

Interactions of larval dynamics and substrate preference have ecological significance for benthic biodiversity and *Ostrea edulis* Linnaeus, 1758 in the presence of *Crepidula fornicata*

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Abstract

1. Populations of the European flat oyster *Ostrea edulis* have experienced catastrophic declines across Europe and subsequent spread of the non-native species *Crepidula fornicata* has led to its occurrence in exceptionally high densities in some areas previously dominated by *O. edulis*.
2. Spatial and temporal concurrence of *C. fornicata* larvae within the zooplankton community occurs throughout the *O. edulis* spawning season. A *C. fornicata* larval peak density of 374.7 ± 96.5 larvae/ml (mean \pm SD) was observed in Langston Harbour sympatrically with *O. edulis* density of 45.7 ± 20.1 larvae/ml in early August. Overall oyster larvae contribution to the zooplankton community was higher in Portsmouth Harbour (12%) than *C. fornicata* contribution (9.6%), whilst the opposite occurred in Langstone (oysters, 11.7%; *C. fornicata*, 12%).
3. Larval abundance is not reflected in recruitment on the seabed, owing to the conspecific substrate preference of *O. edulis*. Settlement of *O. edulis* spat was significantly greater on settlement discs covered with recently deceased oyster shells; 6.7 ± 1.2 (mean \pm SE) spat/disc vs old smooth oyster shells, 2.7 ± 1.3 , *C. fornicata* shell 1.7 ± 0.3 , cemented discs 2 ± 1 or the plastic control disc 0.7 ± 0.7 .
4. Settlement substrate type matters in the presence of high benthic and larval densities of *C. fornicata*. The Solent has become a substrate-limited system for *O. edulis*; substrate management or reef deployment is required to restore a self-recruiting population.
5. Finally, although *C. fornicata* may provide functional equivalence in terms of filtering services, it supports a significantly different and less biodiverse faunal community from that of *O. edulis*. Therefore *C. fornicata* does not provide equivalence as an ecosystem engineer and mechanisms of ecological phase shift are occurring within areas dominated by this invasive species.

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KEYWORDS

biodiversity, ecosystem engineer, habitat complexity, invasive species, limpet, oyster spat, settlement

1 | INTRODUCTION

1.1 | The loss of an ecosystem engineer

The term 'ecosystem engineer' is used to describe any organism that directly or indirectly modulates the availability of resources to other species, by causing physical state changes in biotic or abiotic materials. The European flat oyster *Ostrea edulis* (along with other oyster species), epitomizes the classification of an autogenic engineer, whereby the physical structure provided whilst alive and by the remaining shells when deceased change the environment (Jones, Lawton, & Shackak, 1994). Typically, *O. edulis* inhabits coastal and estuarine environments, which range from the intertidal down to 80 m depth, within a salinity range of 18–40‰ (Jackson, 2007).

Historically, *O. edulis* populated extensive areas of seabed in European waters, equating to over 25,000 km² (Olsen, 1883) of dense aggregations in bed and reef structures. These, once abundant, populations provided a source of sustenance for human populations for centuries, with the earliest shell midden records dating back to the Mesolithic period (Gutiérrez-Zugasti et al., 2011).

Large-scale cultivation and management of the species extend back to the Roman Empire (Günther, 1897) and the continued large-scale extraction throughout the industrial revolution is highlighted by the 120,000-strong fleet of oyster dredgers that, in 1864, supplied 700 million oysters to London alone (Philpots, 1890). The 80 million oysters harvested annually in the Bay of Biscay, prior to 1859, were valued at £10,000 (Sullivan, 1870), equivalent to £1.2 million today. The long-standing impression that the ocean provided an inexhaustible source of fish and shellfish can be seen elsewhere, including the historical shell piles that are estimated to contain 5×10^{12} shells in France (Gruet & Prigent, 1986 as cited by Gouletquer & Heral, 1997). The unsustainable extraction resulted in catastrophic declines across all of Europe; the situation is arguably most severe in Germany where *O. edulis* is now classified as extinct and requires a reintroduction (Pogoda, 2019).

Until recently, the Solent contained one of the largest remaining self-sustaining *O. edulis* fisheries in Europe, with populations forming dense aggregations, predominantly occurring in the areas around Stanswood and Calshot (Key & Davidson, 1981; Palmer & Firmin, 2011). Between 1979 and 1980, 15 million oysters (650–850 tonnes) were landed by 450 vessels and recorded seabed densities were as high as 32/m² (Key & Davidson, 1981). This extraction was not sustainable and resulted in the collapse of the fishery as the biological limit of the species was exceeded, in part owing to the removal of the reproductively viable population but also the settlement substratum for their larvae, provided by those mature oysters.

The availability of suitable substrata is key for the completion of the *O. edulis* life cycle. The veliger larvae display gregarious behaviour, preferentially settling and metamorphosing on conspecifics and other hard, clean substrata that has a high surface heterogeneity (Bayne, 1969; Cole & Knight-Jones, 1939; Cole & Knight-Jones, 1949; Walne, 1964; Walne, 1974). The nature of the settlement surface, biofilm formation and other cues may influence settlement behaviour (Walne, 1974). However, Smyth, Mahon, Roberts, and Kregting (2018) reported the availability of hard substrata rather than its type determined the settlement by *O. edulis* in Strangford Lough. Other research suggests that cultch (disarticulated shell) is an outcome of a self-recruiting oyster reef and the presence of live or box shell (dead but not disarticulated cultch) is key to recruitment for some species of oyster (Powell, Hofmann, & Klinck, 2018). The invasive American slipper limpet *Crepidula fornicata* is also suggested as a suitable substrate for *O. edulis* within fisheries management (T. Cameron, pers. comm., 2019). The large-scale extraction of *O. edulis* habitat and associated substrate remains a serious concern for the recruitment and survival of this species.

1.2 | Ecosystem functions and services of native oyster reefs

Ostrea edulis provides benefits to commercial fisheries, and provides an important ecological role in providing habitat for other organisms (Korringa, 1946; Mistakidis, 1951). Facilitation of increased species diversity and abundance is one of the major and most relevant functions native oysters provide. Korringa (1946) and Mistakidis (1951) conducted studies to detail the associated epibiota. They found numerous species regularly inhabiting shells of *O. edulis*, considered as characteristic epifauna of the native oyster. The three-dimensional structures created by years of successive settlement of oyster larvae on adult shells provide structural complexity in systems dominated by soft, flat-bottom habitats (Bartol, Mann, & Luckenbach, 1999; Micheli & Peterson, 1999). Mobile fish and decapod crustacean species utilize oyster reefs for numerous reasons, consuming the oysters or their associated epibiont community, using oyster shells as surfaces for spawning and finding refuge from predation within the oyster reef (Tolley & Volety, 2005), whereas sessile species use the reefs for settlement and attachment (Boudreaux, Stiner, & Walters, 2006). Fish produced on oyster reefs have significant economic value to coastal communities (Grabowski & Peterson, 2007). The lost habitats caused by decline in oyster reefs have a negative economic impact as they are linked to decreases in overall coastal and shelf sea biodiversity (Airoldi, Balata, & Beck, 2008; Lotze et al., 2006). Although there is an increasing acknowledgement that oyster reefs provide multiple ecosystem

services, management objectives beyond harvest are not yet widespread (Beck et al., 2011). Many European oyster restoration projects go beyond biodiversity conservation as their focus; the Native Oyster Network, UK, and Ireland (2020) and European Native Oyster Restoration Alliance (2020) are jointly creating monitoring guidelines that include metrics that quantify ecosystem functions and services.

