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Broad-scale impacts of salmon farms on temperate macroalgal assemblages on rocky reefs



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ABSTRACT

Intensive fish culture in open sea pens delivers large amounts of nutrients to coastal environments. Relative to particulate waste impacts, the ecological impacts of dissolved wastes are poorly known despite their potential to substantially affect nutrient-assimilating components of surrounding ecosystems. Broad-scale enrichment effects of salmonid farms on Tasmanian reef communities were assessed by comparing macroalgal cover at four fixed distances from active fish farm leases across 44 sites. Macroalgal assemblages differed significantly between sites immediately adjacent (100 m) to fish farms and reference sites at 5 km distance, while sites at 400 m and 1 km exhibited intermediate characteristics. Epiphyte cover varied consistently with fish farm impacts in both sheltered and exposed locations. The green algae *Chaetomorpha* spp. predominated near fish farms at swell-exposed sites, whereas filamentous green algae showed elevated densities near sheltered farms. Cover of canopy-forming perennial algae appeared unaffected by fish farm impacts.

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1. Introduction

Nutrient and sediment inputs from anthropogenic activities can cause changes to habitat structure and diversity in temperate reef ecosystems (Airoldi, 2003; Connell et al., 2008; Krause-Jensen et al., 2008; Worm et al., 1999b). These changes may affect delivery of ecosystem services to society, as well as marine conservation objectives for reef areas, which are disproportionately rich in species compared with other habitats. Whilst much attention has been focussed on terrestrial-derived pollution on reef (Arevalo et al., 2007; Connell et al., 2008; Costanzo et al., 2001; Giordani et al., 2009; Littler and Murray, 1975), eutrophication from marine fish farms may also pose a threat. Most previous monitoring and research programs relating to fish farm aquaculture have focused solely on changes to the soft sediments below and adjacent to farm lease areas, in-water nutrients levels, and phytoplankton populations (Edgar et al., 2010a).

Farmed salmon are commonly grown to maturity in net cages, situated in unpolluted sheltered temperate coastal waters. Salmon rely on nutrient-rich compound aquafeeds as an external food source (Tacon and Metian, 2008). Although improved feeding

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technology has provided a reduction in wasted feed input, Sanderson et al. (2008) suggested that about 70% of the nitrogen and 80% of the phosphorus input to a salmon farm is released to the environment as feed wastage, fish excretion, faeces production and respiration. The majority of these nutrients dissipate in dissolved form. Approximately 87% of nitrogen released from fish farms in the Huon Estuary, Tasmania, is estimated to be in dissolved form and 13% as particulate matter (HEST, 2000b, a). The impact of particulate fish farm waste on sediment communities is variable, according to interactions between depth, current speed, current direction, sediment type, and latitude (Kalantzi and Karakassis, 2006). Commonly impacts are found to be relatively localised (Borja et al., 2009; Grego et al., 2009; Ye, 1991), with meta-analyses reporting benthic community change extending 40-70 m on average (Giles, 2008), although instances of impacts to 145 m have also been recorded (Hamoutene et al., 2015). The extent of impact of dissolved wastes is poorly known, but may extend further (HEST, 2000a).

The effects of fish farm derived nutrients on the diversity and composition of macroalgal-dominated reef communities are likely to be similar to those already observed in eutrophic systems affected by terrestrial derived organic pollution, such as sewage and runoff from fertilised landscapes. A well-documented consequence of excessive nutrients in coastal reef environments is the over-abundant growth of certain types of productive, fast growing macroalgae (Bokn et al., 2003b; Krause-Jensen et al., 2008;





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Teichberg et al., 2008) at the expense of habitat-forming perennial species (Gorgula and Connell, 2004; Valiela et al., 1997; Worm and Sommer, 2000). These fast growing algae have been termed, 'opportunistic', 'bloom forming' or 'nuisance' macroalgae (Krause-Jensen, 2007a; Littler and Littler, 1980; McGlathery, 2001; Valiela et al., 1997).

