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Use of environmental DNA (eDNA) and water quality data to predict protozoan parasites outbreaks in fish farms

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ABSTRACT

Globally, disease accounts for around 40% of lost aquaculture production. Commonly, disease occurs due to an inability of farm managers to accurately quantify disease risk due to the abundance of the pathogenic agent in their production systems, along with a poor understanding of how outbreaks are linked to changes in water quality parameters. Environmental DNA (eDNA) sampling methodology associated with molecular techniques is suited to rapidly assess the background presence of pathogens in fish farms, thereby providing managers with critical information that can be used to mitigate disease threats. Adopting the ciliate protozoan *Chilodonella hexasticha* as a model, this study examined the relationship between environmental DNA of *C. hexasticha*, critical water parameters and the occurrence of disease outbreaks on a commercial barramundi *Lates calcarifer* farm, where water was sampled monthly over a 1 year timeframe. A qPCR assay based on the small subunit ribosomal DNA gene was used to monitor the abundance of *C. hexasticha* in pond water (SSU-rDNA copies/μL). Increased *C. hexasticha* eDNA levels were found to be highly correlated with occurrence of later fish mortality events ($r = 0.402$; $P < 0.001$) and also with size of fish ($r = -0.189$; $P < 0.05$); smaller fish were more prone to being impacted by an epizootic of the parasite. However, no correlations were found between any of the water quality parameters measured (rainfall, water temperature and dissolved oxygen) and abundance of this parasite, although there were significantly more fish mortalities observed during the warmer, wetter monsoonal season compared to the cooler, dry season (1280 vs. 135 mortalities, respectively; $P < 0.05$). This study highlights the potential of an eDNA approach as a management tool to quickly assess parasite loads in water and minimise the risk of disease outbreaks in aquaculture systems.

1. Introduction

Food security is predicted to be a global challenge as the human population reaches nine billion people (FAO, 2014). Aquaculture, which is currently the fastest growing agribusiness, will be a major supplier of future animal protein requirements for this expanding population (FAO, 2016). However, disease currently results in approximately 40% of lost production potential (~USD$102 billion), from direct (e.g. mortalities) and indirect (e.g. additions of chemicals, waste of feed) factors (FAO, 2012). Consequently, early disease detection and management is critically important for future food production from aquatic farm environments.

Environmental DNA, also known as eDNA, is a novel front-line molecular tool that has the potential to change the way detection and monitoring of disease occurs in aquaculture (Bass et al., 2015). The technique enables non-invasive sampling and detection based purely on the collection of water samples when there is no visible presence of the target organism (Robson et al., 2016). The eDNA approach was first applied by Ogram et al. (1987) to understand microbial communities in sediments. However, it was not until Ficetola et al. (2008), who used eDNA on the American bullfrog, *Rana catesbeiana*, as a model for invasive species studies, that this technique started to grow in popularity as a tool to detect biodiversity in aquatic systems. Due to its detection power and relative ease application, environmental DNA has now been applied to address questions related to microbial community diversity, evolution, ecology and even interactions between hosts and pathogens

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(Goldberg et al., 2015; Taberlet et al., 2012; Bass et al., 2015).

Environmental DNA methodologies offer potential to improve animal health monitoring systems in aquaculture, as many pathogens are microscopic, have waterborne life-stages, are hard to directly detect, and clinical signs of diseases are often only observed in advanced stages of infection where subsequent treatment options become ineffective (Bass et al., 2015). Traditional diagnostics, such as histopathology and morphological identification, are time consuming and often lack detection sensitivity unless large numbers of samples from animals are processed, or only detect the presence of a parasite on the species under culture once a widespread epizootic outbreak is in progress (Adrian-Kalchhauser and Burkhardt-Holm, 2016; Bastos Gomes et al., 2016). Through monitoring levels of parasites in water, however, eDNA methodologies may offer a simple early detection and disease risk assessment technique before animals became clinically symptomatic, merely through the sampling of water from ponds or the surrounding aquatic ecosystem. Furthermore, coupling routine eDNA sampling, quantitative PCR (qPCR), fish production data and environmental water parameters may lead to new understandings of the key environmental drivers of disease outbreaks and quantitative prediction of the likelihood of fish mortalities.