Oyster reef habitat provides a wide range of ecosystem services including water filtration, food, shoreline stabilization, coastal defence and fisheries (Grabowski & Peterson, 2007; Newell, Fisher, Holyoke, & Cornwell, 2005; NRC, 2010). As filter-feeders, particulate matter resuspended by tidal currents and storms is an important food source to *O. edulis* (Grant, Enright, & Griswold, 1990). By removing suspended solids from the water, the oysters increase water clarity. Although difficult to quantify in large bodies of water, localized effects of filtration, such as reduced turbidity, have been observed (Coen et al., 2007; Grabowski & Peterson, 2007). Indeed, oysters are able to reduce the volume of suspended solids and phytoplankton (Grizzle, Greene, Luckenbach, & Coen, 2006; Nelson, Leonard, Posey, Alphin, & Mallin, 2004). Healthy oyster reefs can therefore reduce the likelihood of harmful algal blooms occurring and prevent the negative economic and ecological impacts associated with harmful algal blooms, especially at the local scale (Cerrato, Caron, Lonsdale, Rose, & Schaffner, 2004; Newell & Koch, 2004). The improvement to water quality can increase recreational activities such as sport fisheries and tourism to the area (Lipton, 2004). Shellfish are also associated with nutrient remediation in coastal bays via denitrification in surrounding sediments (Newell et al., 2005). The nutrient remediation potential of oysters could translate into a high economic value (Watson, Preston, Beaumont, & Watson, 2020) since nutrient removal and achieving nitrate neutrality is a high priority for coastal stakeholders, including public bodies, housing developers and policy makers (Natural England, 2020).

Oyster reefs serve as natural coastal defences absorbing wave energy thus reducing erosion caused by boat waves, sea-level rise and storms (Meyer, Townsend, & Thayer, 1997; Piazza, Banks, & La Peyre, 2005). Currently ecosystem services provided by *O. edulis* are yet to be quantified. The potential services of a healthy oyster reef are widely understood from the quantification of ecosystem services of other oyster species. Quantifying services and functions of *O. edulis* reefs will be a key step in shifting the focus of management objectives.

1.3 | Ecological invasion by the American slipper limpet

Non-native marine species are of special concern when they become invasive and displace native species. Negative impacts include biotic homogenization, modification of habitats and alteration of community structures and ecosystem functions (Bax, Williamson, Agüero, Gonzalez, & Geeves, 2003; Katsanevakis et al., 2014; Viard, David, & Darling, 2016). When these impacts impede the provision of ecosystem services it can detrimentally affect human health and cause substantial economic losses (Grosholz, 2002; Perrings, 2002; Wallentinus & Nyberg, 2007).

The American slipper limpet *C. fornicata* was accidentally introduced with imports of the Eastern oyster *Crassostrea virginica* (Dodd, 1893; Hoagland, 1985; McMillan, 1938; Minchin, McGrath, & Duggan, 1995; Utting & Spencer, 1992) and the Pacific oyster *Crassostrea gigas* (Blanchard, 1997). First appearing in Liverpool during the 1880s (Moore, 1880 in McMillan, 1938) and the east coast and Thames estuary in the 1890s (Cole, 1915; Crouch, 1893), *C. fornicata* is now a well-established invasive non-native species. The loss of oyster habitat has further exacerbated the spread and the abundance of *C. fornicata* and is a major concern across Europe (Blanchard, 1997; Boyle, 1981), particularly in the Solent (Helmer et al., 2019). In rare instances *C. fornicata* 'stimulates zoobenthic community diversity and abundance' in muddy sediments (de Montaudouin & Sauriau, 1999). However, its rapid expansion throughout the UK (Barnes, Goughlan, & Holmes, 1973; Chipperfield, 1951; Cole & Baird, 1953; Minchin et al., 1995; Orton, 1950) and Europe (Blanchard, 1997, 2009; Davis & Thompson, 2000; Thieltges, Strasser, & Reise, 2003), including oyster beds (Crouch, 1893), has had serious ecological and economic impacts (see Blanchard, 1997).

In contrast to the wide range of ecological benefits provided by *O. edulis*, *C. fornicata* has been shown to be detrimental to habitat suitability for juvenile fish (Le Pape, Guérault, & Désaunay, 2004; Le Pape et al., 2007) and suprabenthic biodiversity (Vallet, Dauvin, Hamon, & Dupuy, 2001). The shell growth and survival of other commercially important bivalves, such as *Mytilus edulis* (Thieltges, 2005), are also impacted. Habitat modification in the presence of *C. fornicata* is also an issue in many areas. This occurs through the production of mucoidal pseudofaeces, which converts predominantly sandy substrata into mud-dominated substrata with a high organic content that rapidly becomes anoxic and unsuitable for other species (Streftaris & Zenetos, 2006). This includes oysters that prefer less silty and muddy waters (Barnes et al., 1973; Bromley, McGonigle, Ashton, & Roberts, 2016; Fulford, Breitburg, & Luckenbach, 2011; Walne, 1979). *Ostrea edulis* populations are also negatively impacted through a reduction in suitable substrata available for larval settlement (Blanchard, 1997), hindering recruitment and potentially oyster restoration efforts on the seabed.

1.4 | Interspecific competition between *O. edulis* and *C. fornicata*

An association of species characterizes benthic fauna in the Solent, with *C. fornicata* dominating the benthic community in many locations throughout the area, regardless of depth and substratum (Barnes et al., 1973). It is well known that invasive species have detrimental effects on the growth and survival of native species (Thieltges, Strasser, & Reise, 2006), especially if they occupy the same niche. Owing to *C. fornicata*'s suspension feeding regime and preference for similar habitats to *O. edulis*, this invasive species can quickly exert a detrimental effect on oyster populations and habitat (de Montaudouin, Audemard, & Labourg, 1999): 'they have a detrimental effect upon oyster culture' (Chipperfield, 1951); '*Crepidula* is

an oyster-pest' (Korringa, 1951; Walne, 1956). It is essential to understand the ecological interactions between the two species to recognize the negative effects caused by the presence of *C. fornicata*. This will help restoration efforts, by enabling adaptive management strategies in locations where *C. fornicata* are present and informing site selection criteria for restoration projects.

Current research investigating competition between *C. fornicata* and *O. edulis* is limited, especially at the planktonic larval stage, but the topic receives increasing attention for its ecological consequences. Blanchard, Pechenik, Giudicelli, Connan, and Robert (2008) found that *C. fornicata* larvae ingested phytoplankton over a larger range of cell sizes and at increased rates compared with *C. gigas*. This laboratory study was a comparison with *C. gigas*; therefore, interactions with *O. edulis* in the natural environment may vary. However, when larvae of both species are present in summer, intensive grazing by *C. fornicata* larvae could out-compete *O. edulis* larvae, reducing the chances of their survival.

The present study addresses a number of interactions between *O. edulis* and *C. fornicata* within a coastal system home to extensive historical oyster populations and current oyster restoration initiatives: (i) the settlement preference of wild *O. edulis* larvae between conspecific and invasive shells and two common types of artificial hard substrata; (ii) the abundance of oyster and *C. fornicata* larvae within the water column across two harbours within the Solent; and (iii) the faunal community assemblages associated with *in situ* live *O. edulis* and *C. fornicata* assessed as a proxy of their function as benthic ecosystem engineers. By determining if *C. fornicata* populations are detrimental to *O. edulis* and the localized biodiversity, restoration efforts can begin to address the issue by incorporating active management strategies.

2 | MATERIALS AND METHODS

2.1 | Recruitment substrate characterization

Settlement substrata availability for, and preference of, *O. edulis* and *C. fornicata* in the eastern Solent harbours (Portsmouth, Langstone

and Chichester, Figure 1) were assessed. Settlement substratum was recorded for each individual *O. edulis* from the three harbours, all of which were purchased from the commissioned fisheries. Settlement substratum of *C. fornicata* chains was recorded for individuals collected during surveys of the three harbours (see Helmer et al., 2019 for collection methods and locations). The settlement substratum was determined for *O. edulis* as the organism or material the oyster was attached to near the hinge/umbo. When a clear scar was present but no material remained, it was recorded as 'absent'. The attachment substratum for each individual chain of *C. fornicata* was recorded as the substratum that the last living individual at the base of the chain was settled upon. Chains were considered separate when the substratum had multiple chains attached to it and live individuals did not interconnect these chains.