In temperate waters, opportunistic green algae in the genera Ulva (which now includes the genus Enteromorpha), Cladophora, and Chaetomorpha (Lavery and McComb, 1991) are commonly reported to form blooms (Valiela et al., 1997). These algae are typically ephemeral, with a filamentous or sheet-like form, a relatively undifferentiated thallus, and a high thallus area to volume ratio (Littler and Littler, 1980). Such attributes allow for fast growth and rapid reproduction when environmental conditions are suitable (Littler and Littler, 1980). These algae typically also have a high demand for nitrogen (Barr and Rees, 2003), and their growth is favoured under a variety of pollution types (Guinda et al., 2008), such as sewage pollution (Arevalo et al., 2007; Soltan et al., 2001), sedimentation (Eriksson and Johansson, 2005), and pollution from urbanisation (Gorgula and Connell, 2004; Mangialajo et al., 2007). In eutrophic systems, dense blooms of opportunistic algae can develop, influencing nutrient dynamics beyond their role as nutrient sinks (Lavery and McComb, 1991), and substantially altering biotic community structure and ecological functions (Nelson et al., 2008).

In many but not all cases, increased over-growth by opportunistic algae is associated with a decrease in species richness and cover of canopy-forming perennials (Wells et al., 2007). On South Australian temperate reefs, algal turfs (filamentous assemblages of algae <5 mm in height) have replaced canopy-forming algae along urbanised coastlines, with canopy algae declining up to 70% in cover on reefs (Connell et al., 2008). Experimental tests indicate that algal turf can rapidly colonise and retain space at high rates of sedimentation and nutrient enrichment (Gorgula and Connell, 2004). Benthic communities in the Baltic Sea change along a gradient of eutrophication, with canopy-forming algae replaced by bloom forming algae towards pollution sources (Worm and Lotze, 2006; Worm et al., 1999b).

The possibility of impacts to macroalgal communities is of concern, since some species of bloom forming macroalgae are known to effectively uptake fish farm derived nutrients (Hernández et al., 2008), and many macroalgal species have a preference for ammonia–nitrogen which is released from fish as metabolic waste (Sanderson et al., 2008; Sanderson et al., 2012). In polyculture situations, the production of 92 tons of salmon can potentially yield 385 tons (fresh weight) of *Ulva* or 500 tons of red algae through assimilation of nutrient waste (Neori et al., 2004). Given the likelihood of dissolved nutrient waste from salmon farms dissipating well beyond farm boundaries, broad-scale effects of nutrification on macroalgal communities should be considered within fish-farm management frameworks.

Although of great ecological and economic significance, few studies have addressed the issue of fish farming impacts on nearby reef communities (Ruokolahti, 1988). Most of what is known relates to the Baltic, where Ronnberg (1991), Ronnberg et al. (1992) and Hemmi et al. (2005) observed increased growth and biomass of epiphytes on *Fucus* near fish farms, with a shift from brown and red epiphytes to green epiphytes towards farms. Vadas et al. (2004) found increases in the foliose green alga *Ulva* near fish farms in Maine. Boyra et al. (2004) also found significant differences between intertidal macrobenthic assemblages near fish farms and at control locations. In the Mediterranean, significant losses of seagrass communities have been associated with fish farms (Dolenec et al., 2006; Holmer et al., 2008; Perez et al., 2008). Virtually nothing is known on the overall scale and nature of such influences on subtidal macroalgal assemblages.

