Hitchhiking in the East Australian Current: rafting as a dispersal mechanism for harmful epibenthic dinoflagellates

Michaela E. Larsson[1],*, Olivier F. Laczka[1], Iain M. Suthers[2], Penelope A. Ajani[1], Martina A. Doblin[1]

[1]**Climate Change Cluster, University of Technology Sydney, PO Box 123 Broadway, Sydney, NSW 2007, Australia**
[2]**Fisheries and Marine Environmental Research Facility, School of Biological, Earth and Environmental Sciences, University of New South Wales, Sydney, NSW 2052, Australia**

ABSTRACT: Due to their small size, planktonic marine microorganisms have large dispersal capacity in the global ocean. However, it is not known how epibenthic microalgae disperse across long distances because they are generally associated with a substrate. In this study, we examined a long-term data series (~50 yr) of microalgal composition from a coastal station in southeast Australia for the presence of epibenthic dinoflagellates in the plankton. In addition, we collected drifting macrophytes (i.e. macroalgae and seagrass) and plastic debris from the East Australian Current, identified the associated microalgal assemblage, assessed their viability, and used phylogenetic analyses to taxonomically identify cryptic harmful epibenthic dinoflagellate species. We found no occurrences of epibenthic dinoflagellates from the genera *Gambierdiscus*, *Fukuyoa*, *Ostreopsis*, and *Coolia* at the long-term coastal station, concluding that entrainment of cells in ocean currents is an unlikely mechanism for transport of these taxa. The epibenthic microalgal communities associated with macrophyte rafts and plastic debris were primarily comprised of diatom taxa. However, intact cells of potentially harmful epibenthic dinoflagellates from the genera *Coolia, Amphidinium*, and *Prorocentrum* were also observed, and their viability was confirmed by division of isolated cells and establishment into clonal cultures. Phylogenetic analyses confirmed the presence of *C. palmyrensis* on a drifting *Sargassum* sp. raft, the first report of this potentially harmful epibenthic species in temperate Australian waters. This study shows that epibenthic dinoflagellates can attach to, and remain viable, when associated with macrophyte fragments that drift in the open ocean, therefore revealing rafting as a potential vector for dispersal of these organisms.

KEY WORDS: *Coolia* · Drifting algae · East Australian Current · EAC · Epibenthic · Macrophyte · Rafting · Seagrass

INTRODUCTION

Dinoflagellates are a frequent part of microalgal communities in the photic and benthic zones of the world's coastal waters and oceans. A range of planktonic species, known as 'harmful' dinoflagellates, can produce potent toxins that accumulate in the food web (e.g. filter-feeding shellfish) and, when consumed by humans, can cause food-related illnesses. (Van Dolah 2000) These include paralytic shellfish poisoning (Wiese et al. 2010), diarrhetic shellfish poisoning (Murata et al. 1982), neurotoxic shellfish poisoning (Baden & Adams 2000), amnesic shellfish poisoning (Jeffery et al. 2004), and azaspiracid shellfish poisoning (Satake et al. 1998). Epibenthic dinoflagellates live in close association with the benthos or substrates

*Corresponding author:
michaela.e.larsson@student.uts.edu.au

such as macrophytes. These organisms have also been implicated in several human-related illnesses, ranging from respiratory (Durando et al. 2007) and dermatologic (Tubaro et al. 2011) conditions through to ciguatera fish poisoning (Yasumoto et al. 1979). Toxins produced by epibenthic dinoflagellates are introduced to marine food webs by fish and invertebrates grazing on colonised macrophyte substrates, or through filter feeding (Lewis & Holmes 1993, Yasumoto 2005, Cruz-Rivera & Villareal 2006).

Over the past decade, there has been considerable research focus on understanding the taxonomy of key epibenthic dinoflagellate genera including *Gambierdiscus* Adachi & Fukuyo (Adachi & Fukuyo 1979), *Fukuyoa* Gómez, Qui, Lopes & Lin (Gómez et al. 2015), *Ostreopsis* Schmidt (Schmidt 1901), *Coolia* Meunier (Meunier 1919), *Prorocentrum* Ehrenberg (Ehrenberg 1834), and *Amphidinium* Claparède & Lachmann (Claparède & Lachmann 1859). Each can easily be identified to genus level based exclusively on cell morphology using a light microscope; however, morphological similarities between species within some genera (e.g. *Gambierdiscus*, *Fukuyoa*, *Ostreopsis*, and *Coolia*) necessitates phylogenetic analyses to resolve identification to species level (Litaker et al. 2009, Karafas et al. 2015, Accoroni et al. 2016). More recently, epibenthic dinoflagellate research has shifted to ecological studies aimed at understanding current species distributions and potential range expansions under predicted climate change conditions (e.g. Bomber et al. 1988a, Morton et al. 1992, Kibler et al. 2012, Xu et al. 2016, Sparrow et al. 2017). As a result, there is growing evidence that harmful epibenthic dinoflagellates are expanding their range from tropical to more temperate locations in both the northern and southern hemispheres (Aligizaki et al. 2008, Kohli et al. 2014). However, the natural and human-assisted vectors facilitating this range expansion remain relatively unexplored.

Marine microorganisms have the potential to be dispersed across long distances suspended in ocean currents (McManus & Woodson 2012). It is possible that harmful epibenthic dinoflagellates could also be dispersed in this way; however, their association with a substrate makes planktonic drift of free-living cells a less likely mechanism of transport. A more likely method is 'rafting'. This involves organisms being transported while attached to floating materials and is already a recognised long-distance dispersal mechanism for many sentinel marine organisms (Thiel & Gutow 2005a,b). These floating materials, or rafts, can have a biotic origin (e.g. macrophytes) or an abiotic origin (e.g. litter and plastics) (Thiel & Gutow

2005a,b), and both have the potential to disperse harmful epibenthic dinoflagellates (Besada et al. 1982, Bomber et al. 1988b, Masó et al. 2003, 2016). Rafting as the most likely dispersal mechanism for epibenthic dinoflagellates was first proposed by Besada et al. (1982) following observations of the poor swimming ability of cells and their nonplanktonic habit. This hypothesis was given support by epibenthic dinoflagellates documented on drifting fragments of macrophytes collected in the Florida Straits (Bomber et al. 1988b), and marine plastic debris collected along the Catalan coast in the northwestern Mediterranean (Masó et al. 2003, 2016). However, whether epibenthic dinoflagellates could remain associated with, and be transported by, rafts in open-ocean environments is not known.

The East Australian Current (EAC) is the western boundary current of the South Pacific Ocean, originating in the tropical Coral Sea and flowing southward along the edge of the eastern Australian continental shelf (Ridgway 2007). Marine organisms such as reef fish are frequently transported from tropical to temperate latitudes in eastern Australia via the EAC (Booth et al. 2007). Like other western boundary currents in the global ocean (Wu et al. 2012), the EAC is increasing its poleward extension as the climate changes (Suthers et al. 2011, Yang et al. 2016), transporting more tropical water into temperate latitudes. Range expansions facilitated by this strengthening of the EAC have already been reported for a number of marine organisms including coastal fish (Last et al. 2011), invertebrates (Banks et al. 2007, Ling et al. 2009), zooplankton (Johnson et al. 2011), and kelp (Coleman et al. 2011) and could also aid range expansions of epibenthic dinoflagellates. Therefore, we investigated the potential of the EAC to facilitate long-distance dispersal of epibenthic dinoflagellates, both suspended in the plankton and via rafting. The influence of the EAC on the temperate southeast Australian pelagic ecosystem has been monitored at a historic time-series station, Port Hacking (PH100), for over 70 yr (Thompson et al. 2009). PH100 is located ~5 km from the coast at a depth of 100 m (Fig. 1a), and is routinely sampled for hydrographic and biological parameters, including planktonic microalgal diversity and abundance (http://imos.org.au). Given that the fraction of EAC water at PH100 increased during the decade 1997–2007 (Thompson et al. 2009), this station provides a relevant point of reference to assess the abundance of epibenthic dinoflagellates in the water column versus their abundance on rafts.

