**Cladophora ruchingeri** (C. Agardh) Kützing, 1845 (Cladophorales, Chlorophyta): a new biofouling pest of green-lipped mussel *Perna canaliculus* (Gmelin, 1791) farms in New Zealand

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**Abstract**

*Cladophora* is a genus of branched filamentous green algae (Ulvophyceae). It contains many species that are challenging to differentiate based on morphology because of the scarcity of diagnostic characters and extensive phenotypic plasticity. Within the past five years, *Cladophora* blooms have been observed on the ropes of green-lipped mussel farms in the Marlborough Sounds, New Zealand. When *Cladophora* reaches high biomass, it can clog mussel-harvesting equipment; thus, it is considered a nuisance organism in the region. This study used morphological and molecular techniques to identify the species responsible for the blooms, and to investigate whether this might be a recent incursion. *Cladophora* samples (*n* = 21) were collected from nine mussel farms, one salmon farm, and a marina. Morphological and phylogenetic analyses (partial large subunit and internally transcribed spacer regions 1 and 2 of the nuclear ribosomal cistron), revealed the identity of the bloom forming species as *Cladophora ruchingeri* (C.Agardh) Kützing, 1845. This represents the first report of this species in the Southern Hemisphere and Pacific region. Given the distinct morphology of *C. ruchingeri* (when mature), its absence from previous surveys of macro-algae from this region, and increasing reports of blooms, our findings suggest that this species has only recently been introduced to New Zealand. This study provides a robust taxonomic identification and initial baseline data. Further directed studies on *Cladophora* are required to advance knowledge on its ecology and distribution in New Zealand, and assist in the development of mitigation strategies.

**Key words:** aquaculture, green tide, marine pest, nuisance algae, ribosomal internal transcribed spacer

**Introduction**

Biofouling represents an important biosecurity risk for the aquaculture industry. Negative effects include direct impacts on cultured species (e.g. smothering, competition for space and food), deterioration of farm infrastructure (immersed structures such as cages, netting and pontoons), and effects on natural ecosystem functioning of adjacent areas (Fitridge et al. 2012; Fletcher et al. 2013). Biofouling assemblages often include a large proportion of non-indigenous species (Carlton 1989; Ruiz et al. 2009), which represent an important threat to coastal ecosystems globally (Molnar et al. 2008; Ruiz et al. 1999). Non-indigenous species can cause economic and ecological harm (Falk-Petersen et al. 2006), and there are multiple examples of high-profile species (e.g., *Ciona intestinalis* (Linnaeus, 1767)) that have severely affected aquaculture regions globally (Ramsay et al. 2008).

In New Zealand, the green-lipped mussel (*Perna canaliculus* Gmelin, 1791) industry is the largest aquaculture sector, producing US$ 218 million in annual exports (AQNZ 2012). The Pelorus Sounds, located in the Marlborough region at the top of New Zealand’s South Island (Figure 1), supports 69% of the annual production of
green-lipped mussel (AQNZ 2012). Green-lipped mussels are cultured on long-lines of a rough fibrous and porous texture, which is favourable for larval settlement of fouling organisms. These lines are suspended from backbone ropes above the seabed and supported by a series of buoys (Anderson and Underwood 1994). A typical three hectare mussel farm has nine backbone ropes, measuring 110 m each, and each rope supports a crop long-line of 3,750 metres (Marine Farming Association Inc., Blenheim, New Zealand, pers. comm.). Mussel production occurs over 12 to 24 month cycles from initial spat settlement to the harvesting of adult mussels (Woods et al. 2012). During intermediate and final seed crop stages, culture ropes are stripped and reseeded to reduce the density of the mussels for grow-out to market size and to reduce mussel biofouling (Woods et al. 2012).

Previous studies have recorded > 70 biofouling taxa growing on culture ropes, representing a large proportion of the total biomass (Woods et al. 2012). Since 2009, mussel culture structures have been fouled by a mat-forming green filamentous macroalga, which was tentatively identified as *Cladophora* sp. (Figure 1). When it reaches high biomass, it can clog the mussel harvesting equipment, resulting in costly delays. It has also been observed growing on other artificial structures (e.g. floating pontoons, floats and wharf piles) and on natural substrates (e.g. shallow rocky reefs) in the region.

