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Trophic responses to nutrient enrichment in a temperate seagrass food chain

Paul H. York^{1,*}, Brendan P. Kelaher^{1,2}, David J. Booth¹, Melanie J. Bishop³

¹School of the Environment, University of Technology, Sydney, Broadway, New South Wales 2007, Australia

²Batemans Marine Park, Narooma, New South Wales 2546, Australia

³Department of Biological Sciences, Macquarie University, North Ryde, New South Wales 2109, Australia

ABSTRACT: Simple ecological models that predict trophic responses to bottom-up forcing are valuable tools for ecosystem managers. Traditionally, theoretical ecologists have used resource-dependent functional responses to explain the modification of food chains exposed to bottom-up perturbations. These models predict alternating positive, negative and zero responses at each trophic level. More recently, ratio-dependent functional response models that predict proportional increases at each level have challenged this paradigm. The present study tested the predictions of the 2 hypotheses empirically by comparing the relative biomasses of 4 trophic levels of an estuarine seagrass food chain in relatively undisturbed, low-nutrient catchments and 'developed' catchments subjected to a prolonged period of nutrient enrichment. We found that nutrient-enriched sites had significantly greater biomass of both epiphytic algae and grazing invertebrates; however, the bottom-up forcing of nutrients was attenuated at higher trophic levels (occupied by juvenile and piscivorous fish), with no significant effect of catchment development. This disconnect in the upward cascade of energy may be due to a number of possible reasons including high levels of diversity and omnivory, trophic subsidy within the system or the strength or nature of perturbations. Although the predictions of both hypotheses failed to hold across all trophic groups, ratio dependence was prevalent at the lower levels of the food chain, which has implications for catchment management.

KEY WORDS: Trophic dynamics · Resource dependence · Ratio dependence · Functional response · Seagrass · Epiphytes · Grazers · Fish

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INTRODUCTION

A key goal of theoretical ecology is to explain complex ecosystem processes with straightforward mathematical models (Bascompte & Melián 2005). A classic example has been models of the movement of energy in food chains, through the relationships among consumers and their resources. Models of trophic dynamics are often based on differential predator–prey equations that determine the abundance and biomass at each trophic level in a food chain. The output of these models largely depends on their functional response, which describes the rate at which an individual predator can consume its prey as a function of prey density (Abrams & Ginzburg 2000).

Early trophic models were based on resource-dependent functional responses, where changes in the rate of predation by an average predator are determined by the abundance of prey (Lotka 1925, Volterra 1926). More recently, ratio-dependent trophic functions (Arditi & Ginzburg 1989), where predation rates depend on the ratio of predators to prey, have been proposed as being more representative of ecological systems. A vigorous debate has since followed (e.g. Ginzburg & Akcakaya 1992, Abrams 1994, Bishop et al. 2006, Kratina et al. 2009) with proponents on each side claiming that their concept's predictions best mirror natural systems. There is, however, general consensus that empirical tests of each of the hypotheses are scarce (Abrams & Ginz-

*Email: paul.h.york@uts.edu.au

burg 2000). The clearest difference between the 2 models occurs in predictions of community responses to sustained bottom-up pressure in a system with 4 trophic levels (Table 1).

Coastal systems, such as estuaries, provide an ideal platform to conduct field tests of resource- and ratio-dependent hypotheses by correlating trophic level biomass to nutrient loading. In these systems there are generally 4 trophic levels (Vander Zanden & Fetzer 2007), linkages between trophic levels are strong (Strong 1992) and, in recent decades, anthropogenically sourced nutrients have led to increasing enrichment (Paerl 1997). Generally, the availability of limiting nutrients is the driving bottom-up force controlling primary productivity in aquatic systems (Cloern 2001). A comparison in a phytoplankton dominated food web between sites close to and away from a sewage outfall revealed increased abundances of all 4 trophic levels proportional to nitrogen loading, therefore supporting ratio dependence (Bishop et al. 2006).