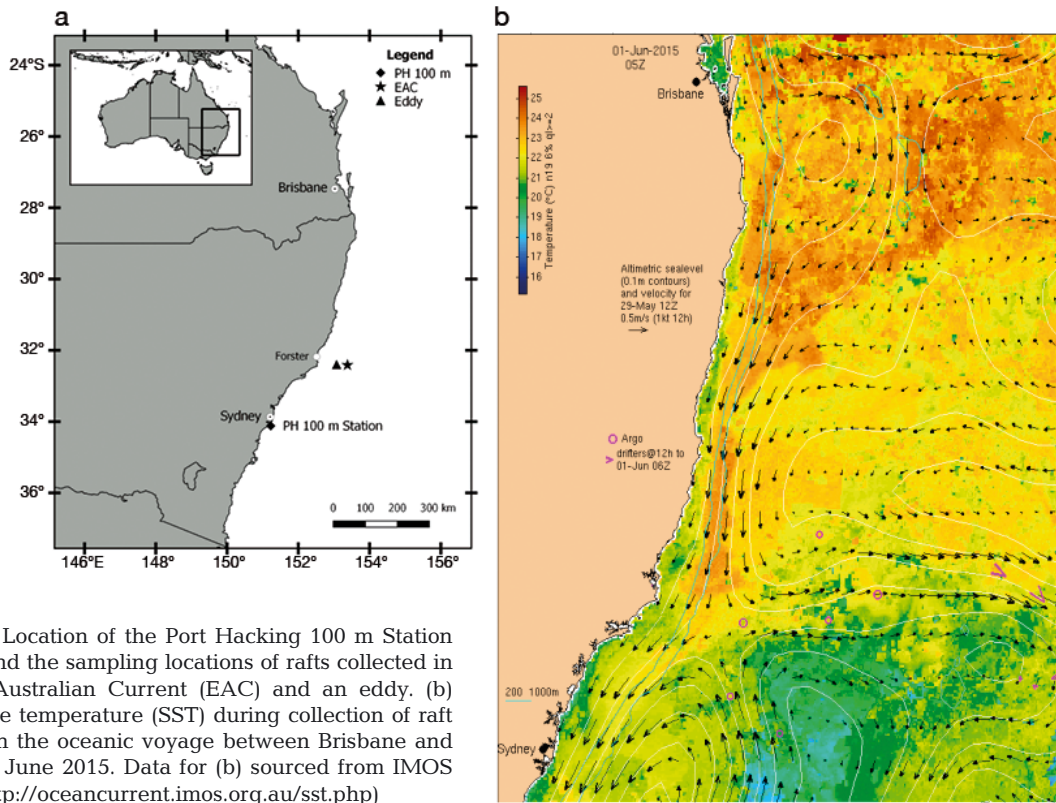In this study, our objective was to evaluate rafting as a natural dispersal vector for epibenthic dinofla-

Fig. 1. (a) Location of the Port Hacking 100 m Station (PH100) and the sampling locations of rafts collected in the East Australian Current (EAC) and an eddy. (b) Sea surface temperature (SST) during collection of raft samples on the oceanic voyage between Brisbane and Sydney in June 2015. Data for (b) sourced from IMOS (http://oceancurrent.imos.org.au/sst.php)

gellates. We sampled rafts of biotic and abiotic origin during an oceanographic voyage and quantified their capacity to transport viable cells of epibenthic dinoflagellate species. We then assessed this against the backdrop of epibenthic dinoflagellate diversity and abundance detected in the plankton at the PH100 station.

MATERIALS AND METHODS

Presence of epibenthic dinoflagellates in the PH100 time series (1965−2013)

PH100 is a long-term observing station located on the continental shelf of southeast Australia (34.120° S, 151.224° E; Fig. 1a). This station is regularly influenced by the EAC and can therefore be used to investigate microalgal assemblages and transport in this dynamic western boundary current system (Thompson et al. 2009, Ajani et al. 2014a,b). The time series used in this study was a combination of datasets from the Port Hacking National Reference Station (1965−2009) and the Integrated Marine Observing System (IMOS) National Reference Station (NRS) Network (same location, 2009−2013), and was combined and curated as described by Ajani et al. (2016). The 5

datasets included weekly sampling between April 1965 and April 1966 (Grant & Kerr 1970); weekly sampling between April 1978 and April 1979 (Hallegraeff 1981); weekly sampling between April 1997 and April 1998 (Ajani et al. 2001); monthly sampling between September 1998 and December 2009 (Ajani et al. 2014a,b); and monthly sampling between February 2009 and December 2013 (imos.aodn.org.au). For a detailed description of the sample collection methodology, see Ajani et al. (2016). Briefly, samples were collected either as discrete bottle samples from 0−50 m (Grant & Kerr 1970, Hallegraeff 1981, Hallegraeff & Reid 1986) or by 50 or 100 m vertical hauls with a 37 or 20 µm mesh net (Ajani et al. 2001, 2014a,b). Samples were preserved, and microscopic examination was performed to identify and enumerate microalgal taxa.

We searched this extensive time series, consisting of 267 individual samples, for occurrences of epibenthic dinoflagellate cells from the genera *Amphidinium*, *Ostreopsis*, *Coolia*, *Prorocentrum*, *Gambierdiscus*, and *Fukuyoa*. Their presence in the plankton at PH100 would suggest that cells had either been advected to the site from local shallow-water habitats after being suspended from the benthos or transported from more distant sites via the plankton. As the genus *Prorocentrum* comprises some species

which are pelagic and others which are epibenthic, only species which were described as benthic/epibenthic by Hoppenrath et al. (2014) or Hallegraeff et al. (2010) (e.g. *P. lima*, *P. rhathymum*, and *P. clipeus*), or were not defined as being either benthic/epibenthic or pelagic (*Prorocentrum* spp.), were included in the analyses. The percent occurrence rate of benthic dinoflagellates was calculated by dividing the number of samples where the genus occurred by the total number of samples in the time series and multiplying by 100.

Presence of epibenthic dinoflagellates on ocean rafts

Raft collection and sample processing

Drifting macrophyte and plastic debris were collected in horizontally towed 1 m^2 Neuston nets and, occasionally, from a 1 m^2 towed multiple opening and closing net in the upper 100 m, at sites within the EAC and associated oceanographic features (e.g. mesoscale eddies) during a voyage (IN2015_V03) from Brisbane to Sydney on the RV 'Investigator' in June 2015 (Fig. 1b). Floating debris in a single horizontal net tow, sometimes comprising multiple macrophyte species, was combined into a single sample and recorded as a single raft. Rafts were then processed by being placed into a plastic sealable bag with a volume (150–500 ml) of 0.2 µm filtered seawater. Epiphytes were vigorously shaken from the surface of the rafts for approximately 1 min and poured through a 120 µm sieve into a wide-mouth jar to remove large zooplankton. A 15 ml subsample of the suspended organisms was preserved with glutaraldehyde to a final concentration of 1% (v/v). Macrophyte raft samples were oven-dried for preservation. The remaining suspension was incubated at 20°C, under ~50 µmol photons m^{-2} s^{-1} light until further processing.

To evaluate the relationship between the type and size of a raft and associated microalgae abundance and diversity, raft samples were graded with a size and complexity score to consider differences in surface area and texture. Each sample was given a score ranging from 1 to 4, describing the raft size (1 = very small [<5 cm^2], through to 4 = large [>20 cm^2]), and a score describing raft complexity (1 = single species with flat blades, through to 4 = complex blade structure such as *Sargassum* sp.). Size and complexity scores for individual rafts were then combined to get a total raft index score.