In Australia, farmed salmonids are, by gross value, the most valuable fisheries product and now account for the largest quantity of fish produced in Australia, surpassing the Australian sardine in 2011 (ABARE, 2014). Almost all (97%) of Australia's \$497 million of salmonid production occurs in Tasmania (ABARE, 2014), and between 1996/97 and 2011/12, Tasmanian salmon production levels increased sixfold from 7647 to 43,989 tonnes (ABARE, 2013). The most concentrated region of fish farming in Tasmania occurs in the D'Entrecasteaux Channel and adjoining Huon Estuary – a 40-km long bifurcated and semi-enclosed water body. Salmon farming is considered the major source of anthropogenic nutrient input to the waters of the region (Macleod and Helidoniotis, 2005). In 2000, The Huon Estuary Study Team estimated that of the nitrogen contained in fish feed, 36% is retained as harvested fish, and the remaining 64% released into the estuary through metabolic waste or uneaten feed. While seasonal variation in biogeochemical attributes of the area are well enough understood to have motivated a voluntary moratorium on increased use of fish feed in the Huon Estuary (Crawford, 2003), significant growth of the industry has continued in the adjacent D'Entrecasteaux Channel, and the relationship between distance from fish farms and the characteristics of reef macroalgae assemblages has not been investigated locally.

In this project, we investigate scale and nature of the ecological impacts of fish farming on temperate macroalgal communities in the D'Entrecasteaux Channel, with the ultimate aim to improve monitoring of reef assemblages in fish farming areas and inform the adaptive management of current and future impacts. We address two specific research hypotheses: (1) macroalgal assemblages are locally influenced by nutrient inputs to fish farms, and as a consequence vary with distance from farms; (2) patterns of variation with distance from farm are also influenced by reef depth and exposure.

2. Methods

2.1. Reef selection

To locate potential study sites, spatial data on the distribution of marine farm lease areas and benthic marine habitats in the Bruny Marine Bioregion were collated in ArcGIS 9.3 (ESRI), and reef sites 0.1, 0.4, 2 or 5+ km from the boundaries of fish farm leases were identified (Fig. 1). These distances were chosen because effects of nutrient drift were likely to decrease exponentially with distance from fish farms. Any reefs close to an onshore source of marine pollution (determined by a spatial dataset for foreshore pollution sources (Migus, 2008)) were then excluded from the set available for selection. Ten spatially well-separated reefs were chosen from those available in each distance class, which varied in their exposure to swell and wind. Alternative site locations were also identified in case some selected sites were not rocky reef.

2.2. Field data collection

Spatial coordinates extracted from ArcGIS were used to locate predetermined field sites. Each site was first scoped with a depth sounder to determine the reef depth and extent. If the site was determined unsuitable, an alternative *a priori* identified site was surveyed or the number of sites investigated was reduced. The final 44 sites were surveyed between 17 November and 17 December, 2008.

As the effect of fish farms on macroalgal composition was expected to vary with depth, reefs were sampled at two depths (2 m and 5 m) where possible. A transect tape of 50 m length



Fig. 1. Relationship between sites sampled, exposure, fish farm leases and the distribution of subtidal reef habitats in the study region.

was initially laid out along the reef following the 2 m depth contour line. Photographs encompassing 50 cm of transect tape (approximately 0.25 cm² quadrat size) were taken at 5 m intervals (n = 10 photos per transect). This process was repeated along the 5 m contour at sites where the reef extended to this depth. Photographs were taken with an 8 mega-pixel digital Olympus camera, with a 28 mm wide angle lens and strobe.

2.3. Data processing and analysis

Photos were cropped and adjusted for brightness and contrast before being imported into CPCe (Coral Point Count with excel extension) for analysis. A point count method was used, where points are overlayed over an image and the species beneath each point visually identified and recorded in a database (Kohler and Gill, 2006). For each image, a grid of 56 points was overlayed with an image border of 100 pixels. Points where the underlying algae could not be identified to species were lumped into a higher category, such as 'Sargassum spp.', or 'foliose red algae'.

Results for each transect were exported, and percentage cover (per transect) calculated for each cover type. Adjustments were made so that obstruction by ephemeral epiphytic algae did not bias the coverage estimate for the more permanent underlying algal community. Percentage cover for the underlying community was calculated as:

Percentage(i) = points covered by type i * 100/(total number of points

- the points attributed to ephemeral coverage – shadow)

Percentage data for ephemeral species was calculated as: Percentage(i) = points covered by type i * 100/(total number of points - shadow)

2.4. Community composition

The percentage cover data of macroalgae and sessile invertebrates on each transect sample were square root transformed for multivariate procedures to reduce the influence of dominant species. We used Bray--Curtis as the distance measure. Both non-metric multidimensional scaling (MDS) and principal coordinate analysis (PCO) ordinations were examined for 2D and 3D solutions.