2.2 | *Ostrea edulis* larval settlement

Settlement plates deployed in May 2016 consisted of 15 discs with three replicates of five alternative substrata: (i) plain plastic discs (control); (ii) plastic discs dipped in cement (Blue Circle Mastercrete); (iii) plastic discs covered in old and smooth *O. edulis* shells (collected from Langstone Harbour intertidal zone); (iv) plastic discs covered in recently deceased and rough *O. edulis* shells (sourced from mortalities in broodstock cage system trials; Helmer et al., 2019); and (v) plastic discs covered in *C. fornicata* shells collected from Langstone Harbour (Figure 2). The settlement plates were deployed for one year, enabling any oyster larvae to settle and develop to a size whereby adult morphological features could be used to distinguish between *O. edulis* and *C. gigas* spat. Samples were fixed in 4% formalin in seawater (borax buffered 5 g/L) for 2 weeks and then transferred to 70% ethanol prior to analysis. A Leica EZ4W stereo microscope with camera mounting was used for identification of oyster spat and image collection. Measurements were taken from the images using the open source software ImageJ (Rueden et al., 2017).

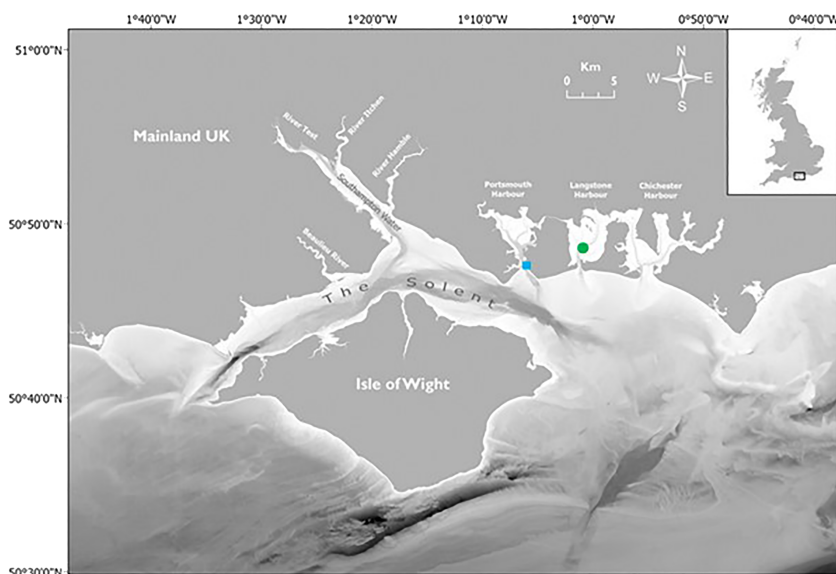
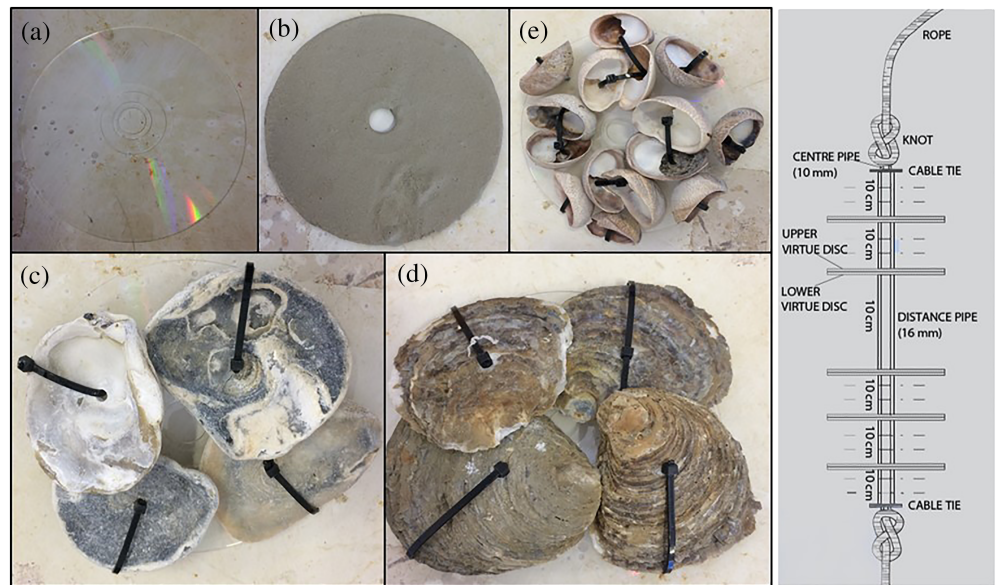


FIGURE 1 The wider Solent, including locations within the eastern Solent harbours (Portsmouth, Langstone and Chichester) from which seabed samples were collected as well as cage sampling locations in the Camber Dock (blue square) and on the University of Portsmouth research platform (green circle)

FIGURE 2 Settlement plates deployed in 2016 comprising (a) blank plastic discs, (b) plastic discs dipped in cement, (c) plastic discs covered in old, smooth *Ostrea edulis* valves, (d) plastic discs covered in recently deceased *O. edulis* valves and (e) plastic discs covered in *Crepidula fornicata* shells. (f) Each structure contained three replicates of each substratum placed in random order. Photos: Luke Helmer. Schematic of disc deployment provided on the right



2.3 | Zooplankton community composition

2.3.1 | Sample collection and preservation

Seawater samples were collected using a plankton net (300 mm diameter, 64 μm mesh, NHBS). Surface tows were conducted at high tide ± 1 h at a speed of 1.5 kn for 1 min, with three replicate samples

TABLE 1 Sample collection dates and labels for both Portsmouth and Langstone harbours

Sample	Langstone	Portsmouth
1	31/05/16	
2	08/06/16	
3	23/06/16	
4	30/06/16	30/06/16
5	07/07/16	07/07/16
6	28/07/16	28/07/16
7	04/08/16	04/08/16
8	12/08/16	12/08/16
9	19/08/16	
10	24/08/16	

collected at each location. Using this method, a volume of 3.27 m³ of seawater was filtered by the plankton net during each tow. Plankton sampling was carried out at two locations (Langstone and Portsmouth) at approximately weekly intervals throughout the spawning season (May to August 2016; Table 1). Immediately after collection samples were filtered across a 64 μm sieve and fixed in 4% formalin in seawater (borax-buffered 5 g/L), stained with Rose Bengal (0.05 g/L), then preserved in 70% ethanol after 1 week (Goswami, 2004). Once in ethanol, samples were split into two sub-samples to be used for larval quantification and scanning electron microscopy (SEM).

2.3.2 | Sample analysis

A 1 ml aliquot of the first sub-sample from each collection date/location was placed onto a S50 plastic Sedgewick-Rafter Counting Cell and viewed under a compound microscope (Leica, Germany). Larval abundance was recorded for 100 randomly selected squares on each slide (Conway, 2012a, 2012b, 2015), for both oyster species, *O. edulis* and *C. gigas* (Hu, Fuller, Castagna, Vrisenhoek, & Lutz, 1993; Le Pennec, 1980; Loosanoff, Davis, & Chanley, 1966; Pascual, 1972; Tanaka, 1981; Waller, 1981) and *C. fornicata* (Figure 3). This procedure was replicated in triplicate for each sampling

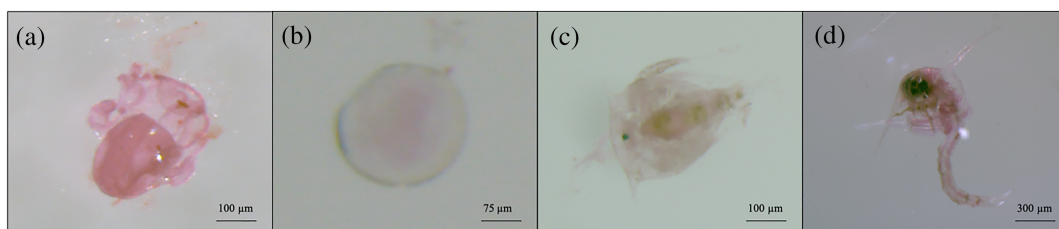


FIGURE 3 Planktonic larvae; (a) *C. fornicata* (slipper limpet), (b) oyster veliger, (c) barnacle nauplius, (d) Decapoda (*Carcinus maenas*)

period/location. The values of larval abundance were then averaged and used to calculate the larval density (larvae/ml).