Identification and enumeration of microalgal community associated with rafts

Glutaraldehyde-preserved samples were concentrated from 15 to 2 ml by sedimentation for 48 h, and microalgal taxa were identified to the lowest possible taxonomic level using a Sedgewick Rafter counting chamber under an inverted light microscope (maximum magnification 1000×) fitted with phase contrast (Nikon Eclipse TS100). Cells were counted to a threshold of 100 and the entire chamber scanned for the presence of rare taxa. The number of cells raft^{-1} was calculated from the initial volume of filtered seawater added and broadly categorised into functional groups (i.e. diatoms, dinoflagellates, other). Taxa were determined to be benthic/epibenthic based on classification by Hoppenrath et al. (2014) and Hallegraeff et al. (2010). Differences among the epibenthic microalgal assemblage associated with raft samples were statistically analysed using multi-dimensional scaling (MDS) in Primer v6.1.16 (Clarke & Warwick 2001), and significance was tested using ANOSIM (p < 0.01).

To examine whether epibenthic dinoflagellate cells associated with rafts were viable, single cells were isolated from a *Sargassum* sp. raft sample using the micropipette technique (Andersen & Kawachi 2005) under an inverted light microscope (Nikon Eclipse TS100). Established monoclonal cultures were maintained in modified K medium (Litaker et al. 2009) made from sterile aged natural seawater at a salinity of 32 ppt at 24°C, under ~100 µmol photons m^{-2} s^{-1} on a 12:12 h light:dark cycle.

Phylogenetic analysis of cryptic harmful epibenthic dinoflagellate species associated with a macroalgal raft

Where possible, monoclonal isolates were identified by light microscopy using an inverted light microscope (Nikon Eclipse TS100); however, phylogenetic analysis was conducted to verify the identity of an established strain of *Coolia* due to strong morphological similarities amongst species within the genus. Cells from 100 ml of the *Coolia* culture (UTSR7) were pelleted by centrifugation at 1000 × *g* (10 min), and DNA was extracted using a PowerSoil DNA Extraction Kit (Mo Bio), following the manufacturer's instructions. Extracted DNA was sent to a commercial service (Australian Genomic Research Facility, Queensland, Australia) where the D1–D3 region of the large subunit (LSU) rDNA was amplified using

primers D1R-F (Scholin et al. 1994) and D3-R (Nunn et al. 1996), following the conditions described by Rhodes et al. (2014). Amplification products (~950 bp) were purified and sequenced in both directions.

Phylogenetic analyses were conducted in Geneious v9.1.5 (Kearse et al. 2012). Publicly available sequences of *Coolia* spp. and *Ostreopsis* spp. were downloaded from GenBank (www.ncbi.nlm.nih.gov) and aligned with the sequences obtained from this study using the MUSCLE algorithm (maximum number of iterations = 8) (Edgar 2004), with *Ostreopsis* spp. sequences used as outgroups. Sequences were truncated to 756 bp, and a maximum likelihood phylogenetic tree was generated using PHYML with 1000 bootstraps (Guindon & Gascuel 2003) using a GTR substitution model and an estimated gamma distribution. Bayesian analyses were performed using MrBayes 3.2.6 (Huelsenbeck & Ronquist 2001) by means of the GTR+G (general-time reversible with gamma-shaped among-site variation) model using 4 simultaneous runs with 4 chains each for 3.1×10^6 generations, sampling every 1000 trees, and the first 1000 trees were discarded as burn-in.

RESULTS

Presence of epibenthic dinoflagellates in the PH100 time series (1965–2013)

There were no occurrences of epibenthic dinoflagellates from the genera *Gambierdiscus*, *Fukuyoa*, *Ostreopsis*, or *Coolia* in the PH100 microalgal time series; however, *Amphidinium* and *Prorocentrum,* which include both pelagic and epibenthic species, were detected. *Amphidinium* spp. occurred in 17 of 267 (6%) samples and *Prorocentrum* spp. occurred in 78 of 267 (30%) samples. Exclusively epibenthic *Prorocentrum* taxa (e.g. *P. lima*) occurred in 3 of 267 samples (1%).

Presence of epibenthic dinoflagellates on ocean rafts

Fifteen raft samples were collected from the oceanographic region influenced by the EAC and associated ocean water masses off the continental shelf of New South Wales, Australia (i.e. >200 m isobath). Thirteen of the fifteen rafts were collected in an eddy and the remaining 2 were collected in the EAC (see Table S1 in the Supplement at www.int-res.com/articles/suppl/m596p049_supp.pdf). Twelve

samples were macrophytes, including seagrasses (*Posidonia* spp. and *Zostera* spp.) and macroalgae (*Hormosira* spp. and *Sargassum* spp.). We also found 1 sample of pumice, a terrestrial plant fragment (small tree branch), and a sheet of plastic.

The composition and abundance of microalgal assemblages associated with rafts of seagrass, macroalgae, and the plastic sheet were very similar, but distinct from the assemblages present on the pumice and small tree branch (ANOSIM, p < 0.01; Fig. 2). The top 6 taxa driving these differences included the diatoms *Grammatophora marina*, *Licmorpha* cf. *abbreviata*, *Navicula* spp., *Plagiotropis* sp., *Leptocylindrus* sp., and the dinoflagellate *Amphidinium* spp.

Raft size and complexity were positively correlated with both the abundance ($R^2 = 0.27$) and diversity ($R^2 = 0.38$) of associated microalgal taxa, with larger and more complex rafts (of any type) harbouring more abundant and diverse microalgal assemblages (Fig. 3a,b).

Overall, the microalgal assemblage associated with rafts was dominated by diatoms (Fig. 4a), with many taxa classified as epibenthic (e.g. *Bacillaria paxillifera*, *Cocconeis* sp., *Cylindrotheca closterium*, *G. marina*, *L.* cf. *abbreviata*, *Navicula* spp., and *Striatella* spp.) (Table 1). Some pelagic taxa were also present, possibly due to their entrainment by the complex 3-dimensional structures of some rafts. Potentially harmful epibenthic dinoflagellate taxa were the next most abundant group, with *Prorocentrum* cf. *clipeus*, *P. lima*, *P. rhathymum*, and *Coolia* spp. associated with 5 of 15 rafts (33%), and *Amphidinium* spp. associated with all rafts. As clonal isolates of microalgae were only established from 1 of 15 raft samples, the overall proportion of rafts from which we confirmed a
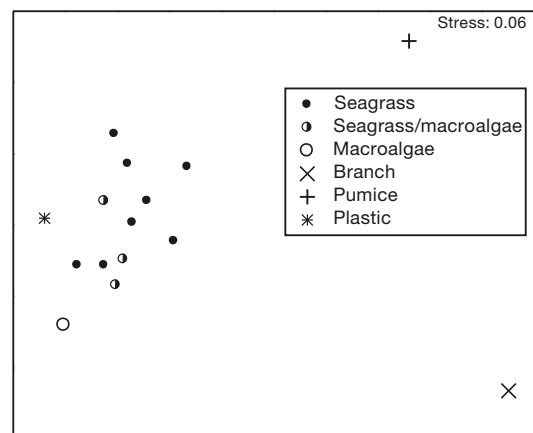

