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Facilitation effects of invasive and farmed bivalves on native populations of the sea slug *Pleurobranchaea maculata*

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ABSTRACT: Invasive and native bivalves can facilitate higher trophic levels through habitat provision and food subsidies. In New Zealand, interest in the predatory sea slug *Pleurobranchaea maculata* increased when 10 dogs died after contact with beach-cast slugs on Narrow Neck Beach (Hauraki Gulf, Auckland) in August 2009. Investigations identified large populations of native *P. maculata* containing the deadly neurotoxin tetrodotoxin on nearshore beds of the invasive mussel *Arcuatula* (*Musculista*) *senhousia*. Subsequent studies revealed extensive populations of *P. maculata* beneath native *Perna canaliculus* mussel farms in Tasman Bay (Nelson, New Zealand). This study investigated whether *P. maculata* benefit from the trophic subsidy and/or habitat complexity provided by introduced and farmed mussels. Isotopic analysis suggested that *P. maculata* from Hauraki Gulf and Tasman Bay were most likely feeding on filter-feeding bivalves. Analysis of stomach contents using real-time PCR confirmed *A. senhousia* as a dietary source for *P. maculata* at one Hauraki Gulf site, and *Perna canaliculus* and *Mytilus edulis* (blue mussel) as dietary sources at Tasman Bay. Artificial habitat experiments in the Hauraki Gulf were confounded by a die-back of *A. senhousia* beds, but in their absence, *P. maculata* also disappeared. In Tasman Bay, *P. maculata* laid eggs on artificial mussel shell treatments beneath mussel farms, but no recruitment was recorded. Subsequent recruitment (ca. 2.7 *P. maculata* recruits per linear metre) was observed on overlying suspended mussel lines. Spatial and temporal changes in the availability of the food subsidy and habitat provided by native and invasive bivalves clearly have facilitative effects on *P. maculata* populations.

KEY WORDS: Facilitation · Invasive species · Bivalve · Ecosystem engineering · *Pleurobranchaea maculata* · *Arcuatula senhousia*

INTRODUCTION

Facilitative interactions between native communities and invasive species are more widespread than once thought (Bruno et al. 2003, Rodriguez 2006, Sellheim et al. 2010). Bivalves, in particular, have a propensity for facilitative effects within ecosystems and often become dominant habitat-formers. They achieve this via high densities, and by providing 3-dimensional habitat structure and a potential food source. These traits have seen them described as ecosystem engineers (e.g. Sousa et al. 2009, Jones et al. 2010). The role played by invasive bivalve species compared to native bivalve species in their ecosystems is complicated by a combination of antagonistic (relative vulnerability to predation and competition) and facilitative (food and habitat provision) processes (Crooks 2001). In general, invasive species that

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occupy lower trophic levels appear more likely to have significant facilitative effects on the biodiversity of higher trophic levels within native communities (Thomsen et al. 2014). However, the direct positive and negative effects of invasive and native bivalves on higher trophic levels is complex. The strength of these interactions depends on the feeding preferences of native predators (Dudas et al. 2005, Lopez et al. 2010), the physical attributes of the native and invasive bivalves (e.g. relative size and shell strength), and the level of competition, abundance, and the physiological limitations of each bivalve species (Shinen et al. 2009). Rodriguez (2006) proposed 3 models that can be adapted to describe why certain native species are facilitated by the arrival of an invasive mussel species: (1) novel facilitation, where no native mussel species previously existed; (2) substitutive facilitation, where an invasive species functionally replaces a native mussel; and (3) indirect facilitation, which occurs when an invasive species releases or reduces the predation or competition on a native mussel.

The notaspidean opisthobranch *Pleurobranchaea maculata* (grey side-gilled sea slug; Family: Pleurobranchidae) is a native New Zealand species, but is also found in southeastern Australia. Interest in *P. maculata* increased in 2009 when populations in the Auckland region (New Zealand) were found to contain significant concentrations (up to 1400 mg kg^{-1}) of the deadly neurotoxin tetrodotoxin (TTX; McNabb et al. 2010). Large numbers of *P. maculata* were washed on to popular beaches, and contact with them caused the death of 10 dogs (McNabb et al. 2010, Wood et al. 2012a,b). Surveys to find the source of beach-cast *P. maculata* identified large populations associated with extensive subtidal beds of the invasive mussel *Arcuatula senhousia* (previously *Musculista senhousia*) (Taylor et al. 2011). Subsequently, increasing numbers of *P. maculata* were also observed on the seabed beneath offshore *Perna canaliculus* farms in Tasman Bay (Clark et al. 2012), but these southern populations did not contain TTX (Wood et al. 2012b). Little is known about the ecology of *P. maculata*, despite it being found throughout New Zealand, from the shallow subtidal to depths of 300 m. *P. maculata* produces opaque tubular egg masses, which have been observed on attached and drifting brown algae, rope, shell and rocky outcrops (D. I. Taylor pers. obs.). Eggs hatch into planktotrophic veligers that last up to 21 d before settling on biofilmed surfaces and metamorphosing into a benthic-dwelling recruit stage (Gibson 2003). *P. maculata* is a carnivorous predator and has been shown to selectively prey upon and potentially affect the vertical distribution of the anemone *Anthothoe albocincta* in New Zealand (Ottaway 1977). They were once common on subtidal native mussel beds (*P. canaliculus* and blue mussel *Mytilus edulis*) that have been greatly depleted by anthropogenic disturbance in the last century (e.g. Paul 2012). Interestingly, since 2009, an outbreak of what appears to be an invasive population of toxic *P. maculata* has also been reported in Argentina (Farias et al. 2015). The community processes determining these recent increases in *P. maculata* abundance are not well described.