Ciliate protozoans are considered among the most economically important parasites for finfish aquaculture (Lom and Dyková, 1992; Bastos Gomes et al., 2016), with *Chilodonella* spp. (Phyllopharyngea:Chilodonellidae) being particularly harmful to farmed freshwater fishes (Padua et al., 2013; Bastos Gomes et al., 2016). The presence of large numbers of *Chilodonella* cells in water predispose their attachment to fish gills and skin epithelial cells, causing severe pathology (Noga, 2010; Mitra et al., 2013; Padua et al., 2013). Chilodonellosis (disease caused by *Chilodonella* spp.) progresses rapidly, causing mortalities within two to three days of infection and losses of 50–95% in fish stocks (Paperna and Van As, 1983; Karvonen et al., 2010). Rapid detection of *Chilodonella* spp. can be challenging as epidemics often occur without warning (Bastos Gomes et al., 2016). The use of eDNA-based technologies offer a novel approach for the early detection of increasing numbers of *Chilodonella* spp. in water and can alert farmers to implement pre-emptive, rather than reactive management to minimise production losses.

The aim of this study was to demonstrate the applicability of integrating eDNA-based techniques and farm water quality parameters as a management tool for predicting future parasite epizootics in commercial fish farms. Here quantitative real-time PCR (qPCR) was used to determine the presence and abundance of *C. hexasticha* in aquaculture ponds and relationships between parasite loads in water, environmental conditions and large-scale fish mortality events were examined to highlight how eDNA methodology may help understand environmental drivers of disease outbreaks.

2. Material and methods

2.1. Pond selection and collection of water samples

Water samples were collected from ~1.4 ha earthen ponds (~20 Megalitres; ML) within a commercial freshwater barramundi, *Lates calcarifer* (Bloch), farm near Innisfail, north Queensland, Australia, monthly for one year (from October 2013 to September 2014, except March 2014) (Fig. 1). Four ponds were selected for regular sampling which had a history of *Chilodonella* infections and another four ponds were opportunistically chosen based on the farm's health reports on the presence of stressed fish and no considerable reduction of ciliate numbers post chemical treatment (CC, personal communication). Triplicate 15 mL pond water samples were collected approximately 1 m from the edge of ponds (30 cm below the water surface) using individual plastic cups attached to a 1 m long pole in three different sites within each pond (i.e. total of nine water samples per pond) and carefully poured into 50 mL centrifuge tubes containing 1.5 mL of sodium acetate (3 M) and 33.5 mL of absolute ethanol for DNA preservation (modified from Ficetola et al., 2008). To ensure there was no cross contamination among tubes during sampling, or DNA extraction, negative control samples (i.e. distilled water transported to the field from our laboratory) were also taken at each sampling site. Immediately following collection, tubes were stored on ice until transportation to the laboratory for processing.

2.2. Collection of *Chilodonella hexasticha* cells, environmental parameters and mortality data

Parasite cells were collected between October 2013 and September 2014 for species identification and validation of a quantitative PCR (qPCR) assay. Barramundi showing behavioural signs of infection (ranging from loss of appetite to gasping at pond edges) were selected for sampling. Animals were sedated using AQUI-S (AQUI-S, New Zealand Ltd) and samples from the skin and gills were scraped gently using the blunt edge of a scalpel blade to prevent bleeding. Mucus was spread on a glass slide and the fresh gill mount examined under a light microscope (200 ×) to confirm infection by *C. hexasticha*. Sixty slides containing *C. hexasticha* cells were selected for silver staining and morphological description. *Chilodonella hexasticha* was identified on the slides using comparative morphology techniques (Bastos Gomes et al., 2017). Sixty samples containing part of the gill or mucus with *Chilodonella* spp. were preserved in 80% ethanol for species characterisation through genetic tests as outlined below.

Environmental and biological parameters were monitored on the farm throughout the study. Dissolved oxygen and water temperature (maximum and minimum) measurements were taken three times a day with an YSI Pro20 meter with a galvanic dissolved oxygen sensor (YSI Inc). Daily rainfall data was obtained from the Australian Government Bureau of Meteorology website (http://www.bom.gov.au/climate/data/index.shtml?bookmark=136), based on readings from Mena Creek Post Office station (8.6 km away from farm). Mean fish weight was obtained by measurements of 200 animals/pond three times over the year and also during fish manipulation (e.g. fish transfer from one pond to another). Mean fish weight in each pond was based on phenotypic records and growth curve algorithms based on feed consumption and water temperature (Glencross, 2008). Fish mortalities were recorded by farm technicians who visually surveyed and removed dead fish from ponds three times per day.