Fig. 2. Non-metric multi-dimensional scaling plot showing differences in the composition of the microalgal assemblage associated with different rafts based on raft type
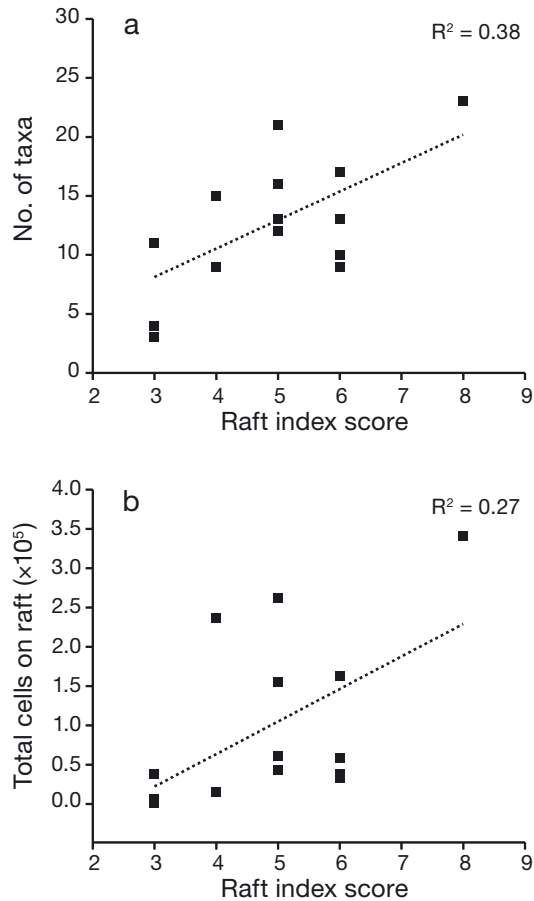
Fig. 3. Relationship between (a) richness of microalgal taxa and (b) microalgal abundance associated with rafts according to the raft size and complexity (raft index score)
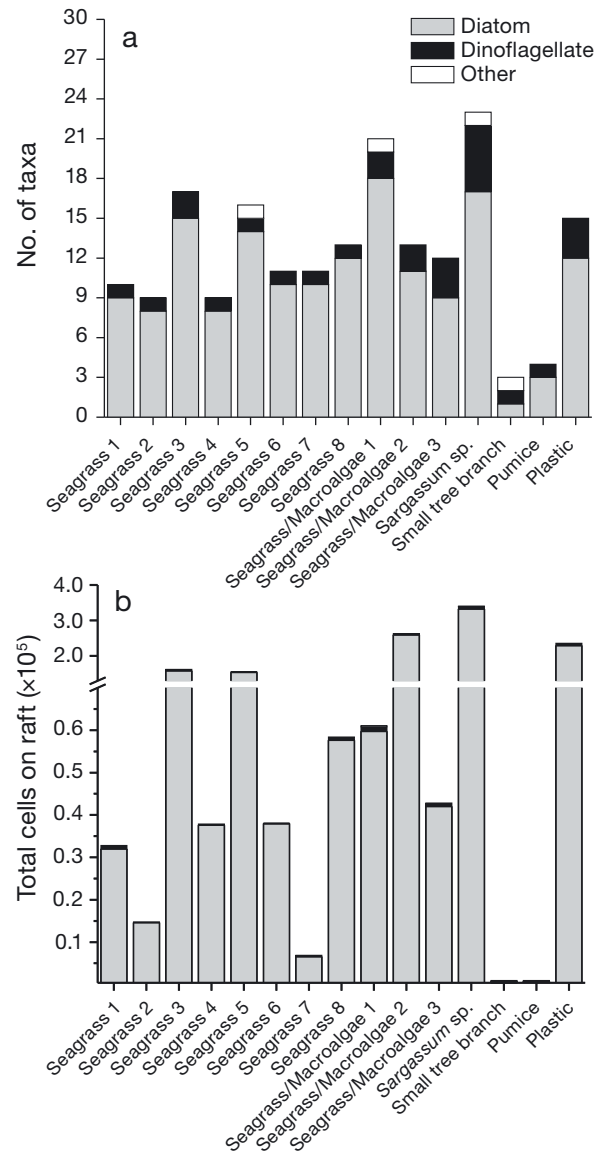

Fig. 4. (a) Richness and (b) abundance of the microalgal communities associated with rafts (see Supplement) drifting in the East Australian Current (EAC) and associated water masses. Note that 86% of dinoflagellate taxa are epibenthic (Table 1)

potentially toxic epibenthic dinoflagellate species was 6%. 'Other' taxa (cyanobacteria) were also present in some samples (Table 1, Fig. 4), contributing the least to the total microalgae abundance (Fig. 4b).

The viability of microalgal cells was confirmed by the establishment of 4 clonal isolates from raft samples. Cells of *Amphidinium* cf. *carterae*, *A.* cf. *operculatum*, *P. lima*, and *Coolia* sp. (Fig. 5) were established into culture after being isolated from a macroalgal raft of *Sargassum* sp. found drifting in the EAC.

The identity of the *Coolia* isolate was confirmed using the LSU D1-D3 region, a widely used marker region of the rDNA capable of distinguishing *Coolia* species (Leaw et al. 2010, Jeong et al. 2012, Karafas et al. 2015). Phylogenetic analysis of the LSU D1-D3 rDNA revealed that the raft strain grouped with other strains of *C. palmyrensis* from tropical locations around the world (Fig. 6), confirming its identity and likely low-latitude origin.

DISCUSSION

Planktonic microorganisms are not typically considered to be dispersion-limited in the ocean, due to their small size, vast population sizes, and pelagic lifestyle, all of which facilitate their transport in ocean currents (McManus & Woodson 2012). However, epibenthic dinoflagellates are closely associated with the benthos or substrates such as macrophytes, and therefore, planktonic dispersal of free-living (unattached) cells is less likely. In this study, we used 2

Table 1. Microalgal taxa associated with rafts collected in the East Australian Current (EAC) and associated water masses. +: epibenthic; –: planktonic; NA: not available or not applicable

Class	Genus/species	Habitat preference
Bacillario-phyceae	*Achnanthes longipes* C. Agardh, nom. illeg.	NA
	Amphora spp. Ehrenberg ex Kützing, 1844	+
	Bacillaria paxillifera (O.F. Müller) T.Marsson	+
	Climacosphenia moniligera Ehrenberg	NA
	Cocconeis sp. Ehrenberg, 1836	+
	Cylindrotheca closterium (Ehrenberg) Reimann & J.C. Lewin	+
	Grammatophora marina (Lyngbye) Kützing	+
	Haslea sp. Simonsen, 1974	+
	Lauderia annulata Cleve	–
	Leptocylindrus spp. Cleve, 1889	–
	Licmophora cf. *abbreviata* C. Agardh	+
	Licmophora spp. C. Agardh, 1827, nom. et typ. cons	+
	Melosira sp. C. Agardh, 1824, nom. cons.	+
	Melosira varians C. Agardh	+
	Navicula spp. Bory, 1822	+
	Nitzschia longissima (Brébisson) Ralfs	+
	Plagiotropis sp. Pfitzer, 1871	NA
	Pleurosigma W. Smith, 1852, nom. et typ. cons.	+
	Striatella sp. C. Agardh, 1832	+
	Striatella cf. *unipunctata* (Lyngbye) C. Agardh	+
	Synedra sp. Ehrenberg, 1830	NA
	Unidentified pennate	NA
Dinophyceae	*Amphidinium* cf. *operculatum*	+
	Amphidinium spp. É. Claperède & J. Lachmann, 1859	+
	Heterocapsa sp. F. Stein, 1883	–
	Prorocentrum cf. *clipeus* Hoppenrath	+
	Prorocentrum lima (Ehrenberg) F. Stein	+
	Prorocentrum rhathymum A. R. Loeblich III, Sherley & Schmidt	+
	Coolia palmyrensis Karafas, Tomas & York	+
Cyanophyceae	Unidentified Oscillatoriales	NA

lines of evidence to show that rafting is a natural mechanism for long-distance transport of epibenthic dinoflagellates: (1) the relatively high prevalence of epibenthic taxa associated with drifting macrophytes collected on an oceanographic voyage; and (2) their virtual absence in the water column at a coastal site over ~50 yr.