The invasive bivalve *A. senhousia* established in New Zealand in the late 1970s, probably through ballast water exchange (Willan 1987). It now forms large beds in both intertidal and subtidal sand and mud substrates around the Hauraki Gulf, Manukau Harbour and Whangarei Harbour. *A. senhousia*, a native of Japan, is now a global invader, with large populations established in the Adriatic Sea, Australia, Egypt, the Mediterranean Sea, Southeast Alaska, British Columbia, California and Kenya. They are a habitat-modifier (Crooks 2002a), changing habitats from sand to mud flats through the stabilisation of mud and silt caught in byssal threads, with potential to adversely affect local benthic ecology, particularly in their invaded harbours (Crooks 1998, Mistri 2003, Mistri et al. 2004, Hayward et al. 2008). Dense mats of *A. senhousia* (20–60 mm deep, with up to 28 650 mussels m^{-2}) can also smother and exclude other bivalves. For example, the New Zealand native bivalve *Paphies australis* has been shown to be negatively affected because they are unable to project their siphons through the compact byssus and consolidated muds within *A. senhousia* colonies (Willan 1987, Creese et al. 1997). In contrast, scavenging and predatory epifauna are commonly found in greater abundance on the extensive *A. senhousia* beds (Creese et al. 1997, Crooks 2002b, Mistri 2004). In southern California, for example, the gastropod *Pteropurpura festiva* has become a dominant predator of *A. senhousia* (Kushner & Hovel 2006), to the extent that it may confer invasion resistance to *A. senhousia* in that system (Reusch 1998).

The intensive aquaculture production of bivalves provides extensive habitat for invasive and native epifauna and epiflora (Woods et al. 2012). In New Zealand, *P. canaliculus* is farmed intensively in suspended culture, with >95 000 tonnes produced annually. The effects of bivalve aquaculture on surrounding communities are well described and include: influencing infaunal (Kaspar et al. 1985, Giles et al. 2009) and epifaunal community dynamics through

the deposition of pseudo-faeces, shell material and live bivalves to the seabed (Jeffs et al. 1999, Wong & O'Shea 2011), and providing novel habitat and food for scavenging species.

In this study, we used abundance surveys to quantify changes in populations of *P. maculata* on beds of *A. senhousia* at a site in the Hauraki Gulf (Auckland) and on the seabed beneath large offshore mussel farms in Tasman Bay (Nelson). We then tested 2 hypotheses: (1) that *P. maculata* consume native mussels (*P. canaliculus* and *M. edulis*), and now also benefit from the food subsidy provided by introduced mussels (*A. senhousia*, i.e. substitutive facilitation), and (2) that recruitment of *P. maculata* is increased by the habitat complexity provided by introduced mussel beds (*A. senhousia*) and the drop-off of farmed mussels (*P. canaliculus* and *M. edulis*) to the seabed. These hypotheses were tested using a combination of traditional isotopic analysis ($\delta^{15}N$ and $\delta^{13}C$), molecular analysis (real-time PCR) targeting prey-specific markers in stomach contents and artificial habitat experiments in the Hauraki Gulf and Tasman Bay.

MATERIALS AND METHODS

Population surveys

Population surveys of *Pleurobranchaea maculata* were undertaken using 2 diver transects approximately 500 m apart at Hauraki Gulf site 1 (Fig. 1A). Transects commenced on the 4–5 m depth contour, and divers swam parallel to the shore for 10 min. Divers macroscopically identified and enumerated *P. maculata.* The distance covered in each transect was determined using GPS units attached to floats above the divers, or from GPS marks taken at the start and end of each transect. Visibility was often low, and the transect width was estimated at ca. 0.5–1 m. Diver transects were undertaken in June, July, September, November and December 2010, monthly from January to April 2011, and again in July 2011.

Population surveys of *P. maculata* were also undertaken beneath a 749 ha offshore mussel (*Perna canaliculus*) farm in Tasman Bay (Fig. 1B), using replicate photo-quadrats. The abundance of *P. maculata* was estimated from 20–30 photo-quadrats (0.25 m^2) at 12 sites beneath the farm mussel lines, and at 4 reference sites outside the farm (Fig. 1B). A survey was undertaken in August 2008, before the farm was established, and 3 times following installation in November 2010, February 2011 and August 2012.