Temperate seagrass beds are an important coastal habitat which are increasingly affected by anthropogenic nutrient enrichment (Short & Wyllie-Echeverria 1996). Excessive nutrients in seagrass systems lead to phytoplankton blooms and increased epiphytic algal growth, resulting in shading of seagrass leaves and ultimately habitat collapse (Dennison et al. 1993). The addition of more moderate amounts of nutrients may not be as devastating to the seagrasses themselves, but instead lead to changes in community structure of the epiphytic algal food webs supported by these habitats (Gil et al. 2006, Jorgensen et al. 2007). As seagrass tissue is low in nutrients and not palatable to most grazers, epiphytes contribute a large portion of the trophic support in temperate seagrass ecosystems (Moncreiff & Sullivan 2001, Connolly et al. 2005).

Table 1. Predicted responses of trophic levels for ratio- and resource-dependent hypotheses when exposed to a sustained bottom-up forcing in food chains with 4 levels (source Ginzburg & Akcakaya 1992). ∞: a proportional response

Trophic level	Hypothesis		Seagrass food chain
	Ratio-dependent	Resource-dependent	
1	∞↑	↓	Epiphytic algae
2	∞↑	↑	Mesograzers
3	∞↑	⇒	Juvenile fish
4	∞↑	∞↑	Piscivores

The present study considers the effects of long-term nutrient enrichment on a seagrass food chain that includes 4 trophic levels: epiphytic algae, grazing invertebrates, juvenile fish and piscivorous fish. The biomasses of each of 4 trophic levels were compared among 3 nutrient-enriched and 3 reference sites, to test the hypotheses that sustained bottom-up forcing will produce trophic interactions that permeate upwards through the food chain. It was also determined whether the response of each trophic level to sustained nutrient enrichment supported (1) the resource-dependent hypothesis; (2) the ratio-dependent hypothesis; or (3) neither of the two, suggesting that either an alternate hypothesis needs to be developed or something other than nutrients is driving trophic interactions.

MATERIALS AND METHODS

Study sites

The study was carried out at 6 seagrass *Zostera capricorni* meadows in the Brisbane Water and Hawkesbury River catchments just north of Sydney, Australia (33°S, 151°E). Comparisons of trophic groups were made between catchments enriched in nutrients over decades by surrounding urban industrial and agricultural development (Narara Creek, Kincumber Creek and Erina Creek), and low nutrient catchments dominated by protected native forests (Waterfall Bay, Woy Woy Bay and Patonga Creek); hereafter these are referred to as 'developed' and 'undeveloped' catchments, respectively. A comprehensive investigation of water quality using flow injection analysis (Paterson et al. 2003) had previously found significant increases of NH₃ (6.6- to 8.48-fold) and NO_x (4.6- to 14.2-fold) in developed catchments compared to the undeveloped reference sites. None of the sites were subject to direct commercial fishing and all were considered to have similar levels of top-down pressure from recreational fishing. There were no point sources of pollution (e.g. fish farms, sewage treatment plants, industrial effluent, etc.) in the locality of any sites.

Field sampling

The 6 sites were sampled on 3 separate occasions from October 2005 to May 2006 to compare the biomass of each trophic level within a seagrass food chain between elevated nutrient sites and low nutri-

ent reference sites. On each occasion, 4 replicate samples (0.38 m²) of seagrass, epiphytes and associated epifauna separated by distances of at least 10 m were taken using a specially designed sampler consisting of a metal cylinder (20 cm diameter) with a cutting blade that slices the seagrass at the base and with a sieve (500 µm mesh) at the top that collects the sample when inverted (O'Neill et al. 2007). From each sample, epiphytic algae were scraped off 20 randomly selected seagrass leaves and invertebrates were sorted into functional groups with grazers being identified and included in the analyses. Seagrass, algae and invertebrates were dried at 60°C and used to determine algal load, calculated as algal biomass (g)/seagrass biomass (kg), and grazer biomass.

Four replicate 25 m² sweeps separated by distances of at least 25 m were taken with a seine net (12 m length, 2 m drop, 6 mm mesh) to collect small and juvenile fishes. Species known to prey upon grazing invertebrates (Burchmore et al. 1984, Edgar & Shaw 1995) were enumerated and measured (wet weight). Larger piscivorous fish that prey upon juvenile fish were caught by hook and line using 2 lines tended for a period of 90 min at each site. All piscivorous fish (Baker & Sheaves 2005, Melville & Connolly 2005) that were caught were identified and measured (wet weight) to give catch per unit effort (CPUE) and then immediately returned to the water.