Earlier investigations demonstrated the association of epibenthic dinoflagellates with biotic or abiotic rafts in shallow and sheltered waters (Bomber et al. 1988b, Masó et al. 2003), but it was not known if these organisms could remain associated with and be transported by rafts in open ocean environments. In this study, we found intact cells of 6 taxa of epibenthic dinoflagellates from 3 genera (*Coolia*, *Prorocentrum*, and *Amphidinium*) on macrophyte rafts drifting in a major western boundary current and confirmed their viability by establishing laboratory cultures of species from all 3 genera. We have therefore demonstrated that viable cells of epibenthic dinoflagellates can remain associated with macrophyte rafts as they are transported offshore, confirming that rafting represents a natural means by which these organisms could expand their distribution, particularly from tropical to more temperate locations when entrained in ocean boundary currents.
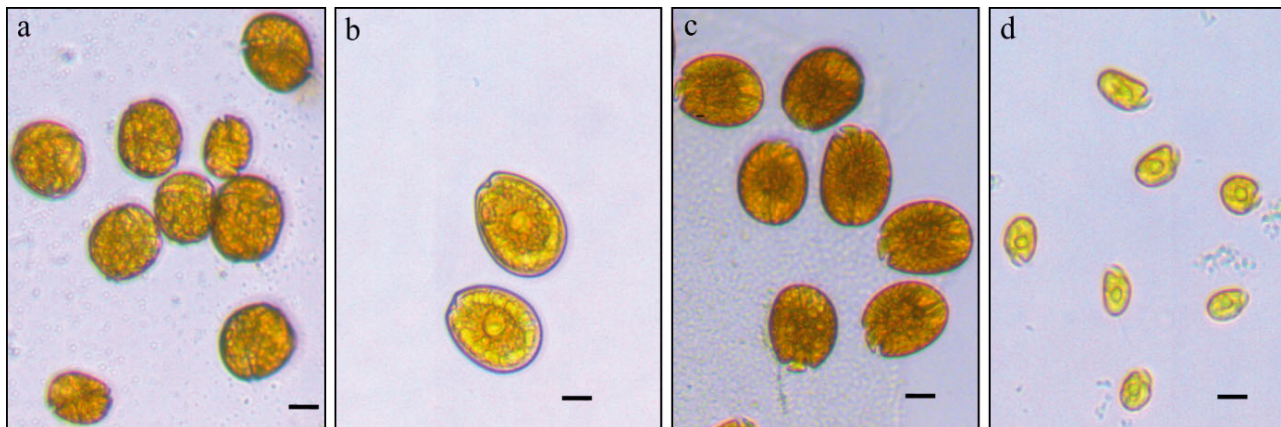

Fig. 5. Images of clonal epibenthic dinoflagellate cultures isolated from a *Sargassum* sp. raft sample collected drifting in the East Australian Current: (a) *Coolia palmyrensis*, (b) *Prorocentrum lima*, (c) *Amphidinium* cf. *operculatum*, and (d) *Amphidinium* cf. *carterae.* Scale bar = 10 µm
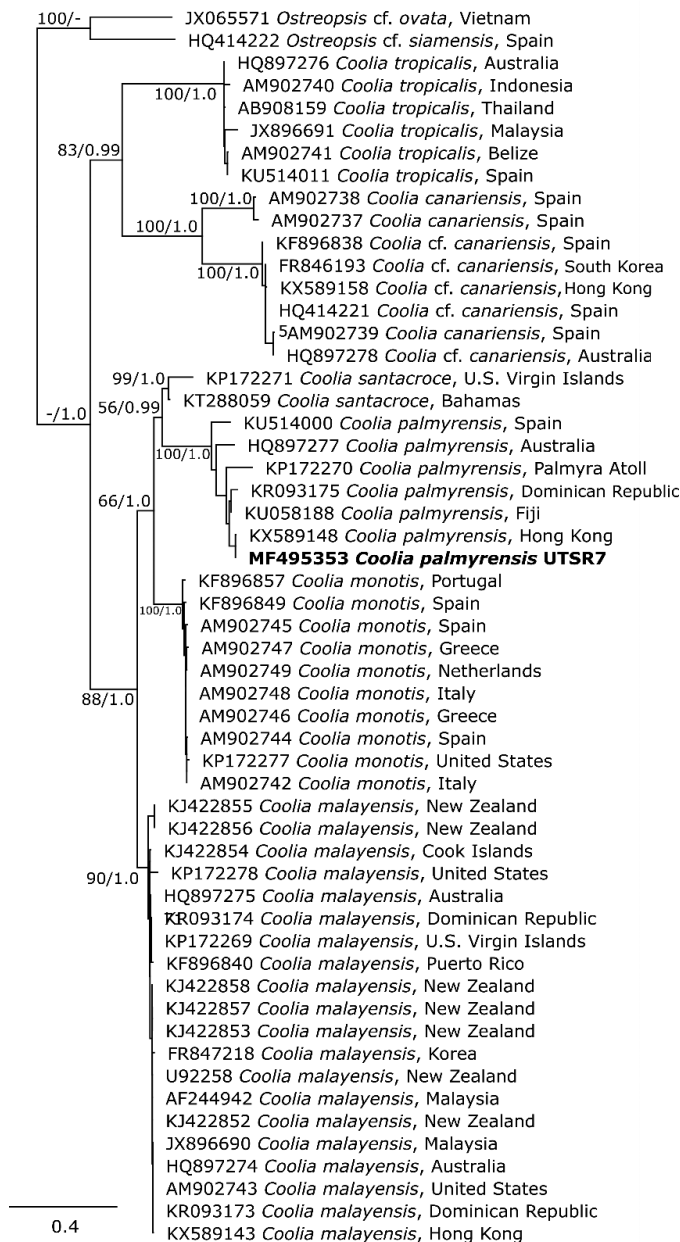
Fig. 6. Maximum likelihood tree of the *Coolia* culture (UTSR7, highlighted in **bold**) isolated from *Sargassum* sp. raft sample collected drifting in the East Australian Current, showing alignment of the partial LSU rDNA sequences (D1–D3 region). Values at the nodes represent maximum likelihood bootstrap and Bayesian posterior probability support

New cultures of epibenthic dinoflagellate species from the genera *Amphidinium*, *Prorocentrum*, and *Coolia* were established from a *Sargassum* sp. raft sample collected in this study. *Prorocentrum* is a very diverse genus that includes both planktonic and epibenthic species (Hoppenrath et al. 2013) and is widely distributed throughout Australia (Morton et

al. 1992, Heil et al. 2004, Murray et al. 2007) and the world (Hoppenrath et al. 2013). Similarly, *Amphidinium* is broadly distributed in Australia (Murray & Patterson 2002, Murray et al. 2004) and globally (Berland et al. 1992, Lee et al. 2003). *Amphidinium* is considered primarily epibenthic but is frequently reported in the plankton, likely due to misidentification or taxonomic uncertainty (Jørgensen et al. 2004, Murray et al. 2004). In comparison, species from the genus *Coolia* have only been found associated with benthic environments and, although the genus has a seemingly global distribution, some species have a more restricted range (Leaw et al. 2016). There are limited records of the distribution and taxonomic identity of *Coolia*, particularly from Australia, motivating the detailed investigation of the *Coolia* isolate in this study.