To test the null hypothesis that community composition was not significantly different between samples with different environmental variables, a PERMANOVA test was conducted (Primer-E 2008). In this case, the model included the fixed categorical factors 'depth', 'distance' and an exposure index (oceanic swell-exposed versus non swell-exposed), and all interaction terms. Calculation of the Pseudo-F ratio and *P* value ($\alpha = 0.05$) was based on 9999 permutations of residuals under a reduced model. The components of variation attributed to each factor were calculated (Anderson et al., 2008). Negative estimates of components of variation were consecutively pooled, starting with the term with the smallest mean-square value, as suggested by Anderson et al. (2008).

A restricted set of *a posteriori* pair-wise tests was also conducted if the term 'distance' or its interaction with another term was found to be significant. This was also done using PERMANOVA with 9999 permutations. First, 100 m sites were tested against 5000 m reference sites, to test for a significant difference. If this test was significant then 400 m sites were tested against 5000 m sites, and so on. Calculations were undertaken separately at the two levels of depth and/or exposure if the interaction term was significant. This method acknowledged use of 5000 m sites as reference sites and also that the magnitude of effect was expected to diminish with distance, thereby reducing the chance of a 'Type I error' occurring. Pair-wise comparisons for interaction effects would otherwise have involved 12 tests unadjusted for Type I error.

In order to identify cover variables that were correlated with the effect of distance, a constrained ordination procedure was conducted using Canonical Analysis of Principal Components (CAP) in PERMANOVA (Primer-E 2008). CAP axes are fitted through the multivariate data cloud to best discriminate between predefined groups. Diagnostics were conducted by permutation, using two test statistics (trace and largest root), and cross-validation of groups by the "leave one out" procedure. Species variables were then correlated with the CAP axes using Pearson correlation.

2.5. Univariate nutrient indicators

Following earlier authors (Juanes et al., 2008; Krause-Jensen et al., 2007b; Steneck and Dethier, 1994), species were separated into categories representing their functional growth habits. 'Opportunistic algae' were algal species known to respond specifically to elevated nutrients with rapid growth. 'Opportunistic green algae' was a sub-category of opportunistic algae which included Chaetomorpha spp. (Juanes et al., 2008), Cladophora spp. (including filamentous green algae) (Juanes et al., 2008), and Ulva spp. (Munda, 1993). 'Epiphytic species' was a sub-category of opportunistic algae included all species known to grow on other macroalgae such as Chaetomorpha billardierii, filamentous algae, Colpomenia spp. and Asparagopsis armata. 'Canopy brown' species comprised perennial brown algae that form a canopy over the mid-storey, under-storey and encrusting species. Individual species that may respond negatively to pollution were not able to be identified a priori on the basis of other studies because most are perennial 'competitive' taxa (Krause-Jensen et al., 2007b; Littler and Littler, 1980), which vary between regions.

Indicator variables and species categories that were identified *a priori* as responding to eutrophication or pollution were grouped

and analysed with univariate tests. Indicators were only tested individually where they had a high rate of occurrence amongst the samples (>19 samples). General linear models were performed in Minitab15, using distance, depth, and exposure categories, and all interaction factors. Variables were transformed and tested for normality and homoscedasticity using the Ryan-Joiner test and model diagnostics. Tukeys pairwise tests were used to determine which classes were significantly different from each other.

