to 10 until the scouring pad is completely clean.
11. Rinse off any tools used (scissors, tweezers etc.) over the sieve.
12. Pour what is left in the bucket over the sieve and rinse the bucket thoroughly over the sieve.
13. Transfer what is in the sieve to a bottle and add 15/20ml of 100% Ethanol.

Appendix 2

Table 3. Statistical output table for Chi-squared likelihood test of negative binomial model (Recruit ~ Date*Site) for *M. galloprovincialis*.

	Df	Deviance Resid.	Df	Resid. Dev	Pr(>Chi)
NULL			597	42040	
Site	2	6518	595	35523	< 2.2e-16 ***
Date	19	31806	576	3716	< 2.2e-16 ***
Site:Date	38	681	538	3036	< 2.2e-16 ***

Table 4. Statistical output table for Chi-squared likelihood test of negative binomial model (Recruit ~ Date*Site) for Ostreidae.

	Df	Deviance Resid.	Df	Resid. Dev	Pr(>Chi)
NULL			597	2940.80	
Date	19	1672.87	578	1267.93	< 2.2e-16 ***
Site	2	557.73	576	710.21	< 2.2e-16 ***
Date:Site	38	144.43	538	565.77	2.56e-14 ***

Table 5. Statistical output table for Chi-squared likelihood test of negative binomial model (Recruit ~ Date*Site) for *Perna perna*.

	Df	Deviance Resid.	Df	Resid. Dev	Pr(>Chi)
NULL			597	2906.25	
Date	19	2058.05	578	848.20	< 2.2e-16 ***
Site	2	150.72	576	697.48	< 2.2e-16 ***
Date:Site	38	151.82	538	545.66	1.538e-15 ***

Table 6. Statistical output table for Chi-squared likelihood test of negative binomial model (Recruit ~ Date*Site) for unidentified bivalve species.

	Df	Deviance Resid.	Df	Resid. Dev	Pr(>Chi)
NULL			597	2434.84	
Date	19	1547.24	578	887.60	< 2.2e-16 ***
Site	2	286.42	576	601.18	< 2.2e-16 ***
Date:Site	38	121.75	538	479.43	1.059e-10 ***

Table 7. Statistical output table for Dunn's test comparing the effect of Site on the size of *M. galloprovincialis.*

	Site	Z	P. unadjusted	P. adjusted
1	M - R	3.241039	1.190950e-03	3.572851e-03
2	M - T	-5.167811	2.368519e-07	7.105556e-07
3	R - T	-7.581535	3.414896e-14	1.024469e-13

Table 8. Statistical output table for Chi-squared likelihood test of negative binomial model (Recruit ~ Time of Year*Tide) for *M. galloprovincialis*.

	Df	Deviance Resid.	Df	Resid. Dev	Pr(>Chi)
NULL			59	108.793	
Time of Year	2	21.0336	57	87.760	2.708e-05 ***
Tide	1	22.8604	56	64.899	1.742e-06 ***
ToY:Tide	2	1.7224	54	63.177	0.4227

Table 9. Statistical output table for Dunn's test comparing *M. galloprovincialis* recruit counts over different lunar phases at Thesen Island Wharf.

Comparison	Z	P. unadjusted	P. adjusted
04/02/18 - 11/02/18 - 12/02/18 – 19/02/18	1.3449479	1.786421e-01	2.233026e-01
04/02/18 - 11/02/18 - 20/02/18 – 27/02/18	-2.1583212	3.090287e-02	5.794288e-02
12/02/18 – 19/02/18 - 20/02/18 – 27/02/18	-3.5032691	4.595850e-04	1.723444e-03
04/02/18 - 11/02/18 - 28/02/18 – 06/03/18	-3.6954045	2.195370e-04	1.097685e-03
12/02/18 – 19/02/18 - 28/02/18 – 06/03/18	-5.0403524	4.646754e-07	6.970131e-06
20/02/18 – 27/02/18 - 28/02/18 – 06/03/18	-1.5370833	1.242729e-01	1.864094e-01
04/02/18 - 11/02/18 - 06/03/18 - 13/03/18	0.3010122	7.634052e-01	7.634052e-01
04/02/18 - 11/02/18 - 06/03/18 - 13/03/18	0.3010122	7.634052e-01	7.634052e-01
04/02/18 - 11/02/18 - 06/03/18 - 13/03/18	0.3010122	7.634052e-01	7.634052e-01
04/02/18 - 11/02/18 - 06/03/18 - 13/03/18	0.3010122	7.634052e-01	7.634052e-01
04/02/18 - 11/02/18 - 14/03/18 – 21/03/18	-1.4410156	1.495803e-01	2.039731e-01
12/02/18 – 19/02/18 - 14/03/18 – 21/03/18	-2.7859635	5.336888e-03	1.601066e-02
20/02/18 – 27/02/18 - 14/03/18 – 21/03/18	0.7173056	4.731856e-01	5.069846e-01
28/02/18 – 06/03/18 - 14/03/18 – 21/03/18	2.2543889	2.417171e-02	5.179653e-02
06/03/18 - 13/03/18 - 14/03/18 – 21/03/18	-1.7420278	8.150359e-02	1.358393e-01

Table 10. Statistical output table for Dunn’s test comparing *M. galloprovincialis* recruit counts over different lunar phases at Railway Bridge.

Comparison	Z	P. unadjusted	P. adjusted
04/02/18 - 11/02/18 - 12/02/18 – 19/02/18	-0.93098992	3.518588e-01	4.398235e-01
04/02/18 - 11/02/18 - 20/02/18 – 27/02/18	-3.34897764	8.111034e-04	2.027759e-03
12/02/18 – 19/02/18 - 20/02/18 – 27/02/18	-2.41798771	1.560660e-02	2.926238e-02
04/02/18 - 11/02/18 - 28/02/18 – 06/03/18	-4.31875881	1.569091e-05	1.176819e-04
12/02/18 – 19/02/18 - 28/02/18 – 06/03/18	-3.38776888	7.046361e-04	2.113908e-03
20/02/18 – 27/02/18 - 28/02/18 – 06/03/18	-0.96978117	3.321556e-01	4.529394e-01
04/02/18 - 11/02/18 - 06/03/18 - 13/03/18	0.47196017	6.369552e-01	6.824520e-01
04/02/18 - 11/02/18 - 06/03/18 - 13/03/18	1.40295009	1.606317e-01	2.677195e-01
04/02/18 - 11/02/18 - 06/03/18 - 13/03/18	3.82093781	1.329452e-04	6.647259e-04
04/02/18 - 11/02/18 - 06/03/18 - 13/03/18	4.79071897	1.661847e-06	2.492771e-05
04/02/18 - 11/02/18 - 14/03/18 – 21/03/18	-0.87280305	3.827704e-01	4.416582e-01
12/02/18 – 19/02/18 - 14/03/18 – 21/03/18	0.05818687	9.535998e-01	9.535998e-01
20/02/18 – 27/02/18 - 14/03/18 – 21/03/18	2.47617458	1.327986e-02	2.845685e-02
28/02/18 – 06/03/18 - 14/03/18 – 21/03/18	3.44595575	5.690435e-04	2.133913e-03
06/03/18 - 13/03/18 - 14/03/18 – 21/03/18	-1.34476322	1.787017e-01	2.680526e-01

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