Water samples were collected from estuarine waters above seagrass beds during each sampling period and were sent to Sydney Water Analytical and Field Services laboratory for analysis of total nitrogen using the method APHA 4500 – PH & NO₃ – I (APHA et al. 2005).

Statistical analyses

Hypotheses about the effect of bottom-up forcing on the biomass of each trophic level were tested to determine if changes matched the predictions of either resource- or ratio-dependent hypotheses or neither. Because the models are based on steady-state systems, data were collected across 3 different time periods to allow for the long-term relationship among trophic groups to be used as an approximation of ecosystem equilibrium (Ginzburg & Akcakaya 1992). Prior to analysis, data were averaged across the 3 sampling periods for each site to reduce the influence of small-scale spatio-temporal variation, which was not the focus of the hypotheses under evaluation (Keough et al. 2007). Analysis was done

using *t*-tests comparing enriched versus controls with *n* = 3 sites. A separate *t*-test was performed on total nitrogen levels in the water column to quantify differences in nutrient levels among treatments. Prior to each test, Levene's test and graphical examination of the data using boxplots was used to check for homogeneity of variance.

To assess the prediction of increases in biomass among trophic levels proportional to the bottom-up force in the ratio-dependent hypotheses (Table 1), a single one-way ANOVA was conducted using the magnitude of change after bottom-up forcing, expressed as the ratio of nutrient-enriched sites to reference sites for the bottom-up force (nutrients) and the 4 trophic groups: epiphytes, mesograzers, juvenile fishes, and piscivorous fishes. In the event that trophic groups respond proportionately to nutrient input, no significant difference in ratios among the 5 groups should be detected. This ANOVA was followed by *a posteriori* Student-Newman-Keuls (SNK) tests to identify any group means that differed significantly.

RESULTS

Diverse assemblages of invertebrates, and juvenile and larger fish were collected within samples. A total of 42 taxa known to graze on algae were included in the analysis. Dominant mesograzers common among all sites included gammarid amphipods (families Maeridae, Amphilochidae and Corophiidae) and small gastropods (*Diala megapicalis*, *Alaba* sp., and *Aplysiidae* sp.). Of the small or juvenile fishes (<80 mm total length) sampled, 4 species (*Acanthopagrus australis*, and *Rhabdosargus sarba* Sparidae, *Pelates sexlineatus* Tetrapontidae and *Gerres subfasciatus* Gerreidae) were included in the analysis as the dominant species consuming mesograzers on seagrass leaves. Large piscivorous fishes were low in abundance and patchily distributed in space and time. Dominant species included large yellow-fin bream *A. australis* and long toms *Strongylura leiura*.

Epiphytic algal load ($t = 14.17$, $p < 0.001$, Fig. 1a), and mesograzer biomass ($t = 6.49$, $p = 0.003$, Fig. 1b) were significantly higher at nutrient-enriched sites compared to reference sites. Juvenile fish biomass ($t = 0.13$, $p = 0.902$, Fig. 1c), predatory fish biomass ($t = -0.49$, $p = 0.647$, Fig. 1d) and total nitrogen levels in water samples ($t = 1.13$, $p = 0.321$), however, showed no significant differences between treatments or across sites. The biomass ratio between ambient and nutrient-enriched catchments differed ac-