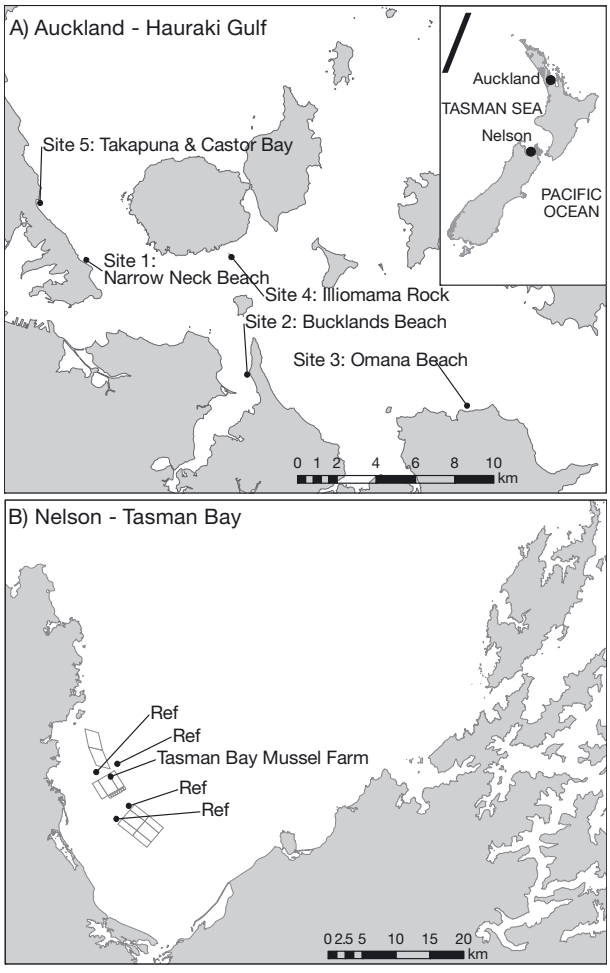

Fig. 1. Field sites in (A) Auckland–Hauraki Gulf (inset: New Zealand) and (B) Nelson–Tasman Bay (Ref = reference site)

Epifauna and *Pleurobranchaea maculata* collection and isotopic analysis

In July 2010, samples of subtidal epifauna from a range of functional feeding groups were collected from *Arcuatula senhousia* beds at Hauraki Gulf site 1 and from a nearby rocky-reef habitat. Between 2 and 5 individuals of the following species were placed in labelled plastic bags and frozen (–20°C): filter-feeders (*A. senhousia*, *Crassostrea gigas*; total n = 10), herbivores (*Evechinus chloroticus*, *Lunella smaragdus*, *Cellana radians*, *Chiton pelliserpentis*; total n = 15), detritivores (*Echinocardium australe*, *Australostichopus mollis*; total n = 8), omnivores (*Macrophthalamus hirtipes*, *Patiriella regularis*, *Pagurus* sp., *Fellaster zelandiae*; total n = 26), and predatory scavengers (*Plagusia chabrus*, *Coscinasterias calamaria*, *Cominella virgata*, *Penion sulcatus*, *Cominella adspersa*, *Cymatium spengleri*; total n = 15).

P. maculata adults (n = 16) were collected at this site on the same date.

To enable a comparison of diets among populations, *P. maculata* specimens (n = 10 per site) were collected from 5 locations around the Hauraki Gulf (sites 1 to 5, Fig. 1) and from Tasman Bay as part of abundance surveys (Wood et al. 2012b). Samples of *P. canaliculus* and *Mytilus edulis* (n = 5) were collected from Tasman Bay in July 2010.

Flesh from each animal was taken using a sterile scalpel and oven-dried overnight at 60°C. Dried samples were finely ground and subjected to carbon and nitrogen stable isotope analysis at the Waikato Stable Isotope Unit (Waikato University, New Zealand). Isotopic abundance analysis was undertaken on a fully automated Europa Scientific 20/20 isotope analyser. All nitrogen $\delta^{15}N$ samples were referenced using a urea standard that was traceable to atmospheric nitrogen ($\delta^{15}N/\delta^{14}N$) reference. This method has a precision of ±1%. All carbon ($\delta^{13}C$) samples were referenced to pre-calibrated C4 sucrose that was cross-referenced to Pee Dee belemnite ($\delta^{13}C/\delta^{12}C$ reference). This method has a precision of ±0.5%.

Molecular analysis of stomach contents

In November 2010, five *P. maculata* from each of Hauraki Gulf sites 1 and 2, and from Tasman Bay (Fig. 1), were collected by divers and placed in individual plastic bags and frozen (–20°C). Frozen *P. maculata* were dissected using a sterile scalpel and stomach contents removed and placed in sterile Eppendorf tubes (1.5 ml). Mussel samples were collected as positive controls from Hauraki Gulf site 1 (*A. senhousia*) and Tasman Bay (*P. canaliculus*, *M. edulis*). Subsamples (ca. 40 mg) of stomach contents or mussels were ground using sterile micro-pestles and DNA extracted using the Bioline ISOLATE Genomic DNA Mini Kit according to the tissue extraction protocol supplied by the manufacturer.