3. Results

A total of 120 algal taxa were identified from 73 transects. The PCO depicting algal relationships between transects indicated a strong relationship between community composition and the wave exposure gradient, with a clear distinction between swell-affected sites and sheltered sites (Fig. 2). The effect of distance from fish farm was independent from that of wave exposure. An interaction term between distance and exposure also achieved a high correlation with the first two PCO axes (Pearson's correlation: -.43 (PCO axis 1), 0.33 (PCO axis 2) (Fig. 2).

Exposure, distance, depth and the interaction factors 'exposure × distance', and 'exposure × depth' were significant in the PERMANOVA model for species composition (Table 1). Exposure explained the most variation within the data (34.8%), followed by the interaction factor exposure × distance (16.4%). Distance alone explained a further 11.6%. Pair-wise comparisons for the interaction factor exposure × distance showed that macroalgal composition at 100 m sites was clearly different from the 5000 m reference sites (Sheltered- t: 1.704, P: 0.002. Exposed- t: 1.596, P: 0.028). Sites 400 m metres away from fish farms were not significantly different from sites 5000 m away from fish farms (Sheltered- t: 1.213, P: 0.165. Exposed- t: 1.291, P: 0.113).

A CAP analysis revealed significant differences between distance groups by permutation tests (Trace statistic = 0.74883, P = 0.0004, and First squared canonical correlation = 0.46613, P = 0.0005) under 9999 permutations. The best separation among groups along the distance continuum was achieved along CAP axis 1 (Fig. 3). Correlation of the species variables with CAP axis1 indicated that *Chaetomorpha billardierii*, *Ulva* spp. and *Chaetomorpha coliformis* decreased with increasing distance from fish farms. Species that increased in abundance with increasing distance were all red algae, mainly consisting of understorey and mid-storey species (Fig. 3).



Fig. 2. PCO ordination of the total algal assemblage per transect, showing distance and exposure categories. Ordination is based on Bray Curtis similarity matrix of square root transformed data. Fitted environmental vectors were calculated using Pearson correlation. The circle represents perfect correlation of 1.

Table 1 Table of results, and estimates of components of variation for PERMANOVA with square root density data and Bray-Curtis distance matrix.										
Source	Degrees of freedom	Sum of squares	Mean Square	Pseudo-F ratio	P value (by permutation)	Components of variation				
Exposure	1	41,000	4100	25.700	0.001	1210				
Depth	1	4310	4310	2.700	0.001	80				

3850

3930

11 500

3930

Distance

Exposure \times depth

3

1

Total	72	172,000					
Pooled residuals [*]	63	100,000	1600			1595	
Exposure × distance	3	11,500	3840	2.406	0.001	268	

2 4 1 2

2.464

0.001

0.004

* SS and degrees of freedom for terms *depth* × *distance* and *exposure* × *depth* × *distance* were pooled with the residuals as they had negative estimates of components of variation.



Fig. 3. (a) CAP ordination from a discriminant analysis by distance, using a Bray-–Curtis matrix of square root species abundance data. (b) Fitted vectors of species variables correlated with CAP axis 1 (Pearsons correlation coefficient >0.25).

The 'leave one out' allocation procedure showed that sites 100 m from fish farms shared the most consistent macroalgal community composition, achieving correct classification for 76.5% of the samples in that group (Table 2). Sites from other distances, particularly 400 m sites, were much more variable. Misclassification of 400 m sites into the 100 m category occurred in 5 cases. Misclassification of 400 m sites respectively. Sites 2000 m and 5000 m from fish farms had low misclassification rates for the 100 m group (Table 2).

All of the groups identified *a priori* as potential nutrient indicators had high frequencies of occurrence amongst the transect samples. Canopy brown algae dominated most sites, averaging 46% cover. Filamentous brown algae and algal turf were also widespread and abundant on many transects. The high cover of filamentous brown algae meant that it was the dominant component of the epiphytes.

Most groups responded significantly to exposure, or an interaction between exposure and distance (Table 3). There were several trends in cover over the four distance categories (Fig. 4). The cover

Table 2Leave-one-out allocation of observations to groups, for the choice of m = 9.