The relationship between water quality parameters and the abundance of *C. hexasticha* SSU-rDNA copies/μL (eDNA) was assessed using the mean maximum water temperature, minimum dissolved oxygen levels and rainfall over a consecutive 5 day period prior to water sampling for each pond. Likewise, the relationship between *C. hexasticha* SSU-rDNA copies/μL in pond water and fish mortality was examined using the mean number of dead fish recorded over the 5 day period following water sampling in each pond.

2.3. DNA extractions

A direct eDNA precipitation and extraction method was adopted, whereby above mentioned sample tubes containing pond water and preservative were centrifuged at 3200*g* for 60 min at 6 °C and the resulting supernatant discarded. The genetic material then present in the resulting pellet was extracted using a CTAB (cetyl trimethyl ammonium bromide) DNA extraction protocol (modified from Edwards et al., 1991) and resuspended in 60 μL of 1 × TE buffer. DNA quality was checked using a 0.8% agarose gel and DNA quantified with a Nanodrop (ND-1000 Spectrophotometer, Thermofisher Scientific). Pond water samples yielded relatively high concentrations of genetic material (mean ± S.D. = 28.88 ± 17.63 μg DNA/μL). Environmental DNA extracted from the three 15 mL water samples taken at each collection site were pooled together, resulting in three eDNA samples analysed per pond.

CTAB (cetyl trimethyl ammonium bromide) extracted DNA from *C.*

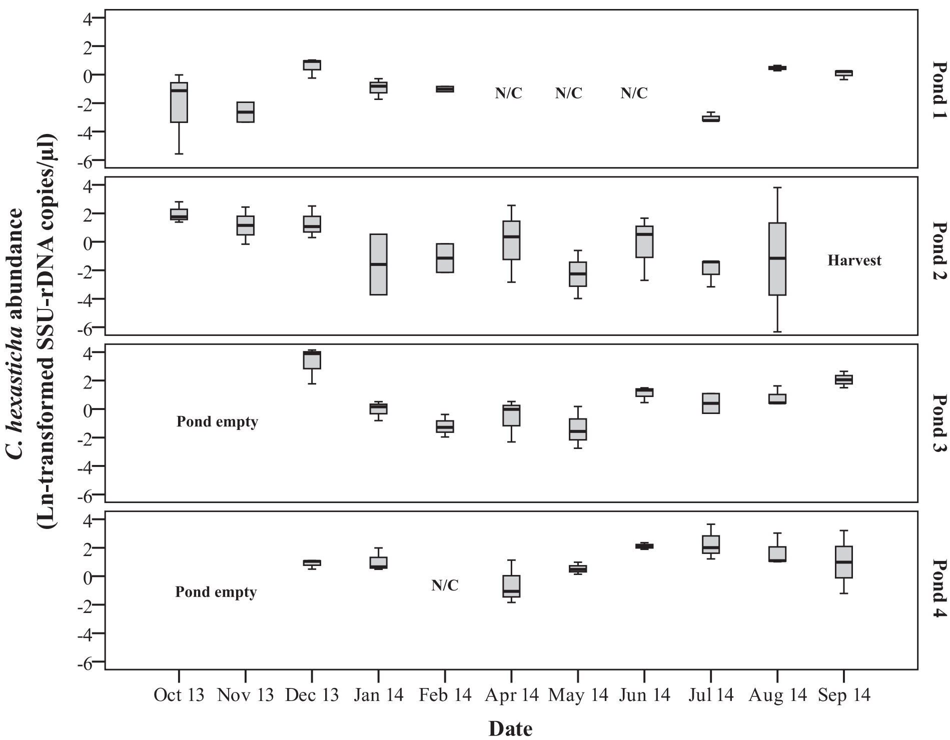

Fig. 1. Variation of *Chilodonella hexasticha* abundance (Ln-transformed SSU-rDNA copies/µL) in pond water between Oct 2013 and September 2014, as assessed by the eDNA technique. Ponds 1 to 4 were selected for regular sampling due to history of *Chilodonella* outbreaks. N/C, sample not collected due to chemical treatment applied to ponds.