An aliquot of the DNA was spiked with 15 ng salmon sperm DNA as an exogenous positive control. Salmon sperm real-time PCR assays were conducted in duplicate in a final volume of 10 µl containing approximately 10 ng of DNA template, 1× Rotor-Gene™ Probe PCR Kit master-mix (Qiagen), 400 nM of each primer and 200 nM of TaqMan® minor groove binder probe (Applied Biosystems; Haugland et al. 2005). The real-time PCR assays were performed on a Rotor-Gene Q (Qiagen) using a cycling profile of 95°C for 3 min, followed by 50 cycles of 95°C for 3 s and 58°C for 10 s. If inhibition was observed, DNA samples were diluted 10-fold and the assays re-analysed. Each DNA sample was then analysed in duplicate using 3 different real-time PCR assays specific for *A. senhousia*, *P. canaliculus* and *M. edulis*. The *A. senhousia* and *P. canaliculus* real-time PCR assays were conducted using the conditions described for the salmon sperm assay. The primers and probe for both of these assays were gifted by Dr. Nathan Bott (SARDI Aquatic Sciences). The sequences of these are proprietary information and are not available for publication. The *A. senhousia* real-time PCR assays were conducted in a final volume of 20 µl containing approximately 10 ng of DNA template, 1× SYBR® GreenER™ supermix (Invitrogen), 500 nM of each primer (Dias et al. 2008; Integrated DNA Technologies) and 0.8 µg non-acetylated BSA (Sigma). The real-time PCR assays were performed on a Rotor-Gene Q using a cycling profile of 50°C for 2 min, 95°C for 10 min followed by 50 cycles of 94°C for 15 s and 60°C for 60 s.

Recruitment habitat experiments

Hauraki Gulf site 1

At 2 subtidal sites (5 m depth and 700 m apart), 5 replicates of 4 recruitment substratum treatments were deployed. Recruitment habitat treatments were (1) 0.5 m × 0.7 m hessian and stainless-steel wire doormats, intended to mimic the dense byssus mats of *A. senhousia*; (2) 0.5 m × 0.7 m *A. senhousia* shell on open substratum; (3) 0.5 m × 0.7 m of transplanted *A. senhousia* bed; and (4) 0.5 m × 0.7 m area of open substratum. The experiment was started on 11 November 2010, and *P. maculata* abundance was recorded using diver visual surveys on 26 January, 9 February, 24 March, 13 April, 13 July and 24 August 2011. No statistical analyses were performed on this experiment.

Tasman Bay mussel farm

At 2 sites (20 m depth and 500 m apart), 5 replicates of 5 substratum treatments were deployed. Recruitment habitat treatments were (1) 0.5 m × 0.5 m concrete tiles with clumps of *P. canaliculus* shell attached by glue (tiles and shell); (2) clumps of *P. canaliculus* shell on 0.5 m × 0.5 m seabed (shell); (3) live mussel clumps on 0.5 m × 0.5 m seabed (mussel clumps); (4) 0.5 m × 0.5 m concrete tiles (tiles); and (5) 0.5 m × 0.5 m of open substratum (seabed). The

experiment was started on 20 February 2011, and *P. maculata* adult abundance, egg-laying and recruitment was recorded on 24 April 2011, 5 July 2011, 30 August 2011, 19 January 2012 and 8 February 2012. Likelihood ratio testing, based on data-driven maximum likelihood parameterisation of Poisson distributions, was undertaken to compare the mean abundance of adults and egg sacs across sites and the different treatments using R (R Core Team 2015). In January 2012, *P. maculata* recruitment onto mussel lines was quantified by counting animals from 5 randomly chosen 1 m lengths of mussel dropper line within the Tasman Bay farm.

RESULTS

Population surveys

Hauraki Gulf site 1

Populations of *Pleurobranchaea maculata* at Hauraki Gulf site 1 reached a peak abundance (±1 SE) of 0.74 ± 0.15 ind. m^{-2} in early September 2010 (Fig. 2). Die-off of *Arcuatula senhousia* beds began between September and October 2010. By November 2010, the percentage cover of *A. senhousia* had reduced by 75%. A large swathe, approximately 1 m wide and 100 m long, of starfish (*Coscinasterias* sp.) and whelks (*Cominella* sp.) were observed feeding on the *A. senhousia* beds as they died back. By January 2011, only mounds of fine sediment and empty *A. senhousia* shell remained. Populations of *P. maculata* declined rapidly, and after December 2010, no *P. maculata* were recorded at the site (Fig. 2).