*Cladophora* is a large genus of branched, uniseriate, filamentous green algae (Ulvophyceae) characterized by multinucleate cells. It is found worldwide in marine, brackish, and freshwater environments and is known to form conspicuous algal mats, sometimes referred to as green tides or macroalgal blooms, where nutrients accumulate and competition is reduced (Flindt et al. 1997; Curiel et al. 2004; Gubelit and Kovalchuk 2010; Zuilikfly et al. 2013). Molecular studies show the genus is polyphyletic (Bakker et al. 1994; Hanyuda et al. 2002; Leliaert et al. 2003), leading to the recent description of new genera (Boedeker et al. 2012). Due to the limited number of morphological features and the pronounced phenotypic plasticity (van den Hoek 1963; Leliaert and Boedeker 2007), species identification is difficult. In addition, cryptic diversity has been revealed in several morphospecies (Bakker et al. 1995; Bakker et al. 1995a, b; Leliaert et al. 2007). Thus, molecular identification and phylogenetics are important tools for assessing evolutionary relationships and diversity within *Cladophora* (e.g., Leliaert et al. 2009; Hayakawa et al. 2012).

The diversity of *Cladophora* in New Zealand has not yet been fully documented. Chapman (1956) lists 25 species, including a number of dubious taxa. Currently, 15 accepted names of *Cladophora* are listed in AlgaeBase for New Zealand (Guiry and Guiry 2014). Of these, five have been documented as marine species occurring around the North and South Islands of New Zealand (Adams 1994; Nelson 2013). However, the true diversity and the correct taxonomic identities of *Cladophora* in New Zealand are unknown (see also Hurd et al. 2004; Nelson 2013), and a comprehensive study including large-scale DNA sequencing is clearly required.

![Figure 1. Photos of *Cladophora ruchingeri* mats (see black arrows) overgrowing (A) green-lipped mussel farm structures and (B) crop lines in the Marlborough Sounds, New Zealand. Photos AshleighWatts.](image)
Invasion of Cladophora in mussel farms

In the interim, the aim of this study was to identify the Cladophora species that forms blooms in Pelorus Sound, using both morphological examinations and molecular techniques. It was also of interest to evaluate whether the Cladophora species responsible for these algal blooms might represent a new incursion to New Zealand.

Methods

Sample collection

Between March and June 2013, 21 specimens of Cladophora spp. were collected by hand from 9 mussel farms in the Pelorus Sound, the Havelock marina, and a salmon farm in the Queen Charlotte Sound, Marlborough, New Zealand (Figure 2). In Pelorus Sound, samples were collected from the top 3 m of mussel long-lines and from wharf piles in Havelock marina. In Queen Charlotte Sound, samples were collected from the top 3 m of a salmon cage net. Samples were cleaned of epiphytes using filtered sea water, and fragments from each specimen were preserved in 70% ethanol for future morphological analyses and stored at -20°C for later DNA extraction.

Morphological analyses

The ethanol-preserved samples were identified as morphospecies using several taxonomic treatments of the genus Cladophora (e.g., Söderström 1963; van den Hoek 1963, 1982; van den Hoek and Womersley 1984; van den Hoek and Chihara 2000; Kraft 2007; Leliaert and Boedeker 2007).

All identifications were performed using a light microscope (Olympus BH2, Olympus Optical Co. GmbH, Germany) and a stereo microscope (Leica MZ9.5, Leica Microsystems, Germany), and images were taken with a digital camera (ColorView Illu, Olympus Soft Imaging Systems, Germany). Voucher specimens were deposited in the Te Papa Tongarewa National Museum of New Zealand herbarium (WELT, see Table 1).