2.3.3 | SEM of oyster larvae

Larval analysis by SEM was used to confirm the light microscope identification of oyster larvae and monitor *O. edulis* larval survival and growth in the column water. Larval measurements were used to calculate the percentage frequency of each shell size class in both locations. The two species, *O. edulis* and *C. gigas*, were distinguished using morphological features clearly visible from the micrographs. Five to 10 oyster larvae were selected from each sample and placed in sodium hypochlorite (5%) for 48 h to disarticulate the two valves of each individual (Rees, 1950). Samples were then dehydrated through a series of increasing ethanol concentrations (50, 60, 70, 80, 90 and 100%), followed by submersion in hexamethyldisilazane (100% HMDS solution).

Samples were mounted on 12 mm SEM stubs, which were then coated in gold/palladium (Leica EM ACE600; Turner & Boyle, 1975). Electron micrographs of the larval shells were obtained using a Zeiss Evo MA10 SEM. Identification was confirmed morphologically using the features of the D veliger, umbo and left valve hinge, according to Hu et al. (1993) and Waller (1981). The maximum shell length and height (μm) of each larva were calculated using ImageJ software (Figure 4). The percentage frequency of each shell size was also calculated for each location and each time point.

2.3.4 | Associated epibiont diversity and abundance

The epibiont succession and community assemblage supported by *O. edulis* and *C. fornicata* populations were assessed using *in situ* benthic experiments. Live samples of *O. edulis* and *C. fornicata* were collected, cleaned of any epibiont and placed into cages composed of 35.5 × 9 cm circular plastic sieves (5 mm mesh) enclosed with 2" deer fencing mesh. Two population densities were trialled; high-density (1,000–1,050 g of *O. edulis* or *C. fornicata*/cage) and low-density (500–550 g of *O. edulis* or *C. fornicata*/cage), with three replicates of each density and species. The experiment was deployed at two locations: the Camber Dock, Portsmouth Harbour (50°47'32.52"N, 1°6'25.93"W) and University of Portsmouth research platform in Langstone Harbour (50°48'23.73"N, 1°1'20.56"W; Figure 1). Oysters were obtained from the Solent fishery

and were selected according to the minimum landing size, using a 70 mm diameter ring (Helmer et al., 2019; Southern Inshore Fisheries and Conservation Authority (IFCA), 2018a). *Crepidula fornicata* were also collected from Langstone Harbour (Figure 1), using a 0.1 m² Van Veen grab onboard the 10.6 m University of Portsmouth research vessel *Chinook II* (Offshore 105 Pilothouse). The epibiont was monitored bi-weekly at each site between May and August 2016; all organisms were identified to species level.

2.4 | DATA ANALYSES

2.4.1 | Planktonic larval abundance

Univariate analysis on two different variables (V_1 , larval density of *O. edulis*; V_2 , larval density of *C. fornicata*) was performed using a general linear model of two-way ANOVA with two factors: site (two levels – Langstone and Portsmouth) and date (six shared date levels – 30 June, 7 and 28 July and 4, 12 and 24 August). The data were transformed by square root and the *post-hoc* Tukey's pairwise tests performed (Minitab® v.18). Multivariate analysis of planktonic larval communities was performed using Primer 6.1.10 and PERMANOVA β 20 (Primer-E Ltd: Plymouth Routines in Multivariate Ecological Research). Taxonomic groups were grouped as barnacle larvae, Bryozoa larvae, Cnidaria, Copepoda larvae and adults, *C. fornicata* larvae, Decapoda larvae, Foraminifera, Nematoda larvae, oyster larvae (*O. edulis* and *C. gigas*), Ostracoda adults, Polychaeta larvae and Tunicata. Data were transformed by the fourth root. The factors site (two levels – Langstone and Portsmouth) and date (six shared levels) were used in a PCO (principal coordinate analysis) with data constrained using an S17 Bray Curtis similarity matrix. A PERMANOVA main test (number of permutations 9,999) was performed to confirm the significance of the dissimilarities illustrated by the PCO, and a *post-hoc* pair-wise test to determine which levels of factors are responsible for the differences. A SIMPER analysis (similarity percentage analysis) was used to assess the degree of similarity within and between levels of both factors, assessing the percentage contribution of each taxonomic group, including *O. edulis* and *C. fornicata* contributions.

Since Langstone Harbour had more time points, the same multivariate analysis was performed but using only the samples from this location. The same taxonomic groups represented multiple variables, with one factor considered: date (10 levels: 31 May, 8, 23 and 30 June, 7 and 28 July and 4, 12, 19 and 24 August).

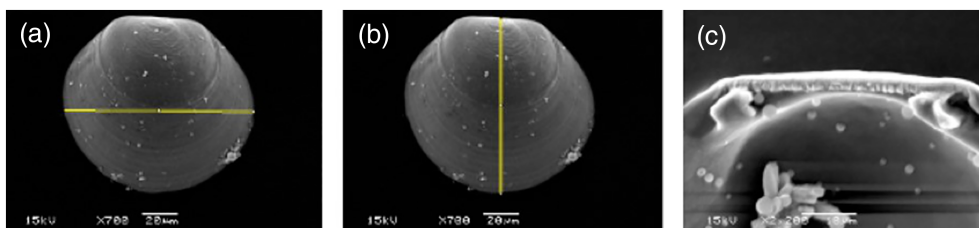


FIGURE 4 Electron micrographs of oyster veliger shells obtained by scanning electron microscopy. Measurements were collected for shell (a) length and (b) height, whilst the (c) umbo was used for species identification

2.5 | *Ostrea edulis* larval settlement

Analysis was conducted in IBM® SPSS® Statistics 25 (IBM Analytics, USA). Data were tested for normality and homogeneity, owing to a non-normal distribution; a Kruskal-Wallis H test was used to analyse the spat settlement between locations and between species and orientation.

2.5.1 | Associated epibiont diversity and abundance

The univariate and non-parametric multivariate techniques using ordination from PCO with S17 Bray Curtis similarity matrices contained in Primer v.6 (Clarke & Gorley, 2006) were used to explore similarities between the two localities, and of faunal communities removed from two habitats: (i) live *O. edulis*; and (ii) live *C. fornicata*. Differences in faunal communities removed from oysters and limpets were tested using SIMPER and ANOSIM tests to determine which faunal communities contributed to each site and habitat. A DIVERSE test was employed to determine which habitat had the greatest number and abundance of fauna, followed by PCO analyses to visualize the results as an ordination, constrained to linear combinations of the localities, habitats and fauna. Similarities of fauna between localities were examined using PERMANOVA main tests and *post-hoc* pairwise tests.

All parametric statistical analyses were performed using Minitab (Minitab Inc, v. 13.20). Spatial differences of faunal abundance were examined using a general linear model (GLM) with site, species and abundance (Portsmouth and Langstone harbours, oysters and limpets, and high and low, respectively) as factors. A one-way ANOVA was used to analyse differences of faunal abundance and numbers of species, and biodiversity (Shannon Wiener) between oysters and limpets, as well as any differences between the number of oyster spat settled on the two substrata. All count data were square root transformed. *Post-hoc* Tukey's pairwise comparison tests separated values into statistically distinct subsets in all ANOVAs.

3 | RESULTS

3.1 | Benthic settlement substrata of *O. edulis* and *C. fornicata*

Of the *O. edulis* assessed within Portsmouth, Langstone and Chichester, 80.3, 90.3 and 65.6%, respectively, did not display any distinguishable attachment point around the hinge area. *Crepidula fornicata* shell accounted for 11.3, 8.6 and 30.4% of attachment points within Portsmouth, Langstone and Chichester harbours, respectively. In all cases, attachment was to a deceased *C. fornicata* with the majority attaching to the ventral surface of the shell, only exposed when no flesh was present. *Ostrea edulis* shell accounted for 8.4, 1.1 and 4.0% of attachment points within the Portsmouth, Langstone and Chichester *O. edulis* populations, respectively (Figure 5a).