Table 1
Specific primers and probes to target SSU-rDNA gene from *C. hexasticha* for Quantitative PCR.

Primer/probe	Primer/probe sequence 5′ → 3′	Length (bp)	Tm of amplicon (°C)	Reference
FSSU Chilo primer	CGCAAGGCTGAAACTTAAAGGA	85	N/R[a]	This study
RSSU Chilo primer	CCCCGTGTTGAATCAAATTGA			
SSU Chilo probe	CTCCTGGTGGTGCCCT-FAM			
985F	GAGTATGGTCGCAAGGCTGAAAC	162	82.75	Bellec and Katz, 2012
1147R	GCACCACCATCCCTCAAATCAAG			

[a] Not relevant for TaqMan chemistry.

hexasticha cells isolated with the aid of a light microscope from gills and mucus of infected fish was used as template for validation and positive controls of the qPCR assay described in the following section. To determine the absolute concentration of *Chilodonella hexasticha* (SSU-rDNA copies/µL) of the DNA template, digital PCR reactions were performed using QuantStudio™ 3D Digital PCR Master Mix v1, forward and reverse primers and a TaqMan probe (specified in Table 1) and a QuantStudio™ 3D Digital PCR Chip v1, in a ProFlex™ 2 × Flat PCR System, following the manufacturer's instructions (Thermofisher Scientific). Thermal cycling conditions were: initial 10 min step at 96 °C, followed by 39 cycles of 60 °C for 2 min and 98 °C for 30 s and final step of 60 °C for 2 min.

2.4. Quantitative PCR (qPCR) assay design

A qPCR assay targeting *C. hexasticha* small subunit ribosomal DNA (SSU-rDNA) gene region was developed to quantify cell copy abundance in water samples. To avoid contamination, dedicated pipettes, tips, plasticware and gloves were UV sterilized in biosafety cabinet for 30 min before use. Five different brands of master mixes (Power SYBR green master mix - Thermofisher Scientific; QuantiFast Probe PCR Master Mix – QIAGEN; TaqMan universal master mix - Thermofisher Scientific; Kapa probe fast universal master mix - Kapabiosystems; SsoFast EvaGreen master mix -Bio-Rad) were tested against our positive control and random eDNA samples at different dilutions to evaluate qPCR reaction efficiencies and potential inhibitory effects of eDNA. A 1:10 dilution of eDNA extracts in ultra-pure distilled water (Invitrogen) was sufficient to overcome potential qPCR inhibitory effects (Bott et al., 2010). Quantitative PCR reactions and cycling conditions were validated using six serial dilutions of the *C. hexasticha* DNA template, run in triplicates at concentrations ranging from 1.1×10^{-1} to 1.1×10^{3} SSU-rDNA copies/µL, as a standard curve with the following parameters was obtained: Efficiency = 1.01 (assessed as $E = (10^{-1/\text{slope}}) - 1$; Bellec and Katz, 2012), slope = 3.31 and $R^2 = 0.9977$. Quantitative PCR reactions comprised of 7.5 µL of SsoFast EvaGreen master mix (Bio-Rad), 0.6 µL of each primer (600 nM of 985F and 1147R; Table 1), 1.3 µL of ultra-pure distilled water (Invitrogen) and 5 µL of 1:10 diluted eDNA on a Corbett Rotor-Gene 6000 system (QIAGEN). Quantitative PCR runs were performed with each sample in triplicate with the addition of two positive control samples of known concentrations derived from the standard curve and a non-template control (ultrapure water). Thermal cycling conditions comprised of an initial 2 min step at 50 °C and 10 min 95 °C, followed by 40 cycles of denaturation at 95 °C for 15 s and annealing/extension at 60 °C for 1 min and a final melt curve analysis with the temperature increasing from 50 °C to 95 °C at a rate of 0.5 °C every 10 s to ensure product specificity (Tm amplicon 82.75 °C). In addition, from a total of 147 water samples used for qPCR amplification and where eDNA was detected, 30% were Sanger sequenced and all confirmed to be within *C. hexasticha* SSU-rDNA gene region with 100% NCBI BLASTN sequence alignment identity to GenBank KY508243.1 (Bastos Gomes et al., 2017). Quantification cycle (Cq) values of each technical triplicate serial dilution were averaged (mean) and multiplied by 10 (dilution factor). Samples with Cq values outside of the linear range of the standard curve and samples with melting profiles different than the expected (Tm amplicon 82.75 °C) were not considered for further analysis. Non-template controls of all qPCR runs were negative. The relationship between the qPCR Cq values and the number of *C. hexasticha* was established by linear regression of the standard curve (SSU-rDNA copies/μL).