DNA extractions, PCR, and sequencing

The DNA from the frozen Cladophora specimens was extracted using the PureLink™ Genomic DNA Kit (Life Technologies, USA) according to the protocol supplied by the manufacturer. All PCR reactions were undertaken on a DNA Engine thermal cycler (Bio-Rad, USA) or an Eppendorf Mastercycler (Eppendorf, Germany). Two regions of the nuclear ribosomal cistron were investigated:

partial large subunit (LSU rDNA) and internally transcribed spacer regions 1 and 2 (ITS rDNA). PCRs for the LSU gene were performed in a total volume of 25 µl using the high-fidelity Platinum® PCR Supermix (Life Technologies), 0.4 µM of each primers (D1R-F 5'-ACCAGCTGAATTTCAGCTTT AACGATA -3' [Scholin et al. 1994] and D3B-R 5'-TCGGAGGGAACCCG TACTA -3' [Nunn et al. 1996]), and template DNA (30–50 ng). PCR cycling conditions were: 94°C for 2 min, followed by 38 cycles of 94°C for 45 s, 54°C for 45 s, 72°C for 60 s, and a final extension of 72°C for 8 min. PCRs for the ITS amplicons were performed using the forward primer ITS1 (5'-GGTGAACCTGAGGAAGGAT-3' [modified from Gottschling et al. 2005]) and ITS4 (5'-TCGGAGGGAACCCGTACTA-3' [White et al. 1990]). Thermocycling conditions were identical to those described for LSU except for the annealing temperature which was lowered to 52°C. PCR products were visualized with 1.5% agarose gel electrophoresis with ethidium bromide staining and UV illumination. Positive PCR products were purified using the AxyPrep PCR...
Table 1. List of 28 Cladophorales species sequenced in this study.

| Species                | Strain ID | Origin                    | Location/Region           | Voucher (LSU) | GenBank (LSU) | Voucher (ITS) | GenBank (ITS) |
|------------------------|-----------|---------------------------|---------------------------|---------------|---------------|---------------|---------------|
| Cladophora albida *    | CAW #21   | New Zealand               | South Bay                 | A033064       | n.i.          | n.i.          | LN679098      |
| Cladophora albida *    | CAW #23   | New Zealand               | Ruakaka (salmon farm)     | A033065       | n.i.          | n.i.          | LN679097      |
| Cladophora glomerata   | K89       | New Zealand               | Wanganui River            | A033066       | LN679067      | LN679077      |
| Cladophora hutchinsioides*| CAW #36     | New Zealand               | Hikapu Reach              | n.i.          | n.i.          | LN679078      |
| Cladophora ruchingeri  | CAW #01   | New Zealand               | Ynceya Bay                | n.i.          | LN679071      | LN679079      |
| Cladophora ruchingeri  | CAW #04   | New Zealand               | South East Bay            | n.i.          | LN679072      | LN679080      |
| Cladophora ruchingeri  | CAW #05   | New Zealand               | Kenepuru                  | n.i.          | LN679073      | LN679081      |
| Cladophora ruchingeri  | CAW #06   | New Zealand               | Clova Bay                 | n.i.          | LN679074      | LN679082      |
| Cladophora ruchingeri  | CAW #07   | New Zealand               | Tawero Point              | n.i.          | LN679085      | LN679085      |
| Cladophora ruchingeri  | CAW #10   | New Zealand               | Forsyth Bay               | n.i.          | LN679086      | LN679086      |
| Cladophora ruchingeri  | CAW #12   | New Zealand               | Beatrix Bay               | A033068       | n.i.          | LN679087      |
| Cladophora ruchingeri  | CAW #14   | New Zealand               | Ynceya Bay                | A033069       | n.i.          | LN679088      |
| Cladophora ruchingeri  | CAW #18   | New Zealand               | South East Bay            | A033070       | n.i.          | LN679089      |
| Cladophora ruchingeri  | CAW #31   | New Zealand               | Scotts Bay                | A033071       | n.i.          | LN679090      |
| Cladophora ruchingeri  | CAW #33   | New Zealand               | Hikapu Reach              | A033072       | n.i.          | LN679091      |
| Cladophora ruchingeri  | CAW #38   | New Zealand               | Ynceya Bay                | A033073       | n.i.          | LN679092      |
| Cladophora ruchingeri  | CAW #39   | New Zealand               | Hikapu Reach              | n.i.          | LN679093      |               |
| Cladophora ruchingeri  | CAW #40   | New Zealand               | Ynceya Bay                | n.i.          | LN679094      |               |
| Cladophora ruchingeri  | CAW #41   | New Zealand               | South East Bay            | n.i.          | LN679095      |               |
| Cladophora ruchingeri  | CAW #42   | New Zealand               | Tawero Point              | n.i.          | LN679096      |               |
| Cladophora vagabunda   | E62       | New Zealand               | Hawke's Bay               | A033074       | LN679068      | n.i.          |
| Rhizoclonium sp.       | C16       | New Zealand               | Havelock                  | A033075       | n.i.          | LN679075      |
| Rhizoclonium sp.       | D88       | South Africa              | Knysna Lagoon             | A033076       | n.i.          | LN679076      |