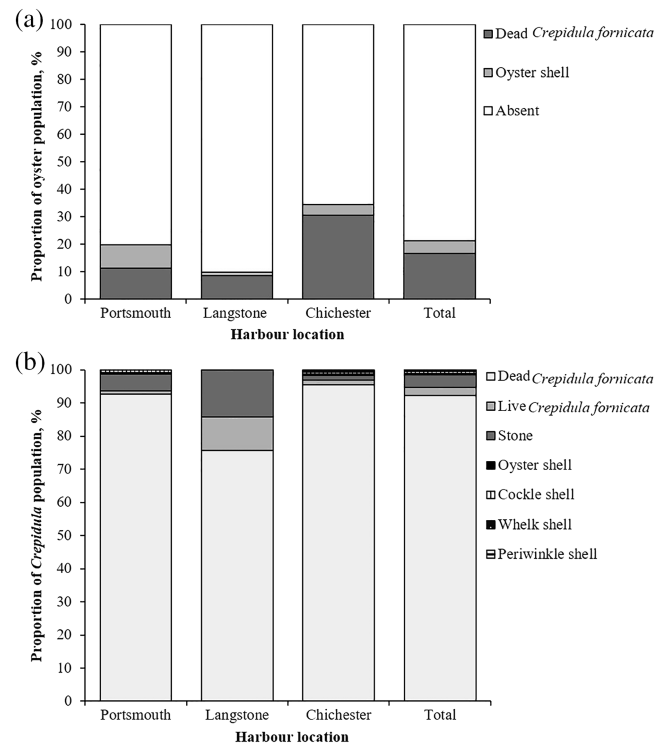


FIGURE 5 (a) Proportion of *O. edulis* ($n/\text{harbour} = 700$) retrieved from the fisheries within Portsmouth, Langstone and Chichester harbours, as well as the total ($n = 2,100$), settled to *C. fornicata* shell, oyster shell or with no obvious attachment point observed. (b) Proportion of attachment substrata for live *C. fornicata* chains observed within Portsmouth ($n = 221$), Langstone ($n = 127$) and Chichester ($n = 584$) harbours, as well as the total for all locations ($n = 932$). Each chain of *C. fornicata* was defined as the individuals attached to one another in a single mass, irrespective of the direction of attachment and excluding any deceased shells used as attachment substratum. Chains were considered separate when the substratum had multiple chains attached to it and these chains were not interconnected by live individuals

The main settlement substratum for *C. fornicata* within all three harbours was found to be dead *C. fornicata* shell with 92.8, 75.6 and 95.5% of chains settled on this substratum within Portsmouth, Langstone and Chichester harbours, respectively (Figure 5b). The percentage of live *C. fornicata* at the base of the chain varied across the harbours, with Portsmouth and Chichester having relatively few, 0.9 and 1.4%, respectively, and Langstone Harbour having 10.2%. Very few chains of limpets, <0.5%, were attached to oyster shells within each harbour. Attachment to stone accounted for the second highest percentage within all three harbours – 5, 14.2 and 1.4% within Portsmouth, Langstone and Chichester, respectively. All other attachments accounted for <1% in each harbour. Settlement on dead *C. fornicata* shell accounted for 92.2% within all harbours (pooled data), with attachment to stone accounting for 4.0%, live *C. fornicata* 2.5%, oyster shell 0.3%, cockle shell 0.6%, whelk shell 0.3% and periwinkle 0.1% (Figure 5b).

3.2 | Planktonic larval densities

Since the percentage contribution of *C. gigas* to the total abundance of oyster larvae was very low in both sites (<5.5%), the overall oyster densities are referred to as *O. edulis* larval densities.

In Langstone Harbour *C. fornicata* larvae dominated, contributing the highest density at 374.7 ± 96.5 (mean \pm SD) larvae/ml, whilst *O. edulis* was lowest at 1 ± 0 larvae/ml (Figure 6a). In Portsmouth Harbour, *O. edulis* occurred at the highest density at 67.7 ± 29.3 larvae/ml and *C. fornicata* was lowest at 6 ± 4.6 larvae/ml (Figure 6b). In Langstone, during the entire spawning season (June to August 2016), *O. edulis* larval density varied between 8 ± 1.7 and 92.3 ± 12.9 larvae/ml. Two possible spawning events are suggested by peaks in *O. edulis*, on 28 July (92.3 ± 12.9 larvae/ml) and 12 August (45.7 ± 20.1 larvae/ml). *Crepidula fornicata* larval density ranged between 6 ± 3 and 374.7 ± 96.5 larvae/ml. This last value indicates that a massive spawning event took place around 12 August. The 65.3 ± 14.2 (mean \pm SD) larvae/ml observed on 19 August could be a second event, or more likely larvae still present in the water column from a previous spawning event.

In Portsmouth, between the end of June and the end of August, *O. edulis* larval density ranged between 6.7 ± 3.5 and 67.7 ± 29.3 (mean \pm SD) larvae/ml. A first spawning event on 30 June and a possible second one on 12 August corresponded to peaks of 67.7 ± 29.3 and 38.7 ± 26.8 larvae/ml respectively. *Crepidula fornicata* larval density was lower in Portsmouth than in Langstone, ranging between 6 ± 4.6 and 44 ± 18.1 larvae/ml, respectively. The only peak in larval

density corresponding to a probable spawning event was found in Portsmouth on 12 August, with 44 ± 18.1 larvae/ml.

From the two-way ANOVA performed on *O. edulis* larval density (variable V_1), no significant differences were found between the two sites, except on the 30 June and 28 July, with significantly higher densities in Portsmouth and Langstone, respectively (Figure 7a). The difference of larval density was significant between dates ($F_{5, 35} = 13.6$, $P \leq 0.001$) and for the combination of factors (site \times date) ($F_{5,35} = 7.8$, $P \leq 0.001$), with levels 6 and 7 of the factor 'Date' (28 July and 4 August) mostly responsible for this significant difference (*post-hoc* Tukey's pairwise test, $P \leq 0.05$). Larval density of *C. fornicata* (variable V_2) did not vary significantly between sites and dates, except on 12 August (Figure 7), when a massive spawning event occurred, particularly in Langstone Harbour. Significant differences were found between sites ($F_{1,35} = 70.3$, $P \leq 0.001$), dates ($F_{5,35} = 71.5$, $P \leq 0.001$) and for the combination of factors (site \times date) ($F_{5,35} = 26.5$, $P \leq 0.001$) (PERMANOVA main test), with level 8 of the factor 'date' (12 August) accounting for these significant differences (*post-hoc* Tukey's pairwise test, $P \leq 0.05$).

The PCO analysis, explaining 64.6% of the total variation between sites and dates, showed a clear separation between the planktonic communities sampled on 12 August (date 8) at both sites and the rest of the samples (Figure 8a – solid circle). Most of the samples from Langstone (date 4, 30 June; date 6, 28 July; date 7, 4 August) could also be grouped into another cluster, revealing a slight difference in planktonic communities between the two locations (Figure 8a – dashed circle). Significant differences in community composition between sites (pseudo- $F_{1,24} = 5.8$, $P \leq 0.001$), dates (pseudo-

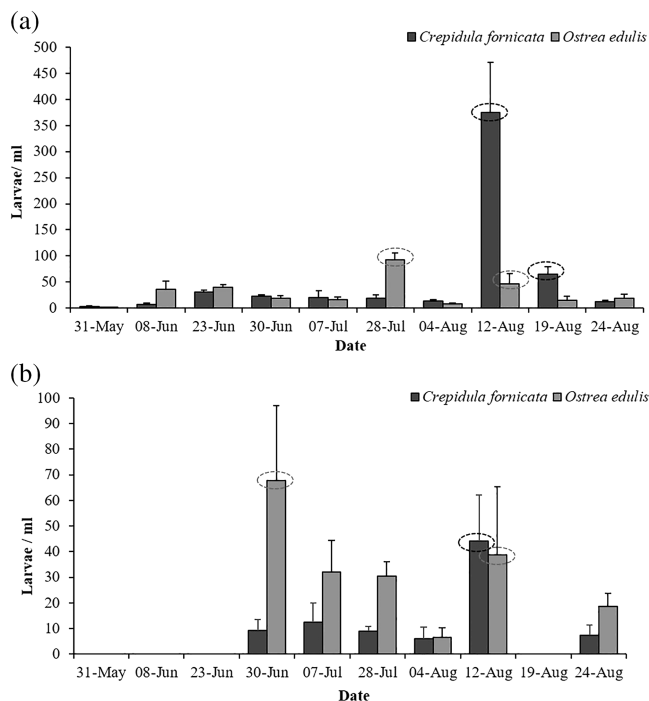


FIGURE 6 Larval densities (mean \pm SD) of *O. edulis* and *C. fornicata* in (a) Langstone Harbour and (b) Portsmouth Harbour during 2016. Dashed circles indicate potential spawning events