	Original group	Classified						
_		100	400	2000	5000	Total	%correct	
	100	13	1	1	2	17	76.5	
	400	5	4	4	6	19	21.1	
	2000	2	4	9	4	19	47.4	
	5000	0	6	4	8	18	44.4	

of epiphytic algae and Ulva spp. were significantly different between distance categories (Table 3). Epiphytic cover was significantly higher for 100 m sites than for 400 m, 2000 m, and 5000 m sites (Fig. 4). Ulva spp. was more dominant at the 100 m sites than 5000 m sites. The interaction factor distance \times exposure was significant for Chaetomorpha spp., filamentous algae and opportunistic green algae. Chaetomorpha spp. were in greatest cover at swell exposed sites 100 m from fish farms, covering an average of 21.4% of each transect. This group of sites was significantly different from all other sites. Filamentous algae were much more abundant at sheltered sites than exposed sites. At sheltered sites, the abundance of these algae was significantly higher at 100 m sites than 2000 m and 5000 m reference sites. However, no significant distance effect was seen at exposed sites. Filamentous green algae showed a pattern of decreasing cover with increasing distance from fish farms, in total and at sheltered sites. Opportunistic greens were dominated by filamentous green algae at sheltered sites, and *Chaetomorpha* spp. at swell exposed sites. At sheltered sites, the cover of opportunistic greens decreased with increasing distance from fish farms.

4. Discussion

Given the consistency of biological response amongst interspersed sites distributed regionally, the most plausible explanation for observed variation in algal community composition with distance from fish farms is impact associated with release of nutrients. The nature of these impacts varied between wave-exposed and sheltered locations in a manner consistent with expectations from previous studies (Barrett et al., 2001; Burrows et al., 2008;

135

138

Table 3

Significant environmental variables affecting the abundance of predicted macroalgal indicators. Adjusted R-squared values, F ratios and significance values are shown (*** = P < 0.001; ** = 0.001 < P < 0.05), calculated from a fully factorial general linear model of the factors distance, depth and exposure against transformed univariate response variables. Non-significant results omitted.

Model response	Distance F	Depth F	Exposure F	Dist * exp F	Depth * exp F	R-sq (adjusted)%
Canopy brown algae		25.81***	23.04***			40.75
Opportunistic green algae	7.87***			3.42*		23.33
Opportunistic algae total	5.56**		64.07***			51.26
Filamentous algae	4.77**	6.04*	171.79***	3.96*	5.35*	73.87
Epiphytic algae	8.1***	5.5*	103.53***		5.2*	63.65
Filamentous green algae			25.46***			22.75
Filamentous brown algae		5.13*	148.37***			68.56
Filamentous red algae						0
Chaetomorpha spp.	9.1***		13.99***	8.04***		39.5
Ulva spp.	4.89**	11.86*	8.71**		7.44**	36.37
Algal turf						0



Fig. 4. Boxplots of percentage cover of indicator variables (raw data) over distance categories. Variables whose cover differed significantly according to an interaction factor between distance and exposure are shown for sheltered sites (1) and swell exposed sites (2) separately. Pair-wise groupings shown on graphs (letters). Significance of the variable is denoted by $^{\circ} = P < 0.001$; $^{\circ} = 0.001 < P < 0.05$). Connect line for mean values shown.

Dayton, 1985; Edgar, 1983a, 1983b, 1984). Robust wave-resistant algae tended to predominate at wave-exposed sites, with high cover of canopy-forming brown algae and encrusting algae (Hill et al., 2010). The cover of filamentous and other delicate algae tended to be low in wave-exposed areas because of swell and whiplash from canopy algae, which cause detachment and export of algal thalli from the system (Kiirikki, 1996; Pihl et al., 1999). Algae that typify sites of high nutrient enrichment are typically epiphytic, opportunistic and delicate (Wallentinus, 1984), and consequently formed higher overall cover at sheltered sites. Regardless, the epiphyte *Chaetomorpha billiarderi* occurred abundantly at sites with moderate exposure to swell, so nutrient indicator species were present at most sites.