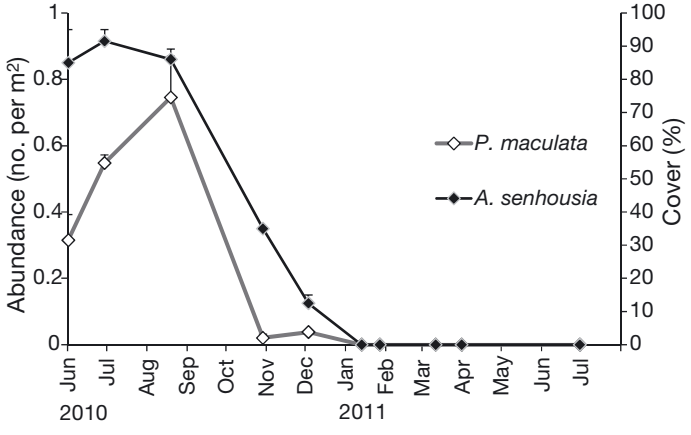

Fig. 2. Average abundance (+1 SE) of *Pleurobranchaea maculata* idividuals and percentage cover (+1 SE) of *Arcuatula senhousia* beds at 2 dive sites (5 m depth, 500 m apart) at Hauraki Gulf site 1 from June 2010 to July 2011

Tasman Bay mussel farm

P. maculata were not observed at any of the 4 reference sites. A single *P. maculata* was recorded in an extensive pre-farm survey of 12 sites within the 749 ha Tasman Bay mussel farm in October 2008 (Fig. 3). By November 2010, the abundance (±1 SE) of *P. maculata* on the seabed beneath the farm averaged 0.5 ± 0.18 ind. m^{-2}. No *P. maculata* were observed in February 2011, but abundance increased to 0.2 ± 0.03 ind. m^{-2} in August 2012 (Fig. 3).

Isotopic analysis of *Pleurobranchaea maculata* and other epifauna

The $\delta^{13}C$ and $\delta^{15}N$ values for *P. maculata* collected during the Hauraki Gulf site 1 survey ranged from −16.43 to −19.65 and 12.86 to 16.03, respectively (Fig. 4), and were relatively similar to that of predatory scavengers (Fig. 4). Omnivores had the most variability in their $\delta^{13}C$ signal, and filter-feeders the lowest values (Fig. 4). The $\delta^{15}N$ for *P. maculata* from various locations in the Hauraki Gulf ranged from 13.64 to 15.4 and for Tasman Bay was slightly lower at 13.11 (Fig. 5). $\delta^{13}C$ values for the Hauraki Gulf ranged from −17.21 to −18.77, and were similar at Tasman Bay (−18.60).

Molecular analysis of stomach contents

All stomach contents from *P. maculata* collected from underneath the Tasman Bay mussel farm were positive with the *Perna canaliculus* real-time PCR

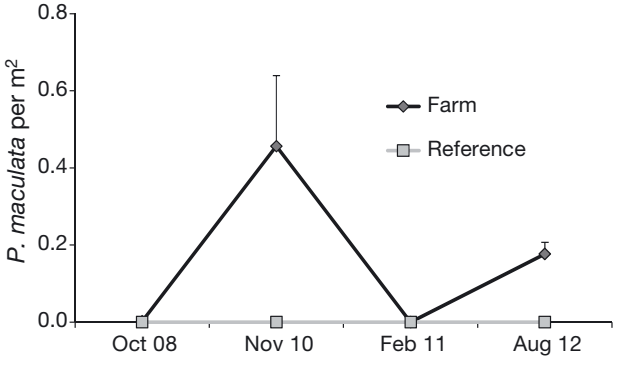

Fig. 3. Average abundance per m^2 (+1 SE) of *Pleurobranchaea maculata* from 20 drop camera images taken from beneath each of 2 Tasman Bay mussel farm sites (Farm) and at each of 4 reference sites (Reference) in October 2008 (pre-farm installation), November 2010, February 2011 and August 2012 (post-farm installation)

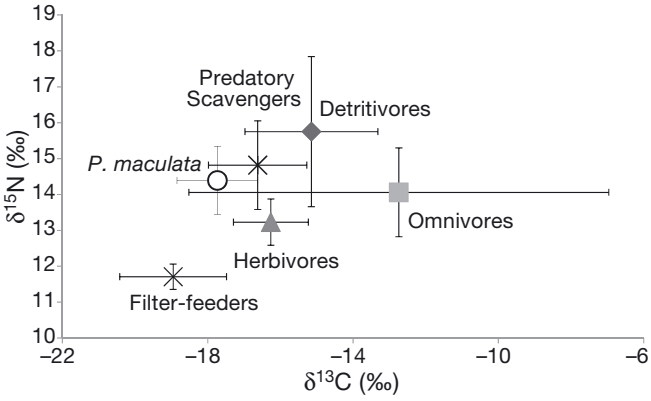

Fig. 4. Average (±1 SD) δ^{13} C and δ^{15}N isotope composition of *Pleurobranchaea maculata* (n = 16) from the Hauraki Gulf area, and filter-feeders (n = 10; 2 spp.), herbivores (n = 15; 4 spp.), detritivores (n = 8; 2 spp.), omnivores (n = 26; 4 spp.) and predatory scavengers (n = 15; 6 spp.) collected from reef habitat and *Arcuatula senhousia* beds at Haurkai Gulf site 1