n.i.: no information; LSU: large subunit; ITS: internal transcribed spacer
*WELT: Te Papa Tongarewa National Museum of New Zealand herbarium collection number
*GENT: Ghent University (Belgium) herbarium collection number
Species found on aquaculture facilities

Clean-up Kit (Axygen Biosciences, CA, USA) and sequenced bi-directionally using the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, USA).

Sequences generated during this work were deposited in the GenBank-EMBL database and additional sequences of Cladophorales from GenBank were included in the analyses (see Table S1 and Table S2). Furthermore, we included a number of ITS sequences from Bakker (1995) and seven additional Cladophora samples from the Boedeker collection (unpublished) were selected (Table 1). DNA sequences were inspected and assembled using Geneious v6.1.4 (Biomatters Ltd, New Zealand) and manually aligned with BioEdit v5.0.9 sequence alignment software (Hall 1999). Uncorrected pairwise distances were calculated using PAUP (Swofford 2002). For the LSU alignment, five species (C. coelothrix Kützing, C. socialis Kützing, C. sibogae Reinbold, C. prolifera (Roth) Kützing, and Siphonooclados tropicus (P.L. Crouon and H.M. Crouon, C. Agardh) were used as outgroup, while Rhizoclonium sp. served as the outgroup for the ITS analysis.

Each DNA alignment (LSU and ITS2 rDNA) was analyzed independently with both Maximum Likelihood (ML) and Bayesian Inference (BI). Best-fit models of evolution were estimated for each alignment using TREEFINDER v1.2.2.0 (Jobb et al. 2004). Maximum Likelihood analyses
were carried out using PhyML v3.0 (Guindon et al. 2009), and the reliability of internal branches was assessed using 1000 bootstraps with subtree pruning-regrafting branch swapping. Bayesian tree reconstructions with posterior probabilities were inferred using MrBayes v3.2.2 (Ronquist et al. 2012), using the same model of DNA evolution as for the ML analyses. Four simultaneous Markov chains were run for 1,000,000 generations with trees sampled every 10 generations, with 25% of initial trees discarded as ‘burn-in’.

**Results**

**Morphological analysis**

Of the 21 Cladophora samples analysed morphologically and genetically, three distinct morphospecies were identified (Table 1). The majority (n = 18) corresponded to *C. ruchingeri* (C.Agardh) Kützing, 1845. Key characteristics for this species include: a sparsely branched thallus growing mainly by intercalary cell divisions, with few long branches originating from pseudo-dichotomies that have the same thickness as the main axis (van den Hoek 1963, 1982 and Figure 3); and terminal branches are short and relatively stiff with cell walls greater than 5 µm, never more than one lateral per cell. Macroscopically, these samples can be described as filamentous green algae displaying very long, sparsely branched thalli that become intertwined and form rope-like strands (see van den Hoek 1963, 1982).

Two samples (CAW#21, CAW#23; Table 1) were characterised by much smaller cell diameter in the terminal branches (10–50 µm), which were more densely branched than *C. ruchingeri*, and were identified as *C. albida* (Nees) Kützing. The remaining sample (CAW#36; Table 1) was not analyzed morphologically but identified genetically (see below).