3. Statistical analyses

Linear regression was used to assess the ability of measured variables (i.e. abundance of *C. hexasticha* (eDNA), rainfall, dissolved oxygen and water temperature and fish weight), to predict fish mortalities observed in the 5 days post water sampling. Furthermore, linear regression was also used to evaluate whether any of the observed environmental variables could predict the abundance of *C. hexasticha* in the water samples. Preliminary analyses were conducted to ensure no violation of the assumptions of normality, linearity, multicollinearity and homoscedasticity. Fish weight, fish mortalities and eDNA (*C. hexasticha* abundance in water samples) data were *Ln* transformed to conform to a normal distribution prior to analysis (Kolmogorov–Smirnov test, $P > 0.05$).

The association between the observed variables, and more importantly, their association with the abundance of parasite cells in pond water, was examined using a principal component analysis (PCA). Here, the original set of six variables of interest were reduced into two principal components for which eigenvalues were > 1 and represented as a two-dimensional plot. To explore the strength, direction and significance of relationships between variables, a correlation matrix based on Spearman rho (r) bivariate analyses was constructed.

4. Results

Environmental parameter data from eight farm ponds sampled between October 2013 and August 2014 were analysed. Table 2 shows the statistical summary for all environmental parameters tested in this study. Quantification of *C. hexasticha* in barramundi farm water varied between ponds, sampling sites (within ponds) and from month to month. Although the abundance of *C. hexasticha* in water samples (SSU-rDNA copies/μL) was extremely variable, the parasite was constantly present in farm water (Fig. 1). The abundance of *C. hexasticha* (SSU-rDNA copies/μL) in pond water was found to be positively correlated with fish mortalities ($r = 0.402$; $P < 0.001$), and negatively correlated with fish size ($r = -0.189$; $P < 0.05$) (Table 3). Fish mortalities were also strongly and positively correlated with warmer water temperatures ($r = 0.448$; $P < 0.001$). As expected in the tropics, warmer water temperatures were also strongly and positively correlated with rainfall ($r = 0.415$; $P < 0.001$) and negatively correlated with dissolved oxygen levels ($r = -0.526$; $P < 0.001$) (Table 3). Results from linear regression indicated that the abundance of the ciliate parasite ($\beta = 0.272$, $P = 0.005$) and fish weight ($\beta = -0.450$, $P < 0.001$) explained 41% of the variance in observed fish mortality ($F_{5,\ 68}$) $= 11.008$, $P < 0.001$), whereas rainfall, water temperature and dissolved oxygen were not significantly associated with parasite abundance ($P > 0.05$). Decreased rainfall ($\beta = -0.200$, $P = 0.026$) and fish weight ($\beta = -0.192$, $P = 0.038$) were also associated with *C. hexasticha* abundance ($F_{DF\ 4,\ 139}$) $= 3.385$, $P = 0.011$), although these parameters explained only 6.3% of the variance observed.

During the tropical wet season (December to February) the warmest water temperatures were observed, along with the highest monthly rainfall and the lowest dissolved oxygen (Figs. 1 and 2). Between December and April, when water temperatures were above 27 °C and daily rainfall were higher than 40 mm, mean fish mortality followed similar trends to the mean abundance of *C. hexasticha* in the water (Fig. 2A, B). During this period (December to April), fish mortality (n = 1280) was significantly higher than between May and November (n = 135) (Mann-Whitney *U* test, $P < 0.05$).

Two principal components derived from PCA explained 66% of the variance among monitored farm parameters (Fig. 3). A positive association was observed between rainfall and water temperature (quadrant II; Fig. 3), which were inversely related to dissolved oxygen levels (quadrant I; Fig. 3). Abundance of *C. hexasticha* in pond water (eDNA) and fish mortalities were directly associated (quadrant II; Fig. 3) and inversely related to fish weight.