This investigation provides the first report of *C. palmyrensis* in temperate Australia. *C. palmyrensis* is a newly described, mildly toxic species (Karafas et al. 2015) and has thus far only been reported to have a tropical distribution, recorded at Palmyra Atoll in the North Pacific Ocean and the Dominican Republic in the Caribbean Sea (Karafas et al. 2015). However, sequences available in GenBank suggest that the distribution might extend to Hong Kong, Fiji, and Spain. *C. palmyrensis* has also been found in a sample from the Central Great Barrier Reef region in Australia (originally described as *Coolia* sp. by Momigliano et al. 2013 but later classified as *C. palmyrensis*; Karafas et al. 2015, Gómez et al. 2016, Leaw et al. 2016). While there has not been a comprehensive survey of the distribution of *C. palmyrensis* in Australia, this species has never been described from temperate locations (Leaw et al. 2016), suggesting the probable tropical origin of the *Sargassum* sp. raft sample from which the *C. palmyrensis* strain was isolated.

Other raft samples in this study were collected in an oceanic eddy located at approximately 32°S. At latitude 30–32°S, the EAC generally separates from the coast to form the eastward flowing Tasman Front, with the remaining southward flow forming a series of mesoscale eddies (Mata et al. 2006). These eddies can entrain coastal water and organisms, and transport rafts of marine macrophytes and debris. The implication is that the raft samples collected in the eddy may have been transported directly offshore from temperate latitudes rather than upstream tropical locations. Prevailing wind direction and strength can also influence the dispersal of floating material (Ruiz-Montoya et al. 2015) and should be considered when assessing the potential source of raft material.

The size and complexity of raft samples collected in this study was positively correlated with both the abundance and diversity of microalgal taxa, as has been commonly found in a wide range of studies considering many different taxa (Thiel & Gutow 2005a,b). *Sargassum* spp., *Posidonia* spp., and *Zostera* spp. were the most common types of rafts, and the general abundance and widespread distribution of these macrophyte taxa in Australia (Christianson et al. 1981, Kirkman 1997) suggest that they may have the highest capacity to act as rafts in this region. Furthermore, if storm intensity and frequency increase (Barnard et al. 2015), the rate of detachment of coastal macrophytes may rise (Witman 1987, Seymour et al. 1989), thereby increasing the supply of rafts and potential for transportation of associated organisms (Macreadie et al. 2011).

Masó et al. (2003) were the first to assess whether rafts of abiotic origin, specifically marine plastic debris, could harbour harmful microalgae taxa. They found the epibenthic dinoflagellates *Ostreopsis* spp. and *Coolia* spp. associated with plastic debris collected along the Catalan coast in the western Mediterranean. We found that the microalgal assemblage associated with the plastic raft sample had a very similar composition to that of seagrass and macroalgae, indicating the likely coastal origin of the plastic. This reveals the capacity of anthropogenic marine debris to act in a manner like that of macrophyte rafts, a concern given that abiotic materials such as plastic have low decomposition rates, can stay afloat for extended periods, and are becoming increasingly more abundant in our oceans (Derraik 2002). The ability of millimetre-sized marine plastics to act as habitat for microorganisms has also recently been investigated (Reisser et al. 2014). Although small, these materials were found to have microalgal cells associated with their surface and may therefore also act as rafts and could also aid in the dispersal of harmful epibenthic dinoflagellates. Pumice was another type of raft collected during our study. Many small clasts of pumice can be generated in a single volcanic event and can travel vast distances in ocean currents, making this material an effective rafting agent (Bryan et al. 2012) that should also be considered in the future.

In this study, we contrasted the abundance of potentially harmful epibenthic dinoflagellates on rafts with those in the plankton at a time-series location. Epibenthic dinoflagellates were virtually absent from the long-term coastal phytoplankton monitoring site located ~5 km from the coast. Although it seems unlikely that an epibenthic species would be ad-vected as free-living cells in the plankton, dispersal of these organisms in ocean currents has been suggested, particularly along the east coast of Australia (Heimann et al. 2011, Murray et al. 2014, Sparrow et al. 2017). Here we found that the epibenthic dinoflagellate *P. lima* was documented in only 3 of 267 samples over approximately a 50 yr period (1978, 1979, and 2008). This occurrence rate is very low, but shows that some unassociated epibenthic dinoflagellate cells can be found in surface waters. Therefore, we cannot rule out that planktonic transport via drifting of free-living cells may occur for these taxa, but it seems far less likely compared to rafting.

CONCLUSION

Here, we have shown for the first time that epibenthic dinoflagellates remain viable when transported offshore while associated with rafts of both biotic and abiotic origin. Rafting is therefore an effective dispersal mechanism for harmful epibenthic dinoflagellates and could facilitate geographic range expansions. However, whether rafted epibenthic dinoflagellate cells can be delivered to shallow waters and successfully colonise new coastal habitats remains an open question and should be a fruitful area of future research.

LITERATURE CITED

Accoroni S, Romagnoli T, Penna A, Capellacci S and others (2016) *Ostreopsis fattorussoi* sp. nov. (Dinophyceae), a new benthic toxic *Ostreopsis* species from the eastern Mediterranean Sea. J Phycol 52:1064–1084

Adachi R, Fukuyo Y (1979) The thecal structure of a marine toxic dinoflagellate *Gambierdiscus toxicus* gen. et sp. nov. collected in a ciguatera-endemic area. Bull Jpn Soc Sci Fish 45:67–71

Ajani P, Lee R, Pritchard T, Krogh M (2001) Phytoplankton dynamics at a long-term coastal station off Sydney, Australia. J Coast Res 34:60–73

Ajani PA, Allen AP, Ingleton T, Armand L (2014a) A decadal decline in relative abundance and a shift in microphytoplankton composition at a long-term coastal station off southeast Australia. Limnol Oceanogr 59:519–531

Ajani PA, Allen AP, Ingleton T, Armand L (2014b) Erratum: A decadal decline in relative abundance and a shift in microphytoplankton composition at a long-term coastal station off southeast Australia. Limnol Oceanogr 59:2240–2242

Ajani PA, Hallegraeff GM, Allen D, Coughlin A and others (2016) Establishing baselines: eighty years of phytoplankton diversity and biomass in south-eastern Australia. Oceanogr Mar Biol Annu Rev 54:387–412

Aligizaki K, Nikolaidis G, Fraga S (2008) Is *Gambierdiscus* expanding to new areas? Harmful Algae News 36:6–7

Andersen RA, Kawachi M (2005) Traditional microalgae isolation techniques. In: Andersen RA (ed) Algal culturing techniques. Elsevier, Amsterdam, p 83–100

Baden DG, Adams DJ (2000) Brevetoxins: chemistry, mechanism of action, and methods of detection. In: Botana LM (ed) Seafood and freshwater toxins: pharmacology, physiology and detection. Marcel Dekker Inc, New York, NY, p 505–532

Banks SC, Piggott MP, Williamson JE, Bové U, Holbrook NJ, Beheregaray LB (2007) Oceanic variability and coastal topography shape genetic structure in a long-dispersing sea urchin. Ecology 88:3055–3064

Barnard PL, Short AD, Harley MD, Splinter KD and others (2015) Coastal vulnerability across the Pacific dominated by El Niño/Southern Oscillation. Nat Geosci 8:801–807

Berland B, Grzebyk D, Thomassin B (1992) Benthic dinoflagellates from the coral reef lagoon of Mayotte Island (SW Indian Ocean); identification, toxicity and preliminary ecophysiological study. Bull Soc Pathol Exot 85:453–456

Besada EG, Loeblich LA, Loeblich AR III (1982) Observations on tropical, benthic dinoflagellates from ciguatera-endemic areas: *Coolia, Gambierdiscus*, and *Ostreopsis.* Bull Mar Sci 32:723–735

Bomber JW, Guillard RR, Nelson WG (1988a) Roles of temperature, salinity, and light in seasonality, growth, and toxicity of ciguatera-causing *Gambierdiscus toxicus* Adachi et Fukuyo (Dinophyceae). J Exp Mar Biol Ecol 115:53–65