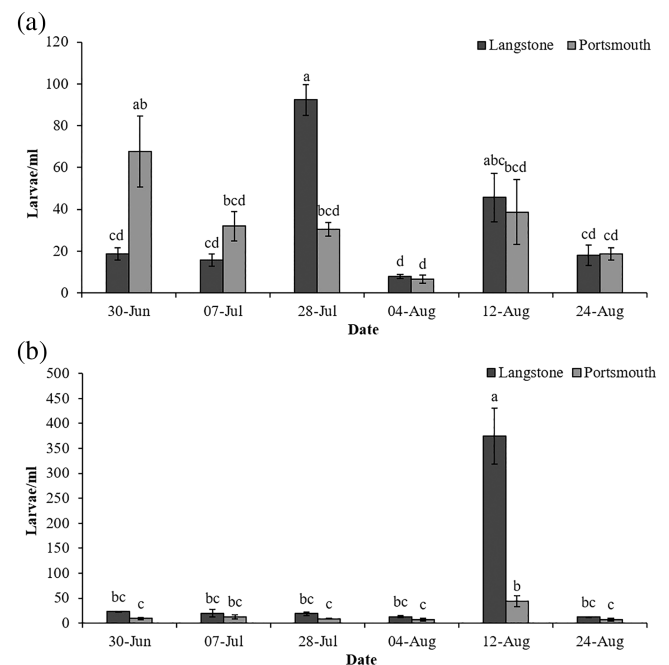
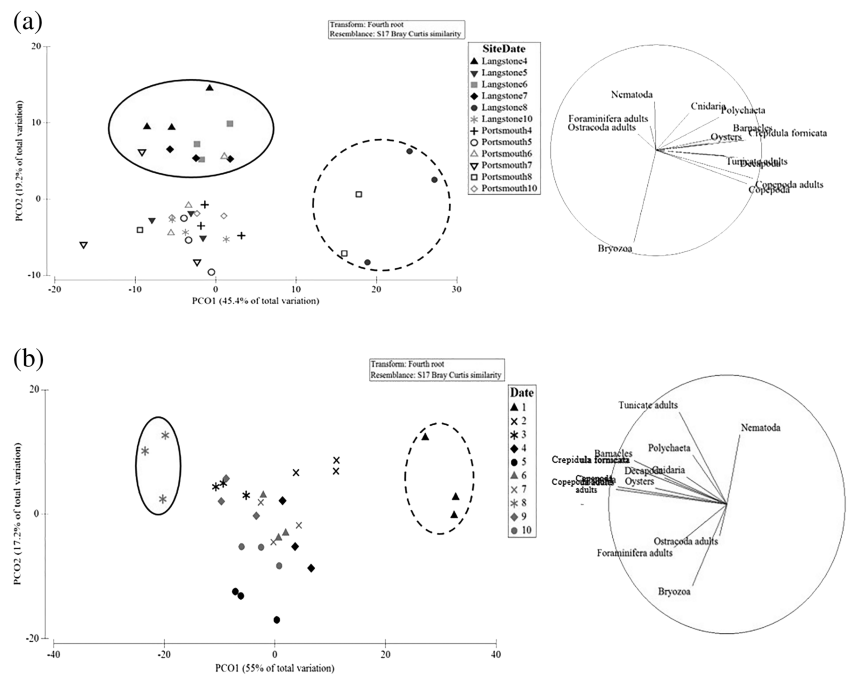


FIGURE 7 Larval densities (mean \pm SE) of (a) *O. edulis* and (b) *C. fornicata* within Langstone and Portsmouth harbours during 2016. Differences between data labels indicate significant differences (two-way ANOVA, $P < 0.05$)

FIGURE 8 Principal coordinate analysis (PCO), S17 Bray Curtis similarity. (a) Distribution of planktonic larval communities in Langstone and Portsmouth harbours and the taxonomic groups that best characterize the planktonic communities. (b) Distribution of planktonic larval communities in Langstone Harbour and the taxonomic groups that best characterize the planktonic communities



$F_{5,24} = 6.9$, $P \leq 0.001$) and for the combination of factors were found ($\text{pseudo-}F_{5,24} = 2.55$, $P \leq 0.05$). No significant differences were found either between sites within each level of factor 'date' and between dates within both levels of factor 'site' (*post-hoc* pair-wise test).

The average similarity of community composition was >80% within each level of both factors (SIMPER analysis). This is mainly due to the presence of Copepoda (larvae and adults), since their contribution to the similarity of each level ranged between 40 and 50%. The dissimilarity between either dates and sites was no higher than 27%. In particular the average dissimilarity percentage between Langstone and Portsmouth was 15.97%, with three taxonomic groups mainly contributing (Barnacle larvae 11.2%, Bryozoa larvae 10.8%, Copepoda larvae 10.4% and adults 10.3%). The same three taxonomic groups contributed to 66–67% of the planktonic community composition at both sites: Copepoda (larvae and adults), *C. formicata* and oysters. In Portsmouth the contribution of oysters (12%) was higher than that of *C. formicata* (9.6%), whilst the opposite occurred in Langstone (oysters, 11.7%; *C. formicata*, 12%).

The PCO analysis, explaining 72.2% of the total variation between dates in Langstone Harbour, showed a separation of the planktonic communities sampled on 31 May and 12 August from the rest of the samples (Figure 8b – solid circles). Significant differences in community composition were found between dates ($F_{9,20} = 11.08$, $P \leq 0.001$) in Langstone (PERMANOVA main test performed with one factor). Nonetheless the *post-hoc* pair-wise test did not produce any significant difference between each level of factor 'date'.

The average dissimilarity was <25% between most of the dates. It was slightly higher (25–35%) between 12 August and the other dates, with three taxonomic groups mainly contributing (~60%): Copepoda (larvae and adults), barnacle larvae and *C. formicata*. Greater dissimilarity (35–45%) was found between 31 May and the other dates, with the main contribution (~60%) coming from Copepoda (larvae and

adults), barnacles and oysters. The greatest dissimilarity was found between 31 May and 12 August (53%). Copepoda (larvae and adults) contributed 40–45% of the planktonic community composition in Langstone Harbour during the whole spawning season. On dates 1, 4 and 7–9 the contribution of *C. formicata* (C) was higher than that of oysters (O) (date 1 – C 14.2%, O 12.9%; date 4 – C 14.1%, O 12.8%; date 7 – C 10.7%, O 9.6%; date 8 – C 15%, O 8.5%; date 9 – C 14%, O 8.8%; SIMPER analyses).

The SEM analysis of planktonic *O. edulis* veligers revealed that in Langstone Harbour the greatest length and height, $125.5 \pm 27 \mu\text{m}$ (all values mean \pm SD) and $147.2 \pm 31.2 \mu\text{m}$, respectively, were found on 4 August, whilst the lowest values, 73 ± 0 and $97 \pm 0 \mu\text{m}$, were found on 31 May. In Portsmouth Harbour the greatest length and height, 115.7 ± 16.8 and $145.2 \pm 13.7 \mu\text{m}$, respectively, were found on 7 July, whilst the lowest values, 94 ± 17.7 and $114.7 \pm 20.6 \mu\text{m}$, were found on 28 July. The length of larval oyster shells varied between 60 and >200 μm in Langstone Harbour with the most frequent size class being 100–110 μm (19.4%). In Portsmouth Harbour the length ranged between 60 and 170 μm , with the most frequent size class also 100–110 μm (25.5%). The height of oyster shells varied between 70 and >200 μm in Langstone Harbour with the most frequent size classes being 100–110 and 110–120 μm (both 16.7%), whilst in Portsmouth Harbour it ranged between 70 and 180 μm , with the most frequent size class being 140–150 μm (25.5%).

3.3 | Epibiont biodiversity and settlement substrate preference of *O. edulis*

In Portsmouth Harbour *Palaemon serratus* (31.2%), *Pomatoceros triquetus* (16.4%) and *Ascidella scabra* (13.7%) accounted for >50% of the community (SIMPER analyses). In Langstone Harbour, 50% of the

species contributed to 70% of the faunal community on *O. edulis*, with *P. serratus*, *P. triqueter*, *A. scabra* and *T. indivisa* contributing 31.2, 16.4, 13.7 and 9.4%, respectively (SIMPER). Two species contributed to 70% of the faunal community associated with *C. fornicata*, with *P. serratus* and *Spirorbis spirorbis* (contributing 53.3 and 15.6%, respectively).