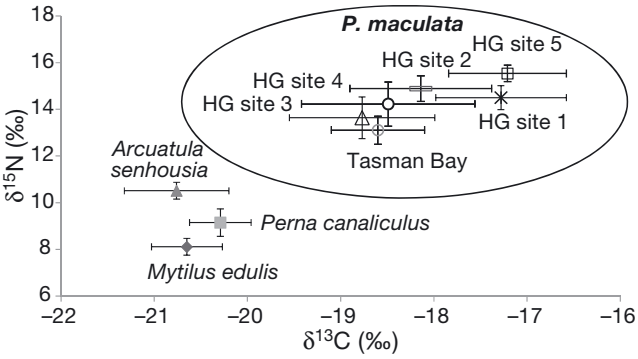

Fig. 5. Average (±1 SD) δ^{13}C and δ^{15}N isotope composition of *Arcuatula senhousia* (Hauraki Gulf site 1), *Perna canaliculus* and *Mytilus edulis* (Tasman Bay), and *Pleurobranchaea maculata* from 6 locations (Tasman Bay and 5 Hauraki Gulf [HG] sites)

assay (Table 1). All samples from Hauraki Gulf sites 1 and 2 were negative for *P. canaliculus* and *Mytilus edulis* (Table 1). Four of the 5 samples from Tasman Bay were tested positive using the *M. edulis* real-time PCR assay, whereas the samples from Hauraki Gulf site 1 and 2 were negative. Four out of 5 samples from Hauraki Gulf site 1 were positive with the *A. senhousia* real-time PCR assay, but all other samples from Hauraki Gulf site 2 and Tasman Bay were negative.

Recruitment habitat experiments

No recruitment of *P. maculata* was recorded on artificial or natural habitat treatments at Hauraki Gulf site 1. The subtidal beds of *A. senhousia* at Hauraki Gulf site 1 died off soon after the recruitment experiment began in November 2010 (see 'Population surveys'), and no adult *P. maculata* or egg sacs were recorded on treatments during the experiment, or across the site after December 2010.

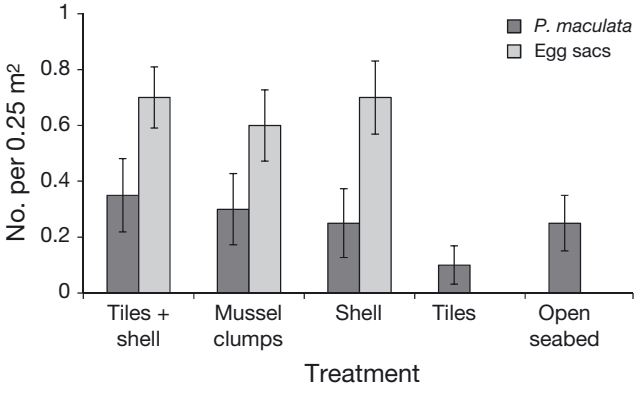

Fig. 6. *Pleurobranchaea maculata* adult and egg-sac abundance per 0.25 m^2 (±1 SE) across all experimental treatments in Tasman Bay. No recruitment, egg-laying or adults were observed in experimental treatments at Hauraki Gulf site 1

In Tasman Bay, beneath the mussel farms, adult *P. maculata* and their egg sacs were recorded in approximately equal abundance across all experimental treatments constructed of mussel shells in July and August 2011 (tiles and shells, shells, and mussel clumps; Fig. 6). However, only adult *P. maculata* was observed on the bare tiles and seabed. No *P. maculata* or egg sacs were recorded in April 2011, or January and February 2012. Likelihood ratio testing suggested no significant difference in *P. maculata* abundance data by site or treatment (p = 0.61). However, the fit to the egg sac distribution data was significantly improved (p = 0.013) by some

Table 1. Number of positive real-time PCR results from stomach contents of *Pleurobranchaea maculata* (n = 5) from the seabed beneath an offshore *Perna canaliculus* farm in Tasman Bay, and subtidal *Arcuatula senhousia* beds at Hauraki Gulf sites 1 and 2. Each sample was analysed in duplicate for the presence of *P. canaliculus*, *Mytilus edulis* and *A. senhousia*. In all cases, the duplicate results were identical

Location	*P. canaliculus*	*M. edulis*	*A. senhousia*
P. canaliculus farm seabed			
Tasman Bay	5	4	0
A. senhousia beds			
Hauraki Gulf site 1	0	0	4
Hauraki Gulf site 2	0	0	0

treatment combinations. Further inspection showed the bare and tile treatments were the source of this difference, because *P. maculata* egg sacs were only found on treatments that included mussel shell. This pattern of egg-laying on only mussel shell was repeated at both experimental sites. While no recruitment was recorded on the experimental treatments over the duration of the experiment, recruitment of *P. maculata* was observed on mussel lines suspended above the experiments. An average (±1 SE) of 2.6 ± 0.7 individual juvenile *P. maculata* per linear metre of mussel line was recorded from 5 replicate 1 m lengths in January 2012.