**Phylogenetic analysis**

The LSU sequence alignment (n=49 sequences) was 553 bp long (including gaps). The model of evolution corresponded to the General Time Reversible (GTR; Lanave et al. 1984) + Gamma model (Gamma value = 0.22). Four *Cladophora* sp. samples collected in four distinct locations of the Marlborough Sounds (CAW #1 to #4; Table 1; Figure 1) yielded identical LSU sequences to previously published sequences of *Cladophora ruchingeri* (Table S1), and clearly grouped within the well-supported clade of marine *Cladophora* species. Other closely related species include:

**Figure 3.** Morphology of *Cladophora ruchingeri*. (A) Upper part of sparsely branched thallus with simultaneous ramifications in the apical region, younger branches intercalated between older branches. (B) Upper part of thallus showing sparsely branched habit, older sidebranches long and unbranched. (C) Lower part of thallus showing sparsely branched habit with long sidebranches. Secondary laterals are rare. (D) Sidebranch twisted around the main axis, a typical feature of *C. ruchingeri* that might contribute to the rope-like strands often formed by this species. (E) Pseudodichotomous branching, the lateral branch being shifted to the apical pole of the cell. Scale bars: (A)–(C) 500 µm, (D) 200 µm, (D) 100 µm.

*C. albida*; *C. laetevirens* (Dillwyn) Kützing; *C. sericea*; and *C. capensis* (C. Agardh) De Toni. This clade of marine *Cladophora* species is sister to a clade containing the brackish species *C. vagabunda* (L.) C. Hoek and the freshwater species *C. glomerata* (L.) Kützing.

The Cladophorales samples (n=21) collected from sites in the Marlborough Sounds (including the four samples analysed using LSU, see Table 1; Figure 2) were investigated phylogenetically using the 5.8S/ITS-2 gene region to increase phylogenetic resolution within the true *Cladophora* clade (see discussion for terminology). The sequence alignment (n = 49 sequences) was 847 bp (including gaps). The model of evolution corresponded to the GTR + Gamma model (Gamma value = 0.56). Eighteen samples clearly corresponded to *C. ruchingeri* in a highly supported clade (Figure 5), with negligible pairwise
distances of maximum 0.6%. Among remaining sequences, we found two specimens of *C. albida* (CAW#21, CAW#23), and one specimen which was a close match (98% sequence similarity; BLASTn e-value of 0) to the ITS sequence JQ308254 of *C. hutchinsioides* Hoek and Womersley (CAW#36, no voucher/morphological identification available), differing by only two bp.

**Discussion**

**Taxonomical and biogeographical considerations**

All *Cladophora* samples from the Marlborough Sounds grouped with marine *Cladophora* species, which are sister to a clade containing the brackish species *C. vagabunda* and the freshwater species *C. glomerata*. Together, they represent the true *Cladophora* clade of this polyphyletic genus. Morphological and phylogenetic analyses revealed the identity of the dominant *Cladophora* species from the mussel farms in the Marlborough Sounds to be *C. ruchingeri*.

The LSU and ITS2 sequences of *C. ruchingeri* were virtually identical to sequences previously identified as *C. flexuosa* (O. F. Müller) Kützing (Cflex84.82, Rua84.60, and Rua84.24 [Bakker 1995] and UTEX LB2875 [Ichihara et al. 2013], Tables S1 and S2), with a maximum difference of only 0.4% in ITS2. According to van den Hoek (1963), *C. flexuosa* should be regarded as a synonym of *C. sericea*. However, based on the genetic data presented here (Figures 4 and 5), it is clear that the ‘*C. flexuosa*’ samples do not represent *C. sericea*, but *C. ruchingeri* instead. Since young individuals of *Cladophora ruchingeri* are morphologically similar to *C. sericea*, we assume that the ‘*C. flexuosa*’ samples corresponded to juvenile individuals that had not been correctly identified and instead represent *C. ruchingeri*.

*Cladophora ruchingeri* has previously been recorded from the European Atlantic and the Mediterranean (van den Hoek 1963) as well as the Atlantic coasts of the USA (van den Hoek 1982). In addition, this species has been confirmed in Ghana (van den Hoek 1982) and has been reported in Venezuela (Ganesan 1990), resulting in a tropical to temperate amphi-Atlantic distribution. The finding of *C. ruchingeri* in New Zealand represents the first record of this species for the Southern Hemisphere and for the Pacific region.