Bomber JW, Morton SL, Babinchak JA, Norris DR, Morton JG (1988b) Epiphytic dinoflagellates of drift algae—another toxigenic community in the ciguatera food chain. Bull Mar Sci 43:204–214

Booth DJ, Figueira WF, Gregson MA, Brown L, Beretta G (2007) Occurrence of tropical fishes in temperate south-eastern Australia: role of the East Australian Current. Estuar Coast Shelf Sci 72:102–114

Bryan SE, Cook AG, Evans JP, Hebden K and others (2012) Rapid, long-distance dispersal by pumice rafting. PLOS ONE 7:e40583

Christianson IG, Clayton MN, Allender BM, Fuhrer BA (1981) Seaweeds of Australia. Reed, Sydney

Claparède É, Lachmann J (1859) Études sur les infusoires et les rhizopodes. Mém Inst Natl Genevois 6:261–482

Clarke K, Warwick R (2001) Change in marine communities: an approach to statistical analysis and interpretation, 2nd edn. PRIMER-E, Plymouth

Coleman MA, Roughan M, Macdonald HS, Connell SD, Gillanders BM, Kelaher BP, Steinberg PD (2011) Variation in the strength of continental boundary currents determines continent wide connectivity in kelp. J Ecol 99:1026–1032

Cruz-Rivera E, Villareal TA (2006) Macroalgal palatability and the flux of ciguatera toxins through marine food webs. Harmful Algae 5:497–525

Derraik JG (2002) The pollution of the marine environment by plastic debris: a review. Mar Pollut Bull 44:842–852

Durando P, Ansaldi F, Oreste P, Moscatelli P and others (2007) *Ostreopsis ovata* and human health: epidemiological and clinical features of respiratory syndrome outbreaks from a two-year syndromic surveillance, 2005–06, in north-west Italy. Euro Surveill 12:E070607.1

Edgar RC (2004) MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res 32:1792–1797

Ehrenberg CG (1834) Dritter Beitrag zur Erkenntnis grosser Organisation in der Richtung des kleinsten Raumes. Abh königl Akad Wiss Berlin 1833:145–336, pls I–XIII, 1–11

Gómez F, Qiu D, Lopes RM, Lin S (2015) *Fukuyoa paulensis* gen. et sp. nov., a new genus for the globular species of the dinoflagellate *Gambierdiscus* (Dinophyceae). PLOS ONE 10:e0119676

Gómez F, Qiu D, Otero Morales E, Lopes RM, Lin S (2016) Circumtropical distribution of the epiphytic dinoflagellate *Coolia malayensis* (Dinophyceae): morphology and molecular phylogeny from Puerto Rico and Brazil. Phycol Res 64:194–199

Grant B, Kerr J (1970) Phytoplankton numbers and species at Port Hacking station and their relationship to the physical environment. Mar Freshw Res 21:35–46

Guindon S, Gascuel O (2003) A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. Syst Biol 52:696–704

Hallegraeff G (1981) Seasonal study of phytoplankton pigments and species at a coastal station off Sydney: importance of diatoms and the nanoplankton. Mar Biol 61: 107–118

Hallegraeff G, Reid D (1986) Phytoplankton species successions and their hydrological environment at a coastal station off Sydney. Mar Freshw Res 37:361–377

Hallegraeff GM, Bolch CJ, Hill DR, Jameson I and others (2010) Algae of Australia: phytoplankton of temperate coastal waters. CSIRO, Melbourne

Heil C, Chaston K, Jones A, Bird P, Longstaff B, Costanzo S, Dennison W (2004) Benthic microalgae in coral reef sediments of the southern Great Barrier Reef, Australia. Coral Reefs 23:336–343

Heimann K, Capper A, Sparrow L (2011) Ocean surface warming: impact on toxic benthic dinoflagellates causing ciguatera. The Encyclopedia of Life Sciences A23373. John Wiley & Sons, Chichester

Hoppenrath M, Chomérat N, Horiguchi T, Schweikert M, Nagahama Y, Murray S (2013) Taxonomy and phylogeny of the benthic *Prorocentrum* species (Dinophyceae)—a proposal and review. Harmful Algae 27:1–28

Hoppenrath M, Murray SA, Chomérat N, Horiguchi T (2014) Marine benthic dinoflagellates—unveiling their worldwide biodiversity. Kleine Senckenberg-Reihe, Band 54. Schweizerbart, Stuttgart

Huelsenbeck JP, Ronquist F (2001) MRBAYES: Bayesian inference of phylogenetic trees. Bioinformatics 17:754–755

Jeffery B, Barlow T, Moizer K, Paul S, Boyle C (2004) Amnesic shellfish poison. Food Chem Toxicol 42:545–557

Jeong HJ, Yih W, Kang NS, Lee SY and others (2012) First report of the epiphytic benthic dinoflagellates *Coolia canari-*

ensis and *Coolia malayensis* in the waters off Jeju Island, Korea: morphology and rDNA sequences. J Eukaryot Microbiol 59:114−133

Johnson CR, Banks SC, Barrett NS, Cazassus F and others (2011) Climate change cascades: shifts in oceanography, species' ranges and subtidal marine community dynamics in eastern Tasmania. J Exp Mar Biol Ecol 400: 17−32

Jørgensen MF, Murray S, Daugbjerg N (2004) *Amphidinium* revisited. I. Redefinition of *Amphidinium* (Dinophyceae) based on cladistic and molecular phylogenetic analyses. J Phycol 40:351−365

Karafas S, York R, Tomas C (2015) Morphological and genetic analysis of the *Coolia monotis* species complex with the introduction of two new species, *Coolia santacroce* sp. nov. and *Coolia palmyrensis* sp. nov. (Dinophyceae). Harmful Algae 46:18−33

Kearse M, Moir R, Wilson A, Stones-Havas S and others (2012) Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics 28:1647−1649

Kibler SR, Litaker RW, Holland WC, Vandersea MW, Tester PA (2012) Growth of eight *Gambierdiscus* (Dinophyceae) species: effects of temperature, salinity and irradiance. Harmful Algae 19:1−14

Kirkman H (1997) Seagrasses of Australia. Environment Australia, Canberra

Kohli GS, Murray SA, Neilan BA, Rhodes LL and others (2014) High abundance of the potentially maitotoxic dinoflagellate *Gambierdiscus carpenteri* in temperate waters of New South Wales, Australia. Harmful Algae 39:134−145

Last PR, White WT, Gledhill DC, Hobday AJ, Brown R, Edgar GJ, Pecl G (2011) Long-term shifts in abundance and distribution of a temperate fish fauna: a response to climate change and fishing practices. Glob Ecol Biogeogr 20:58−72

Leaw CP, Lim PT, Cheng KW, Ng BK, Usup G (2010) Morphology and molecular characterization of a new species of thecate benthic dinoflagellate, *Coolia malayensis* sp. nov. (Dinophyceae). J Phycol 46:162−171

Leaw CP, Tan TH, Lim HC, Teng ST and others (2016) New scenario for speciation in the benthic dinoflagellate genus *Coolia* (Dinophyceae). Harmful Algae 55:137−149

Lee JJ, Olea R, Cevasco M, Pochon X, Correia M, Shpigel M, Pawlowski J (2003) A marine dinoflagellate, *Amphidinium eilatiensis* n. sp., from the benthos of a mariculture sedimentation pond in Eilat, Israel. J Eukaryot Microbiol 50:439−448

Lewis RJ, Holmes MJ (1993) Origin and transfer of toxins involved in ciguatera. Comp Biochem Physiol C Pharmacol Toxicol Endocrinol 106:615−628