DISCUSSION

This study demonstrates that both invasive and native mussel habitats facilitate the abundance of *Pleurobranchaea maculata* in New Zealand. The process of facilitation for this native epifaunal species was similar across invasive and native mussel habitats, but was complicated by temporal changes in mussel habitat abundance and depended on the life stage of *P. maculata*. This study showed that the presence of beds of the invasive mussel *Arcuatula senhousia* provides adult *P. maculata* populations with a food subsidy. We were unable to determine if recruitment of *P. maculata* was facilitated by the dense byssus mats of *A. senhousia*. However, the lack of *P. maculata* recruitment in the years following the disappearance of *A. senhousia* beds at Hauraki Gulf site 1 suggests that increasing *P. maculata* abundance may result from the substitutive facilitation mechanism proposed by Rodriguez (2006), whereby *A. senhousia* beds substitute for native mussel beds. Recruitment of *P. maculata* was also facilitated by suspended cultures of native *Perna canaliculus* on large offshore mussel farms in Tasman Bay, through the provision of recruitment habitat. Adult *P. maculata* were most abundant on the seafloor, but their abundance varied between sampling times. It appears that adult *P. maculata* fall to the seafloor from the mussel farms either naturally due to wave action or during farm operations. The results of the recruitment experiment also suggest they utilise the 3-dimensional habitat provided by the dropped mussels as habitat for egg-laying. Dietary analysis suggests that adult *P. maculata* on the seafloor also benefit from the food subsidy provided by the drop-off of mussels (*P. canaliculus* and *Mytilus edulis*) from the farm lines.

The global invader *A. senhousia* has had considerable effects on the communities it has entered and has been described as an ecosystem engineer (Crooks 1998, Mistri 2003, Rodriguez 2006). Extensive and dense beds of *A. senhousia*, often with >3300 ind. m^{-2} (Willan 1987, Crooks 1996), provide a modified substratum topography and change the transport dynamics and organic matter content of sediments (Crooks & Khim 1999, Sousa et al. 2009). Species inhibited by this invader often include native bivalve populations that are smothered by, or fail to recruit onto, the sediments that accumulate around the web of byssus bags that typify *A. senhousia* mats. Such inhibitive effects have been reported for scallops *Pecten novazealandiae* and pipi *Paphies australis* in New Zealand (Willan 1987, Creese et al. 1997), epifaunal suspension-feeding taxa (e.g. *Hydroides dianthus* and *Ficopomatus enigmaticus*) in Italy (Mistri 2003), and razor clams *Solen rostriformis* in southern California (Crooks 2001). Facilitative effects of *A. senhousia* invasions include positive effects on diversity and density of some macrofaunal species (Crooks 1998, 2002a, Mistri 2003), and increased leaf growth rates in native seagrasses due to sediment enrichment (Reusch 1998, Reusch & Williams 1999). There is also evidence of positive trophic subsidy for native faunal species. For example, Crooks (2002b) provided evidence that native predatory fauna such as birds and fish feed upon and could be responsible for changes in the seasonal abundance of intertidal populations of *A. senhousia* in Mission Bay, San Diego. The real-time PCR methods used in our study clearly showed that *A. senhousia* were consumed by *P. maculata* from Hauraki Gulf site 1. The gut contents of *P. maculata* from Hauraki Gulf site 2 were, however, negative for all mussels tested. This may be due to differences in the abundance of mussels at each site. Hauraki Gulf site 2 is situated at the mouth of a tidal estuary and only sparse patches of mussels were observed. In contrast, at their peak, *A. senhousia* beds at Hauraki Gulf site 1 covered nearly the entire seafloor over ca. 10 ha. Additionally, the real-time PCR method only represents a snapshot of the *P. maculata* diet, and it is plausible that these individuals consumed *A. senhousia* previously and this was not detected with the assay.