A PCO explaining 46.1% of the variation in Langstone Harbour revealed a significant difference between the faunal community associated with *O. edulis* and *C. fornicata* (PERMANOVA main test $F_{1,28} = 5$, $P \leq 0.001$), also corroborated by an ANOSIM test ($R = 0.47$, $P \leq 0.001$; Figure 9b). Twenty-five species characterized the faunal community associated with *O. edulis*, and eight species characterized the faunal communities associated with *C. fornicata* (DIVERSE Test). Five species contributed to 70% of the faunal community associated with *O. edulis*, with *P. serratus*, *T. indivisa*, *A. scabra*, *P. triqueter* and *Dendrodoa grossularia* contributing 25.6, 16.3, 12.9, 10.6 and 5.1%, respectively (SIMPER analyses). Two species contributed almost 70% of the faunal community associated with *C. fornicata*, with *P. serratus* and *T. indivisa* contributing 41.2 and 22.1%, respectively.

Settlement of *O. edulis* spat was significantly greater on settlement discs covered with recently deceased oyster shells, with 6.7 ± 1.2 (mean \pm SE) spat/disc, more than double the number of spat associated with old smooth oyster shells, 2.7 ± 1.3 . No significant difference in the number of settled spat was found between old smooth oyster shells, *C. fornicata* shell (1.7 ± 0.3), cemented discs (2 ± 1) or the plastic control disc (0.7 ± 0.7 ; Figure 10; one-way ANOVA, $F_{4,10} = 5.6$, $P \leq 0.05$).

4 | DISCUSSION

Surveys of the benthic composition combined with data on recruitment substrate utilized by *O. edulis* and *C. fornicata* larvae depict an ecologically concerning picture for the native oyster. A long-term study by Helmer et al. (2019) revealed that, over a 19-year period, *O. edulis* populations within Chichester Harbour (Solent, UK) decreased by 96%, and populations of *C. fornicata* increased by 68.9%. Extremely high densities of *C. fornicata* were found within the Solent [84.1 ± 24.5 , 174.3 ± 34.5 and 306 ± 106 individuals per m^2

(mean \pm SE) for Portsmouth, Langstone and Chichester harbours, respectively]. Both Langstone and Chichester harbours contained significantly more individuals than Portsmouth Harbour. During the survey, no oysters were found in Portsmouth Harbour, two were found in Langstone Harbour and one was found in Chichester Harbour, which led to concern about mechanisms of competitive ecological exclusion of *O. edulis* by the invasive *C. fornicata*.

In the absence of plentiful live oyster substrate (1–8% were found associated with conspecific shells), a relatively low percentage (8–30%) of the Solent *O. edulis* population was observed to have settled on the ventral surface of dead *C. fornicata* shell. No oysters were found settled on live *C. fornicata*. This is in contrast to the 75–98% of *C. fornicata* found settled on conspecific shells. This suggests that there are strong competitive interactions at the settlement stage during which *C. fornicata* outcompetes *O. edulis* larvae for available dominant substrate, thereby perpetuating the negative feedback loop and furthering the exclusion of the native species. This is suggested as one of the main mechanisms of the ecological phase shift occurring in the Solent from mixed sediment featuring *O. edulis* reefs to *C. fornicata*-associated silty mud.

The zooplankton analysis also suggests that in areas where high densities of *C. fornicata* are present, native oyster larvae are facing significant competition for food resources in the water column during the prodissoconch free-swimming feeding stage. Within Portsmouth Harbour, abundances of *C. fornicata* and *O. edulis* were largely similar, with an *O. edulis* spawning event occurring in late June, almost a month earlier than the Langstone spawning event in late July. This supports the occurrence of geographic population structure in *O. edulis* over very small spatial scales observed in the adult populations of these harbours (Helmer et al., 2019). A second spawning event for both species was observed in both harbours in early August.

This was confirmed for *O. edulis* by the demographic analysis of larval size as a proxy for growth using the SEM images, as is the earlier spawning event in Portsmouth Harbour. In the present study plankton sampling was conducted on the surface, allowing the collection of only the small shell size classes (maximum 190 μ m), but it must be considered that oyster larvae usually move deeper in the water during their growth, ending up near the substrate, seeking a suitable settlement surface. Therefore, it is recommended to repeat plankton sampling at different depths in order to collect all shell size classes, and follow the spatio-temporal growth pattern of oyster larvae. A more thorough sampling, including greater depths, and the combination with the recruitment data, could provide a wider overall view on *O. edulis* reproduction in the Solent.

Understanding the spawning phenology of *O. edulis* and *C. fornicata* is of critical importance for the development and implementation of any restoration strategy and management. Of ecological significance are both the timing and magnitude of the *C. fornicata* spawning event that occurred in Langstone Harbour concurrently with the second peak in *O. edulis* larval density. These harbours are eutrophic (Environment Agency, 2016a, 2016b) and therefore food is unlikely to be a limiting factor for feeding planktonic larvae. However,

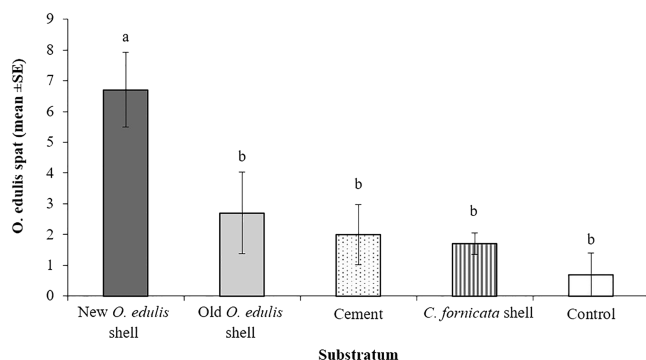


FIGURE 10 Number of *O. edulis* spat (mean \pm SE) settled to the various settlement substrata provided

the effect of simultaneous spawning in these sympatric species on larval energetics, development and food resource partitioning is not currently known. It is feasible that the extremely high densities of *C. fornicata* larvae, eight times greater than the *O. edulis* larval density, could have a negative competitive effect both in the nekton and at the benthic boundary layer during settlement, particularly when the conspecific shell abundance is stacked firmly in favour of *C. fornicata* recruitment. Further investigation is required, but the *O. edulis* larvae size class analysis suggests that larvae are remaining and growing in the water column over 3–4 weeks. The cumulative impact of interspecific competition and lack of substrate availability on larval fitness could lead to delayed onset of pediveliger development and successful metamorphosis. These factors will create a barrier to healthy recruitment in *O. edulis*.

The relatively complex life history of *O. edulis* makes it a particularly vulnerable species. As a viviparous species, the success of wild population reproduction depends on broodstock density to a greater degree than oviparous species reproducing by broadcast spawning. In many areas, broodstock density might be too low to ensure synchronous spawning, leading to sporadic spawning events and insufficient production of larvae. In 2016, spawning events and relatively high densities of *O. edulis* larvae were found in both Langstone and Portsmouth harbours, indicating a successful production of native oyster larvae in the Solent. This could be related to, or enhanced by, the presence of broodstock cages installed during this time, acting as larval pumps.

Despite the *O. edulis* population successfully breeding in 2016 and the presence of larvae in the water column, there has been no substantial recruitment in the Solent since then (Southern Inshore Fisheries and Conservation Authority (IFCA), 2014, 2015, 2017, 2018b; Sussex IFCA, 2018). To increase the chance of successful *O. edulis* spat settlement there needs to be an increase in the presence of either live *O. edulis* or recently deceased empty shells. One current plan for increasing the successful settlement of *O. edulis* involves dredging *C. fornicata* from the seabed and returning the empty shells as a substrate for *O. edulis* settlement (Harding, Nelson, & Glover, 2016). However, this present study suggests that this method would be ineffective as the *O. edulis* spat did not preferentially settle on *C. fornicata* shells; in fact the levels of settlement were not significantly different from those on plain plastic or cement-covered discs.