Whilst percentages were higher in sheltered waters, the cover of epiphytic algae still increased with proximity to fish farms at both levels of wave exposure. No significant interaction was evident between distance from fish farm and exposure for epiphytes. Based on mesocosm experiments, Krauvfelin (2007) also concluded that nutrient enrichment could enhance opportunistic algal growth and export in both sheltered and moderately wave exposed conditions. Nutrients are unlikely to accumulate in exposed waters with high flushing rates, however, assemblages existing within them may also be more susceptible to nutrient inputs than those naturally adapted to withstand enrichment for longer periods (Russell and Connell, 2007; Russell et al., 2005).

Although depth had a significant influence on macroalgal community composition, no significant interaction between depth and distance was found. This result was unexpected since other studies report eutrophication to reduce light levels at depth, due to increased suspended sediments, epiphytic shading or phytoplankton blooms, with flow on effects to benthic macroalgal systems (Krause-Jensen et al., 2007a; Krause-Jensen et al., 2007b). The consistent effect at the two depths investigated in our study indicates that nutrients probably dispersed throughout the depth range sampled on the majority of farm-affected reefs in the area.

As in other studies on nutrient enrichment and macroalgal composition (Arevalo et al., 2007; Juanes et al., 2008; Russell et al., 2005), fish farms impacts were characterised by increases in epiphyte cover. The high cover of opportunistic species suggests that, for the seasonal period sampled, algal growth was nutrient-limited. Thus, the ecosystem was unable to counteract increased growth of opportunistic algae through grazing or physical disturbance. Algal turf did not exhibit a notable increase towards fish farms. However, the ability to detect turf may have been compromised by the use of photo-quadrats, as this algal functional group is associated with the rock substratum and is generally obscured by foliose algae. Similarly, no obvious trend in red filamentous algae was evident, and this group often grows under other filamentous or foliose algae iin reduced light conditions, so may have been obstructed in photos.

Secondary impacts resulting from overgrowth of epiphytic algae were unclear. Canopy algae did not detectably decline near fish farms, as has been reported for other cases of eutrophication (Cebrian et al., 2014). Three potential explanations for this result are that macroalgal communities in the region perhaps display some resilience to extreme effects of nutrient enrichment, nutrient levels were insufficient to generate such responses, or insufficient time had elapsed since establishment of farms for full effects to manifest.

Previous studies on fish farm impacts in Tasmania have largely focussed on benthic soft sediment habitats (Crawford et al., 2002; Edgar et al., 2005; Macleod et al., 2002; Macleod et al., 2004), with subtle or intermediate impacts detected at regulatory compliance sites located 35 m from lease boundaries (Edgar et al., 2010b; Edgar et al., 2005). More recent reports emphasize the need for broad-scale monitoring (Ross and Macleod, 2013). This study indicated that fish farms had a significant effect on benthic reef communities at greater distances than regulatory compliance sites. and combined with the distribution of fish farms, impacts regional in nature are also of concern. Fish farms affected reef benthic communities at both sheltered and exposed sites to at least 100 m from fish farms, at which point macroalgal communities differed significantly from reference sites. Although sites at 400 m distance were not significantly different to reference sites when assessed collectively, effects extended this distance in some areas but not others, generating wide confidence intervals associated with the overall mean. This was indicated by the leave-one-out procedure in CAP, which revealed 5 of the 400 m sites showed characteristics akin to 100 m sites, compared to none of the 5000 m reference sites and two 2000 m sites.