Trophic fractionation for $\delta^{13}C$ generally results in enrichment in the order of 0–1‰ per trophic level, although this can be variable (ranging up to about 3‰), depending on factors associated with an organism's physiology, the tissues sampled, and extent and range of variation in food sources. Greater fractiona-

tion occurs in nitrogen than in carbon, with trophic enrichment of roughly 3‰ (but ranging from 2–5‰) common in a consumer compared to its food source (Vander Zanden & Rasmussen 2001). Based on analysis of a range of organisms in the present study, *P. maculata* across all sites is most likely feeding on filter-feeding bivalves such as oysters and mussels. This was clearest in Tasman Bay, where the signature of *P. maculata* for $\delta^{15}N$ and $\delta^{13}C$ is enriched by 3.9 and 1.7‰, respectively, compared to *P. canaliculus*, which are the most abundant shellfish species in Tasman Bay. The level of enrichment for the data from the Hauraki Gulf sites also suggests that *P. maculata* in this region are also relying primarily on filter-feeding bivalves as a food source, rather than other organisms such as scavengers, herbivores or detritivores. A specific comparison between the abundant *A. senhousia* in comparison to *P. maculata* shows that the level of enrichment for $\delta^{15}N$ is what would be expected if they are a food source (e.g. enrichment in the order of 4‰); however, the enrichment for carbon is higher than typically expected for 1 trophic level at 3.5‰. A possible explanation for this is that *P. maculata* prey on each other. This has been seen numerous times in the laboratory, particularly when *P. maculata* were stocked at a density >2 in aquariums (19 l; Wood et al. 2012a). This cannibalism has also been used to explain how extremely high levels of TTX can accumulate in this species (L. R. Salvitti unpubl.).

The dense monocultures of mussels in the suspended culture provide extensive settlement and recruitment habitat for a diverse range of native and invasive fauna and flora in an otherwise depauperate, mud-dominated habitat (Forrest et al. 2009, McKindsey et al. 2011). In a recent study, during 6 mo of installation of mussel lines, 54% of the biomass on experimental mussel farm lines was biofouling, consisting of 71 taxa (Woods et al. 2012). Our observations of *P. maculata* recruitment into the extensive habitat provided by cultured mussel lines, while never before reported, are not surprising. *P. maculata* larvae are planktonic for up to 21 d (Gibson 2003), and it appears that larvae hatching from egg sacs laid by benthic-dwelling adults recruit almost exclusively to the mussel lines above, as no juvenile *P. maculata* were observed on experimental treatments beneath farms or during other benthic surveys. Several studies have described positive effects for benthic communities beneath mussels farmed in suspended culture. For example, Kaspar et al. (1985) found that the drop-off from mussel farms not only seemed to provide food for a variety of fish, starfish and whelk species, but also allowed for the development of a diverse community of tunicates, polychaetes and sponges that would otherwise only be found on rocky reefs. We found *P. maculata* that fall to the seafloor benefit both from a food subsidy and from the 3-dimensional egg-laying habitat afforded by mussels and shell material that is deposited from the lines above. Egg sacs were only observed on 3-dimensional structures, including our experimental treatments, beneath the farms and did not occur on surrounding muddy sediments.

Populations of *P. maculata* in New Zealand have likely undergone significant change over the last 100 yr. Nearshore marine habitats, such as subtidal *P. canaliculus* beds, were greatly reduced by dredging in the Hauraki Gulf and Tasman Bay in the middle of last century (Handley 2006). The influx of land-derived sedimentation from deforestation and poor land management has increased coastal sedimentation rates by an order of magnitude (Swales et al. 2010), and also continues to smother coastal marine habitats (Schiel et al. 2006, Gibbs 2008). Although likely to have been historically abundant on these coastal habitats, no *P. maculata* were seen in any of the bay-wide benthic surveys completed prior to installation of the large offshore farm in Tasman Bay (Clark et al. 2012). The substitutive facilitation process afforded by invasive (*A. senhousia*) and cultured native mussel (*P. canaliculus*) habitats, as postulated by Rodriguez (2006), however, appears to be substituting *P. maculata* habitats that have been lost to anthropogenic destruction.

The facilitative processes described here have had positive outcomes for *P. maculata* in some parts of New Zealand, but the consequences for humans and their pets in the Hauraki Gulf are less desirable. Large populations of highly toxic *P. maculata* establishing on subtidal beds of *A. senhousia* brings them into close proximity of beaches, where they can become beach-cast. The social, environmental and ecological effects of *A. senhousia* beds on *P. maculata* populations are, however, short-lived, as subtidal beds can die off every 2–3 yr (as observed at our study sites), and intertidal beds are reduced when extreme low tides coincide with hot weather, causing desiccation (Willan 1987, Mistri 2004). With over 7500 ha of offshore mussel farms proposed around the coast of New Zealand, populations of toxic *P. maculata* are likely to increase in some locations. This problem may not be restricted to New Zealand, since 2009 an outbreak of an as-yet unidentified species of *Pleurobranchaea* has been documented in the port of Mar del Plata, Argentina. The new Argentin-

ian pleurobranchid, which is assumed to be a recent invader of the region, closely resembles *P. maculata* and was also found to contain deadly neurotoxins (Farias et al. 2015). With a potentially growing international distribution, the facilitation of *P. maculata* populations by native and invasive bivalves, which can lead to large increases in their abundance, may require close monitoring to ensure toxicity risks to people in nearby coastal communities are not increased.

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