Table 2
Summary statistics for environmental and biological parameters tested from eight ponds from a freshwater barramundi, *Lates calcarifer*, farm from tropical Australia in 2013/14. n = number of observations.

Variables	n	Mean	Minimum	Maximum	SD	SE
Chilodonella hexasticha in water (SSU-rDNA copies/μL)	147	10.97	0.00	412.90	40.01	0.19
Water temperature (°C)	52[a]	26.63	21.3	30.34	2.72	0.22
Dissolved oxygen (mg/L)	52[a]	3.86	1.95	6.73	1.26	0.10
Mortality[c](fish/day)	53[b]	41.19	0.00	1631.00	230.77	19.03
Rainfall (mm)	55[a]	7.53	0.00	85.00	14.81	1.99
Fish weight (g)	53	1213.4	25.5	4147.6	1108.10	91.4

SD = standard deviation; SE = standard error.
[a] Mean data five days prior to eDNA water sampling.
[b] Mean data five days following eDNA water sampling.
[c] Mortality recorded five days following eDNA sampling.

5. Discussion

Environmental DNA (eDNA) analyses are revolutionising how aquatic biodiversity surveys are conducted and also have an application in the detection of pathogens impacting on aquaculture. In the present study, an eDNA approach was used to quantify the abundance of an important parasitic protozoan that causes chilodonellosis in commercial freshwater finfish aquaculture. We show that eDNA is a powerful approach to detect and quantify levels of *C. hexasticha* in commercial barramundi aquaculture ponds and that there were significant correlations between abundance of this parasite and subsequent fish mortality events. Given that severe fish mortalities can occur with no warning and there are practical difficulties in directly identifying ciliate parasites in pond water and/or on cultured fish, the direct quantification of parasite DNA from water samples can provide farm managers with an efficient tool to assess the risk of future disease outbreaks.

As shown in the present study, environmental DNA and qPCR can be used to monitor abundance of *C. hexasticha* in fish farms and to preempt the likelihood of mortalities, particularly during early stages of fish culture. High numbers of *C. hexasticha* in pond water in this study predisposed smaller fish to high mortalities (Table 2, mean = 41.2 fish/day), whereas increased rainfall and decreased dissolved oxygen levels were not directly linked to fish mortalities, or to parasite abundance. Water temperature, however, was shown to be correlated with fish mortality events. Other environmental and biological factors, in addition to those measured and reported in this study, may be promoting increased abundance of *C. hexasticha* in fish ponds. Parameters such as ammonia, pH, the availability of organic matter and the association with other microbial (e.g. bacterial, protozoan, microalgae) communities, can impact ciliate protozoan demographics (Lynn, 2008) and could provide further insights into the population dynamics of *C. hexasticha* in farmed barramundi water ponds.

While the relationship between abundance of *C. hexasticha* and fish mortality was evident, the correlation between fish mortality and high rainfall was not significant. Furthermore, low levels of dissolved oxygen had a negative relationship with higher water temperatures and rainfall. These results are expected within aquaculture ponds, as both dissolved oxygen solubility and saturation decreases with increased water temperatures and reduced algal photosynthetic capacity on overcast

Table 3
Spearman's rho correlation coefficient values among variables monitored in the study (below diagonal). Significance (*P*) values are represented in bold above diagonal ($P < 0.05$). Spearman's Rho value $r = 1$ means a perfect positive correlation and the value $r = -1$ means a perfect negative correlation.

Variables	*Chilodonella* (eDNA)	Fish mortality	Fish weight	Water temperature	Dissolved oxygen	Rainfall
C. hexasticha (eDNA)	–	**0.000**	**0.022**	0.585	0.422	0.341
Fish mortality (fish/day)	0.402	–	**0.000**	**0.000**	0.071	0.370
Fish weight (g)	− 0.189	− 0.561	–	**0.000**	0.501	0.782
Water temperature (°C)	0.046	0.448	− 0.386	–	**0.000**	**0.000**
Dissolved oxygen (mg/L)	0.067	− 0.211	0.057	− 0.526	–	**0.011**
Rainfall (mm)	− 0.079	0.106	0.023	0.415	− 0.212	–