Our results thus suggest nutrient enrichment from fish farms affects subtidal reef communities to a variable distance, and at scales of hundreds of metres, but rarely kilometres. A caveat associated with this conclusion is the assumption that impacts did not extend throughout the whole farming region, in which case reference sites at 5-km distance from farms would not provide appropriate controls for assessing impacts. Regardless, previous studies on the dispersal of nutrients from fish farms have concluded that nutrient enrichment occurred within a range of hundreds of metres. Algal growth in bioassays was significantly elevated to 150 m from fish farms in the Mediterranean Sea (Dalsgaard and Krause-Jensen, 2006), while in Scottish waters ammonium levels at 4 m depth were elevated for extended periods of the day at distances exceeding 200 m (Sanderson et al. 2008). A region-wide study by Husa et al. (2014) assessed macroalgal communites that were more than 1 km from salmonid farms, and found them to be of 'high' or 'good' ecological status under the indices of the European Water Framework Directive.

Distance from fish farm provides a highly simplified metric of the influence of any fish farm due to complexities associated with output and dispersal of nutrients, and susceptibility of the marine environment to nutrient enrichment. Current direction and strength, in particular, greatly affect the footprint of nutrients and particulate wastes dispersing from fish farms (Ali et al., 2011; Giles, 2008; Venayagamoorthy et al., 2011). Nutrient enhancement generally extends much further in the direction of prevailing currents, and in some cases may not be detectable in the opposite direction (Sanderson et al. 2008). However, sites in the present study were identified purely on the basis of distance to nearest fish farm, reef location, a wide spatial distribution of sites, wave exposure, and absence of other pollution types. Including prevailing current directions from each fish farm may have improved models and reduced statistical noise in the effects seen at different distances from farms, if current data were available at appropriate temporal and spatial scales, and adequately captured time integrated patterns. These considerations, as well as fish pen distribution and stocking levels, feeding regimes, and characteristics of the reef habitat studied, such as the abundance of grazers, will be important aspects of future and finer-scale studies. Nevertheless, considering the high density of fish farms, the lack of previous monitoring of aquaculture related reef impacts in the broader area, and the seasonal and inter-annual variability in the hydrodynamics of the study area (Harris et al., 1987), it was considered prudent to focus on overall dominant patterns on a regional scale. Regional scale models can encompass variability in tidal currents and the cumulative impacts of multiple fish farms (Symonds, 2011).

Regardless of this variability, impacts of farm-released nutrients were arguably easier to detect using macroalgae than by monitoring the nutrients themselves (Dalsgaard and Krause-Jensen, 2006). Nutrient concentrations vary greatly through the day, requiring repeated sampling, whilst growth in macroalgae is a cumulative effect of locally-elevated nutrients over a longer time period (Munda, 1993). Nitrogen isotopes in macroalgae have also been used to detect fish farm effluent, however values must be referenced specifically for each region and species studied (Carballeira et al., 2013). Macroalgal composition thus represents an important tool for comprehensive monitoring programs in fish farming regions containing reef habitat.

Our results provide a snapshot of algal communities on reefs at a relatively early stage of regional fish farm development, given that the majority of Australian salmonid production occurs within the region studied, with annual growth of up to 20% in Australian production varying in the five years prior to 2011, and a 50% expansion projected to 2016 (Curtotti et al., 2011). Whilst no major decline in algal canopy cover was detected near farms in our study, ongoing nutrient enrichment from fish farms remains a potential threat to macroalgal community structure and reef diversity through the long-term. The apparent barren-state of a reef 100 m from a fish farm at Port Esperance, which had low canopy cover, a high density of grazers, and a high opportunistic green algal cover, was notable in this regard. This situation may indicate complex relationships involving grazers, nutrients, sedimentation and canopy loss; however, additional research is needed to assess this.

A comprehensive understanding of impacts of fish farm nutrients on reef communities requires knowledge of seasonal relationships between grazers, foliose algal canopy cover, and growth of filamentous and other opportunistic algae. Experimental manipulations of grazers, nutrients, propagule banks, and/or disturbance would usefully add to *in situ* observational studies in providing such information (Bokn et al., 2003a; Kraufvelin, 2007; Worm et al., 1999a; Worm et al., 2002), thereby allowing effects and interactions at reef sites to be much better predicted, ultimately resulting in improved management of marine resources in areas affected by multiple anthropogenic threats.

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