Successful settlement of *O. edulis* larvae depends on the presence of suitable substrata. The significantly higher settlement of *O. edulis* larvae on new conspecific shells than on old eroded shells or *C. fornicata* shells confirms recent findings of the importance of conspecifics in settlement cues (Rodríguez-Perez et al., 2019). Although *O. edulis* larvae will settle on other available hard substrata, they are a gregarious species that prefer to settle on adult shells, especially the new growth (Bayne, 1969; Perry & Tyler-Walters, 2016). The surface heterogeneity could be driving this difference in settlement: *O. edulis* shells are rough and scaly in appearance (Perry & Jackson, 2017), whereas *C. fornicata* shells are much smoother (Rayment, 2008). Differences in CaCO₃ mineral composition may also explain this settlement preference by *O. edulis* larvae; *C. fornicata* shells are

predominantly aragonitic (Pilkey & Goodell, 1964), whereas the dominant component of the outer prismatic foliated shell layers of *O. edulis* is calcite, with traces of aragonite and halite (Medaković, Traverso, Bottino, & Popović, 2006). *Ostrea edulis* valves usually consist of three layers, the first being the periostracum, a thin outer layer which sits on the middle section or prismatic layer of calcite. The innermost layer, normally pearly white in colour, is formed from aragonite (Walne, 1974). This study disputes the finding that settlement is determined by the availability of hard substrata alone (Smyth et al., 2018), but rather the properties of the hard strata are important factors in determining settlement in *O. edulis*.

The diminished seabed *O. edulis* populations in the Solent and throughout Europe mean that there is a lack of suitable settlement substrate even when locations are not recruitment limited. The evidence presented here makes it clear that the native oyster population requires conspecific or other appropriate settlement substrate. The extremely high densities of live *C. fornicata* do not provide suitable substrate for successful settlement of native *O. edulis* larvae and are a barrier to the recovery of the European native oyster. In areas of high slipper limpet densities, deploying recently deceased oyster cultch on top of the *C. fornicata* could be an effective strategy to mitigate the inhibition of *O. edulis* settlement by increasing the quantity of suitable substrate whilst reducing predation and competition.

The lack of suitable settlement sites for *O. edulis* larvae, owing to the presence of *C. fornicata* shells and lack of *O. edulis* shells on the seabed, could lead to delayed metamorphosis of the oyster larvae while they look for a suitable settlement substrate. The delaying of metamorphosis is likely to have negative impacts on the larvae, whether that be degeneration of the foot or starvation, both leading to reduced survival. Withholding suitable settlement sites for *M. edulis* resulted in delayed metamorphosis, during which the velum degenerated and the foot grew larger, there was also a decline in feeding rate and eventually the larvae were no longer able to feed (Bayne, 1965). In the polychaete *Hydroides elegans*, metamorphosis cannot be delayed without measurable negative effects on juvenile survival and growth (Qian & Pechenik, 1998). *Echinometra* larvae that experienced a prolonged delay in metamorphosis also had a reduced chance of survival, metamorphosis success and survival to juvenile stage (Rahman, Boon, Muntohar, Tanim, & Pakrashi, 2014). There is currently no evidence that delayed metamorphosis in *O. edulis* has these adverse effects; however, it is likely that there will be negative effects as observed in other species.

Both *O. edulis* and *C. fornicata* are filter feeding molluscs that potentially offer functional equivalence in their nutrient assimilation or water filtration services. They do not, however, provide ecological equivalence in terms of the ecological niche and suprabenthic communities they support. De Montaudouin et al. (1999) found that the presence of *C. fornicata* had no effect on the benthic community; however, this study demonstrates that the presence of *C. fornicata* has a significant negative effect on the epibiont biodiversity. Specifically, the biodiversity decreased in the presence of *C. fornicata*. As well as supporting a lower total abundance of species, in relation to *O. edulis*, *C. fornicata* also supported a significantly different

community. It is now widely accepted that oyster shells show higher diversity than non-living hard substrata, and as oysters grow older and therefore larger, epibiotic diversity will increase (Smyth & Roberts, 2010). However, this study is one of the first (at least in recent years) to show that *O. edulis* substrate supports higher levels of biodiversity than *C. fornicata*.

As well as an increase in biodiversity, *O. edulis* also provides an increase in overall biomass, which in turn improves the health and quality of an ecosystem. Although increases in biomass and biodiversity themselves do not necessarily make an ecosystem more resilient to change, they are driving factors. The three main factors required to facilitate ecosystem resilience are diversity, connectivity within the ecosystem and adaptive capacity (Bernhardt & Leslie, 2013). Therefore, an increase in trophic complexity associated with *O. edulis*, compared with *C. fornicata*, will also increase the resilience and health of an ecosystem.

Non-native invasive species are a threat to the conservation of biodiversity and can negatively impact ecosystem services, with both ecological and economic impacts (Katsanevakis et al., 2014). Phase-shifts caused by the introduction of invasive species are becoming increasingly common, for example the introduction of *Arcuatula senhousia* (Asian date mussel) to San Diego, USA, changed the entire community composition (Grosholz, 2002; Lambert, Levin, & Berman, 1992). The mats of byssal threads produced by the mussel created a unique habitat that was not present in the otherwise largely unstructured mudflats, which as a result encouraged the development of a new community assemblage (Crooks, 1998; Crooks & Khim, 1999). *Crepidula fornicata* is a threat to native habitats and species; as a habitat engineer it has been reported to cause substantial large-scale changes in the recipient ecosystems, which could lead to phase shifts. These include modification of the trophic structure, changes in phytoplankton composition, enhanced siltation owing to accumulation of faeces and pseudofaeces, and changes in benthic sediments and near-bottom currents (Thieltges et al., 2006). This study demonstrates that the species assemblage of the community associated with *C. fornicata* was significantly different from the community associated with the native keystone species *O. edulis*, causing a shift in the coastal benthic biodiversity and ecosystem structure.

It can be concluded that, as an ecosystem engineer, *O. edulis* provides three-dimensional complex habitat in an otherwise sparse environment, increasing potential ecological niches. The native oyster *O. edulis* facilitates an increase in biodiversity of epibiont communities, especially when compared with the invasive *C. fornicata*. This study finds newly deceased conspecific cultch to be the most suitable for *O. edulis* spat settlement, although surface complexity and material composition are likely to be important drivers. Live *C. fornicata* substrate both inhibits *O. edulis* settlement and significantly changes the benthic community. Settlement substrate type matters in the presence of high benthic and larval densities of *C. fornicata* and is a limiting factor to recruitment of *O. edulis*. It is clear that the Solent is now a substrate-limited system for *O. edulis*, lacking the reef structure to which shellfish larvae can attach (Westby, Geselbracht, & Pogoda, 2019). Substrate management is required to provide reef

substrate in areas of high *C. fornicata* if the aim is to restore self-recruiting populations of *O. edulis*.

Although *C. fornicata* may provide functional equivalence in terms of filtering services, its associated species community is distinct from that of *O. edulis* and it is not equivalent as an ecosystem engineer. This study identifies the mechanisms of ecological phase shift occurring within areas dominated by the invasive species *C. fornicata*. This intensifies the need to manage *C. fornicata* benthic populations to enable recovery of *O. edulis* and its associated ecosystem services and functions.

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AUTHOR CONTRIBUTION

Joanne Preston conceived and designed the study, analysed the data, contributed reagents/materials/analysis tools, authored and reviewed drafts of the manuscript and approved the final draft. Monica Fabra performed the field sampling, performed laboratory sample processing, analysed data, prepared figures and tables, authored and reviewed drafts of the manuscript and approved the final draft. Luke Helmer conceived and designed the study, constructed the sampling apparatus, performed the field sampling, performed the laboratory sample processing, analysed the data, prepared figures and tables, authored and reviewed drafts of the manuscript and approved the final draft. Emma Johnson constructed sampling apparatus, performed field sampling, performed laboratory sample processing, analysed data, prepared figures and tables, authored and reviewed drafts of the manuscript and approved the final draft. Eric Harris-Scott performed laboratory sample processing, reviewed drafts of the manuscript and approved the final draft. Ian Hendy analysed data, reviewed drafts of the manuscript and approved the final draft.

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