Sample CAW#36 differs by only two bp from the published ITS sequence of *C. hutchinsioides* (GenBank no. JQ308254) from China, and must thus be regarded as conspecific. *Cladophora hutchinsioides* has been described from southern Australia (van den Hoek and Womersley 1984), and has also been reported from East Asia (van den Hoek and Chihara 2000; Zeng 2009). This species has been reported to grow on a commercial fishing boat in South Australia (van den Hoek and Womersley 1984), and on fishing nets and ropes as well as oyster cages in Japan (van den Hoek and Chihara 2000). Thus, there is a possibility that this species is spreading globally via fishing vessels and equipment. This is the first record of this species for New Zealand; however, the lack of distribution data means we cannot determine whether it is a recent arrival.

*Cladophora albida* has not been recorded previously in New Zealand, but this species has a cosmopolitan distribution and can be assumed to be a natural part of New Zealand’s algal flora (Nelson 2013). *Cladophora albida* has also been collected in other parts of New Zealand (e.g. Auckland, Taranaki, Wellington, Kaikoura; C. Boedeker, unpublished data).

*Cladophora ruchingeri*, a new marine pest of mussel farms in New Zealand

*Cladophora ruchingeri* is a poorly known species. In its young stages, it is difficult to differentiate from several other marine *Cladophora* species (e.g. van den Hoek 1963). However, the long, sparsely branched thalli of mature individuals that intertwine into rope-like threads are very characteristic (e.g. van den Hoek 1963, 1984). In an earlier, comprehensive, survey of marine algae along the coasts of northern South Island of New Zealand (Nelson et al. 1992), the only dominant species reported from the Marlborough Sounds was *C. subsimplex*, a species that is morphological distinct from *C. ruchingeri*. If present in its mature form, it is unlikely that *C. ruchingeri* would have been overlooked or misidentified in previous surveys of the Marlborough Sounds (Hurd et al. 2004; Nelson 2013).

Chinook salmon (*Oncorhynchus tshawytscha* (Walbaum, 1792)) and green-lipped mussel farms have been present in the Marlborough Sounds region for ca. 30 years, and culture intensity has not increased markedly in the last 10 years (see further discussion below). This in concert with *C. ruchingeri*’s distinctive macroscopic appearance (when mature), its absence from previous Marlborough Sounds surveys, and the fact that it has not previously been reported from the Southern Hemisphere or Pacific region suggest that this species has only recently arrived in New Zealand.
Invasion of Cladophora in mussel farms

Figure 4. Phylogenetic reconstruction of Cladophorales species inferred from partial large subunit (LSU) rDNA based on Maximum Likelihood (ML). The tree includes 49 LSU sequences. For each sequence, the species name is followed by the geographical origin of the specimen and sequence accession number. The numbers at nodes correspond to bootstrap values in ML and posterior probabilities (PP) in MrBayes analyses. Values <70 ML and <0.8 PP are replaced by a dash (-); Branches at nodes displaying both values of <50 ML and <0.5 PP were manually collapsed. Grey area indicates Cladophora ruchingeri specimens collected in the Marlborough Sounds.

Zealand. This is further substantiated by the identification of this species primarily on artificial substrates used in aquaculture, a typical first point of establishment for non-indigenous organisms (Minchin 2007). An assessment of the diversity of New Zealand marine Cladophorales using a polyphasic approach is required to confirm this hypothesis.