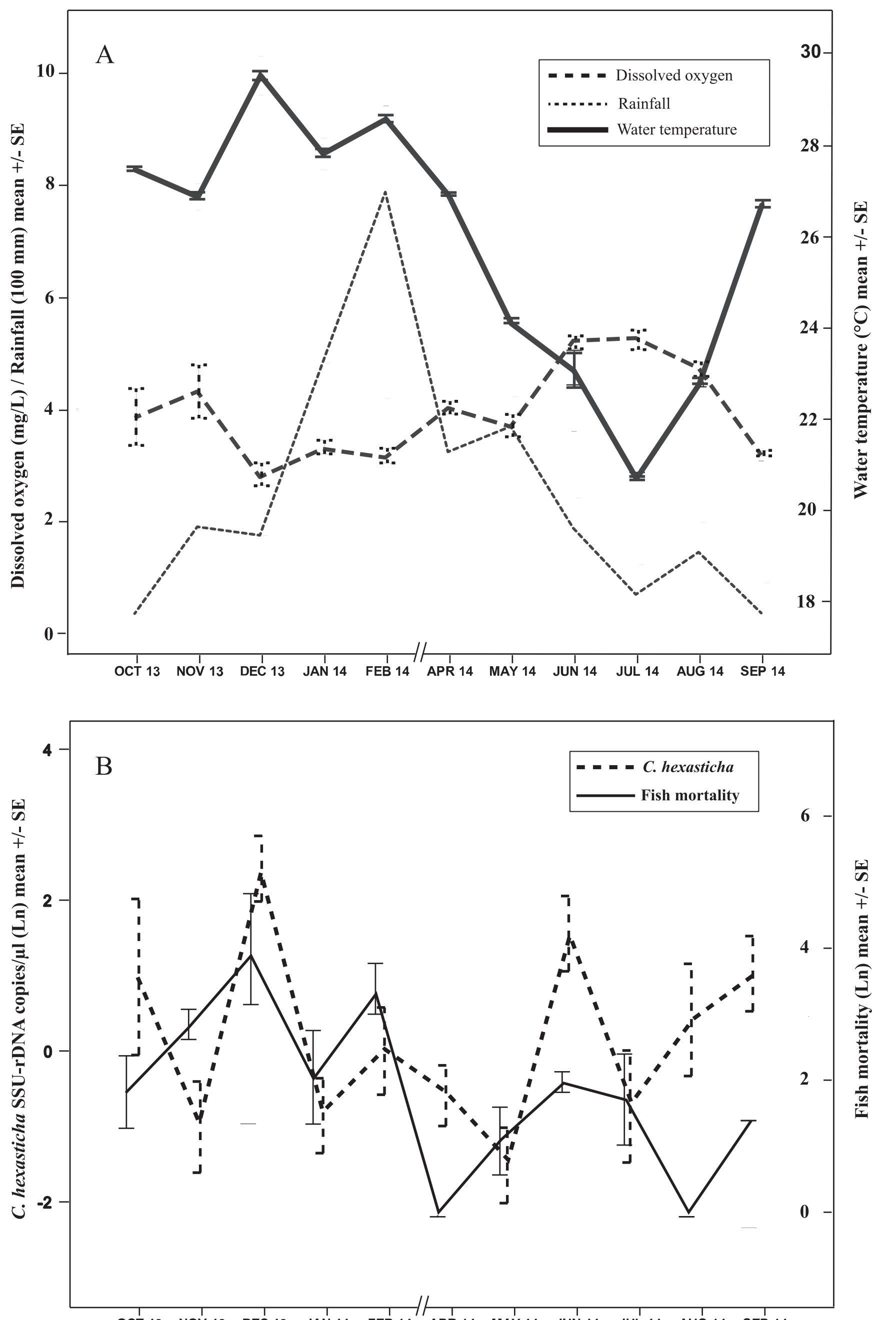

Fig. 2. Environmental and biological parameters from a freshwater barramundi *Lates calcarifer* farm located in North Queensland, Australia in 2013/14. A: mean (SE) dissolved oxygen, water temperature and rainfall; B: mean (SE) abundance of *C. hexasticha* in water (eDNA) and mean fish mortality.

rainy days (Boyd and Tucker, 1998). Smaller fish may have a developing immune system which predisposes them to be more affected by sub-optimal environmental conditions, such as low dissolved oxygen and drastic environmental changes. These changes can cause stress and impair a fish's immune response to pathogens (Alvarez-Pellitero, 2008). Although generally environmental parameters observed alone cannot clearly indicate early problems, when used in an integrated approach with ways to estimate parasite abundance, such as eDNA, environmental variables can help farmers to make the best management decisions to avoid disease outbreaks.