Ling S, Johnson C, Ridgway K, Hobday A, Haddon M (2009) Climate driven range extension of a sea urchin: inferring future trends by analysis of recent population dynamics. Glob Change Biol 15:719−731

Litaker RW, Vandersea MW, Faust MA, Kibler SR and others (2009) Taxonomy of *Gambierdiscus* including four new species, *Gambierdiscus caribaeus*, *Gambierdiscus carolinianus*, *Gambierdiscus carpenteri* and *Gambierdiscus ruetzleri* (Gonyaulacales, Dinophyceae). Phycologia 48: 344−390

Macreadie PI, Bishop MJ, Booth DJ (2011) Implications of climate change for macrophytic rafts and their hitchhikers. Mar Ecol Prog Ser 443:285−292

Masó M, Garcés E, Pagès F, Camp J (2003) Drifting plastic debris as a potential vector for dispersing Harmful Algal Bloom (HAB) species. Sci Mar 67:107−111

Masó M, Fortuño JM, de Juan S, Demestre M (2016) Microfouling communities from pelagic and benthic marine plastic debris sampled across Mediterranean coastal waters. Sci Mar 80:117−127

Mata MM, Wijffels SE, Church JA, Tomczak M (2006) Eddy shedding and energy conversions in the East Australian Current. J Geophys Res 111:C09034

McManus MA, Woodson CB (2012) Plankton distribution and ocean dispersal. J Exp Biol 215:1008−1016

Meunier A (1919) Microplankton de la Mer Flamande. III. Les Peridiniales. Mém Mus R Hist Nat Belgique Bruxelles 8:116

Momigliano P, Sparrow L, Blair D, Heimann K (2013) The diversity of *Coolia* spp. (Dinophyceae Ostreopsidaceae) in the central Great Barrier Reef region. PLOS ONE 8:e79278

Morton SL, Norris DR, Bomber JW (1992) Effect of temperature, salinity and light intensity on the growth and seasonality of toxic dinoflagellates associated with ciguatera. J Exp Mar Biol Ecol 157:79−90

Murata M, Shimatani M, Sugitani H, Oshima Y, Yasumoto T (1982) Isolation and structural elucidation of the causative toxin of the diarrhetic shellfish poisoning. Bull Jpn Soc Sci Fish 48:549−552

Murray S, Patterson D (2002) The benthic dinoflagellate genus *Amphidinium* in south-eastern Australian waters, including three new species. Eur J Phycol 37:279−298

Murray S, Flø Jørgensen M, Daugbjerg N, Rhodes L (2004) *Amphidinium* revisited. II. Resolving species boundaries in the *Amphidinium operculatum* species complex (Dinophyceae), including the descriptions of *Amphidinium trulla* sp. nov. and *Amphidinium gibbosum* comb. nov. J Phycol 40:366−382

Murray S, Nagahama Y, Fukuyo Y (2007) Phylogenetic study of benthic, spine bearing prorocentroids, including *Prorocentrum fukuyoi* sp. nov. Phycological Res 55:91−102

Murray S, Momigliano P, Heimann K, Blair D (2014) Molecular phylogenetics and morphology of *Gambierdiscus yasumotoi* from tropical eastern Australia. Harmful Algae 39:242−252

Nunn GB, Theisen BF, Christensen B, Arctander P (1996) Simplicity-correlated size growth of the nuclear 28S ribosomal RNA D3 expansion segment in the crustacean order Isopoda. J Mol Evol 42:211−223

Reisser J, Shaw J, Hallegraeff G, Proietti M and others (2014) Millimeter-sized marine plastics: a new pelagic habitat for microorganisms and invertebrates. PLOS ONE 9:e100289

Rhodes L, Smith K, Papiol GG, Adamson J, Harwood T, Munday R (2014) Epiphytic dinoflagellates in sub-tropical New Zealand, in particular the genus *Coolia* Meunier. Harmful Algae 34:36−41

Ridgway K (2007) Long term trend and decadal variability of the southward penetration of the East Australian Current. Geophys Res Lett 34:L13613

Ruiz-Montoya L, Lowe RJ, Kendrick GA (2015) Contemporary connectivity is sustained by wind-and current-driven seed dispersal among seagrass meadows. Mov Ecol 3:9

Satake M, Ofuji K, Naoki H, James KJ and others (1998) Azaspiracid, a new marine toxin having unique spiro ring assemblies, isolated from Irish mussels, *Mytilus edulis*. J Am Chem Soc 120:9967−9968

Schmidt J (1901) Flora of Koh Chang. Contributions to the

knowledge of the vegetation in the Gulf of Siam. Peri-
diniales. Bot Tidsskr 24:212–221

Scholin CA, Herzog M, Sogin M, Anderson DM (1994) Iden-
tification of group and strain specific genetic markers for
globally distributed *Alexandrium* (Dinophyceae). II.
Sequence analysis of a fragment of the LSU rRNA gene.
J Phycol 30:999–1011

Seymour R, Tegner M, Dayton P, Parnell P (1989) Storm
wave induced mortality of giant kelp, *Macrocystis
pyrifera*, in southern California. Estuar Coast Shelf Sci
28:277–292

Sparrow L, Momigliano P, Russ GR, Heimann K (2017) Effects
of temperature, salinity and composition of the dinoflagel-
late assemblage on the growth of *Gambierdiscus carpen-
teri* isolated from the Great Barrier Reef. Harmful Algae
65:52–60

Suthers IM, Young JW, Baird ME, Roughan M and others
(2011) The strengthening East Australian Current, its
eddies and biological effects—an introduction and
overview. Deep Sea Res II 58:538–546

Thiel M, Gutow L (2005a) The ecology of rafting in the mar-
ine environment. I. The floating substrata. Oceanogr Mar
Biol Annu Rev 42:181–264

Thiel M, Gutow L (2005b) The ecology of rafting in the mar-
ine environment. II. The rafting organisms and commu-
nity. Oceanogr Mar Biol Annu Rev 43:279–418

Thompson PA, Baird ME, Ingleton T, Doblin MA (2009)
Long-term changes in temperate Australian coastal
waters: implications for phytoplankton. Mar Ecol Prog
Ser 394:1–19

Tubaro A, Durando P, Del Favero G, Ansaldi F, Icardi G,
Deeds J, Sosa S (2011) Case definitions for human poi-
sonings postulated to palytoxins exposure. Toxicon 57:
478–495

Van Dolah FM (2000) Marine algal toxins: origins, health
effects, and their increased occurrence. Environ Health
Perspect 108(Suppl 1):133–141

Wiese M, D'agostino PM, Mihali TK, Moffitt MC, Neilan BA
(2010) Neurotoxic alkaloids: saxitoxin and its analogs.
Mar Drugs 8:2185–2211

Witman JD (1987) Subtidal coexistence: storms, grazing,
mutualism, and the zonation of kelps and mussels. Ecol
Monogr 57:167–187

Wu L, Cai W, Zhang L, Nakamura H and others (2012)
Enhanced warming over the global subtropical western
boundary currents. Nat Clim Chang 2:161–166

Xu Y, Richlen ML, Liefer JD, Robertson A and others (2016)
Influence of environmental variables on *Gambierdiscus*
spp. (Dinophyceae) growth and distribution. PLOS ONE
11:e0153197

Yang H, Lohmann G, Wei W, Dima M, Ionita M, Liu J (2016)
Intensification and poleward shift of subtropical western
boundary currents in a warming climate. J Geophys Res
Oceans 121:4928–4945

Yasumoto T (2005) Chemistry, etiology, and food chain
dynamics of marine toxins. Proc Jpn Acad B 81:43–51

Yasumoto T, Inoue A, Bagnis R, Garcon M (1979) Ecological
survey on a dinoflagellate possibly responsible for the
induction of ciguatera. Nippon Suisan Gakkaishi 45:
395–399