Human activities in the Marlborough Sounds area, particularly aquaculture and vessel movements, are a significant mechanism for the introduction, establishment, and spread of marine pests (Acosta and Forrest 2009; Woods et al. 2012). The large surface area provided by farming floating structures (e.g., ropes, floats, pontoons) provide ideal habitat for biofouling organisms, such as C. ruchingeri,
Figure 5. Phylogenetic reconstruction of Cladophorales species inferred from partial 5.8S and internal transcribed spacer 2 (ITS2) rDNA gene based on Maximum Likelihood (ML). The tree includes 49 sequences. For each sequence, the species name is followed by the geographical origin of the specimen and sequence accession number. The numbers at nodes correspond to bootstrap values in ML and posterior probabilities (PP) in MrBayes analyses. Values <70 ML and <0.8 PP are replaced by a dash (-). Branches at nodes displaying both values of <50 ML and <0.5 PP were manually collapsed. Grey areas indicate Cladophora spp. specimens collected in the Marlborough Sounds.

to proliferate (Forrest and Blakemore 2006; Minchin 2007). Once floating artificial structures become infected, they can act as a reservoir for the spread of the pest into adjacent areas (Bulleri and Airoldi 2005; Carver et al. 2003). For example, high densities of C. ruchingeri on floating structures in the Marlborough Sounds may provide a propagule supply to natural habitats, where it has also been recorded in large abundance, especially during summer months (J. Atalah; pers. observation).

Cladophora ruchingeri has the potential to negatively impact the mussel industry by overgrowing and smothering mussel lines. This
can increase their weight and lead to crop losses, affect seed-stock survival, and result in additional production and processing expenses (Fitridge et al. 2012; Fletcher et al. 2013). In natural habitats, Cladophora species are regarded as ecological engineers, where they have the ability to alter the flow of organic matter and light and provide a structurally complex habitat for benthic microfauna (Zulkify et al. 2013). Several Cladophora species form conspicuous algal mats in coastal and freshwater systems worldwide (Dodds 1991; Zulkify et al. 2013). These mass developments frequently occur in brackish estuaries and lagoons, where nutrients accumulate and competition is reduced (Flindt et al. 1997; Curiel et al. 2004; Gubelit and Kovalchuk 2010).

Many Cladophora species, including C. ruchingeri, have high growth rates, short generation times, broad ecological tolerance, and produce significant quantities of offspring via the release of large amounts of asexual spores, resulting in large seasonal populations (Dodds and Gudder 1992; Zulkify et al. 2013). Several species of Cladophora can form akinetes, thick-walled resting stages that can persist during unfavourable conditions (e.g. van den Hoek 1963; Whiton 1970; Dodds and Gudder 1992). These features provide some species of Cladophora with a competitive advantage over other macroalgae and this may explain why C. ruchingeri has become problematic in the Marlborough Sounds.

An alternative hypothesis for the sudden appearance of C. ruchingeri in recent years is a change in environmental conditions (e.g., nutrient enrichment) that has facilitated the rapid increase in biomass. Most Cladophora species have high nutrient requirements, and phosphorus seems to be the limiting factor for growth (e.g. Whiton 1970; Dodds and Gudder 1992). However, the Marlborough Sounds are considered to be low-mesotrophic, with moderate nutrient concentrations and primary productivity (Gibbs et al. 1992; Zeldis et al. 2008). There has been no evidence of an increase of nutrient concentration in the Marlborough Sounds at a regional scale in recent years (Gibbs et al. 1992; Zeldis et al. 2008). Nevertheless, mussels’ biodepositions (i.e. faeces and pseudofaeces) and ammonium excretion result in localised nutrient enrichment (Kaspar et al. 1985), which may stimulate the mass proliferation of C. ruchingeri attached to farm structures. Further studies of C. ruchingeri blooms and nutrients availability around aquaculture farms are required to test this hypothesis.

**Conclusions**

Within the past five years, large Cladophora blooms have been observed on the ropes of green-lipped mussel farms in the Marlborough Sounds. These blooms represent a nuisance for the mussel farming industry and may alter ecosystem functioning. We identified this green filamentous alga as C. ruchingeri and believe this species has only recently arrived in New Zealand. This study is a crucial first step for understanding C. ruchingeri ecology and distribution, and in the development of management strategies to minimise risks for the aquaculture industry and protect natural values.

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The following supplementary material is available for this article:

**Table S1.** List of 49 Cladophorale species included in the phylogenetic analysis of LSU rDNA gene (Figure 4).

**Table S2.** List of 49 Cladophorale species included in the phylogenetic analysis of ITS rDNA gene (Figure 5).

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