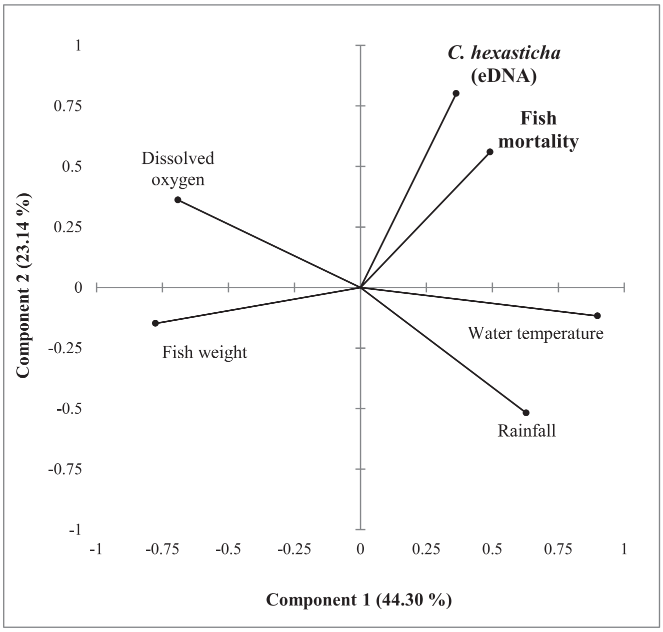

Fig. 3. First and second components of principal component analysis (PCA), showing relationship among variables: abundance of *Chilodonella hexasticha* in *Lates calcarifer* pond water (eDNA), fish mortalities, fish weight, rainfall, dissolved oxygen and average temperature for nine ponds sampled between 2013/14. Mean dissolved oxygen and water temperature were recorded five days prior eDNA collection. Mean fish mortality were recorded five days following eDNA collection.

Abundance of *Chilodonella* in farm water from the fish farm monitored in present study was detected in varied levels across ponds along the year. The specific reasons for variation on *Chilodonella* levels in pond water are difficult to determine based only on our results (Fig. 1). However, parasite density variation may be due to specific pond environmental conditions beyond those monitored in this study (e.g. organic matter, algal blooms, interaction with bacterial community, etc). Furthermore, *Chilodonella* abundance might be influenced by transmission dynamics between parasite and fish, patterns of water exchange in the farm, and physical, chemical and biological characteristics of the water column. It has been reported that in high-stressed aquatic environments (e.g. aquaculture farms) ciliates' population richness tends to be reduced while an increase in resistant species (e.g. potential pathogenic species) is observed (Li et al., 2009).

This study used eDNA to monitor ciliate parasites in a semi-intensive barramundi farm, with focus on the association of environmental parameters and abundance of parasites in water. Molecular genetic quantifications of fish parasites such as *Parvicapsula minibicornis*, *Ceratomyxa shasta*, *Neoparamoeba perurans*, *Kudoa yasunagai* and *Ichthyophthirius multifiliis* have also been used previously to detect parasites in rivers, the open ocean and aquariums (Jousson et al., 2005; Hallett and Bartholomew, 2006; Atkinson and Bartholomew, 2010; Bridle et al., 2010; Hallett et al., 2012; Ishimaru et al., 2014); however, most of these studies primarily focused either on the qPCR assay development itself, or on the seasonal, geographic and temporal distribution of parasites and their association with fish infections, rather than as a predictive tool for day-to-day farm management. If, however, frequent (weekly) water sampling can be performed on farm, potentially stronger correlations between parasite abundance and fish mortalities can be established, allowing fish farmers to promptly adopt preventative health management and early intervention strategies (e.g. such as water exchange, improvement of oxygen levels, stocking timing and size of fish stocked). In our study we collected water samples on a monthly basis with a clear trend evident between parasite abundance and later fish mortality events. More regular sampling than this, however, would be recommended, as water quality parameters can rapidly change over shorter time frames and these may be what was ultimately influencing parasite community structure and downstream fish health.

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