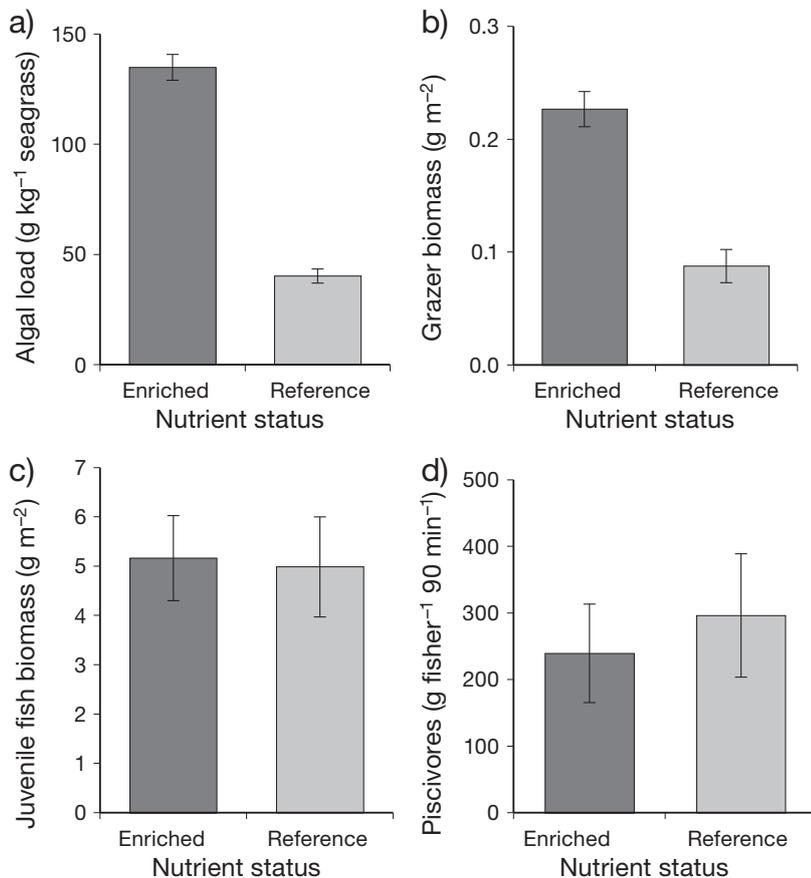


Fig. 1. Mean (± 1 SE) biomass of (a) epiphytes, (b) mesograzers, (c) juvenile fishes, and (d) piscivorous fishes at nutrient-enriched (dark grey bars) and reference (light grey) sites ($n = 3$ in both cases)

cording to trophic level ($F_{4,14} = 7.100$, $p > 0.06$). Ratios for epiphytes (mean \pm SE: 3.7 ± 1.0) and grazers (2.6 ± 1.5) were statistically similar due to the significant increases in biomass for these trophic groups (SNK, $p > 0.05$); however, ratios for nutrients (1.1 ± 0.5), juvenile (1.3 ± 0.6) and piscivorous fish (0.6 ± 0.2) were significantly lower (SNK, $p < 0.05$).

DISCUSSION

We have demonstrated that trophic responses in seagrass beds exposed to long-term nutrient enrichment operate more closely to ratio than resource dependence. The present study builds on the results of Bishop et al. (2006), who showed the presence of ratio dependence in a 4 level planktivorous food chain at a single site enriched by a point source of nutrients from a sewage treatment plant. The present study differed from their work by investigating enrichment from more diffuse sources and by using a

more spatially comprehensive sampling design. While both studies demonstrated increased primary production and algal loads with nutrient enrichment, the present study found that in a seagrass system, secondary production was attenuated above the level of primary consumers and did not cascade through to the 2 highest trophic levels. The alternate resource-dependent hypothesis incorrectly predicts that the bottom trophic level (epiphytes) should be of lesser biomass at nutrient-enriched than reference sites.

The difference in the results from these 2 studies indicates that the strength of upward trophic cascades can vary depending on the type of ecosystem involved and the level and nature of enrichment. A recent meta-analysis of contaminants, such as nitrogen enrichment, found that point sources (e.g. fish farms and sewage treatment plants) had a positive effect on fish abundances, while diffuse sources such as run-off produced negative effects (McKinley & Johnston 2010). It is possible that pollutants other than nutrients that are present in run-off, the main source of enrichment in this study, have negative effects on higher trophic levels, such as fish, that are not apparent in studies from point source enrichment (van der Oost et al. 2003).

The apparent dampening of the bottom-up cascade at higher trophic levels may be due to a variety of reasons. Attenuation of the upward trophic cascade from bottom-up forcing has been demonstrated in many systems (Borer et al. 2006) including seagrasses (Moksnes et al. 2008). It can be demonstrated experimentally in simple, well-understood systems that changes in the density of one trophic level do not necessarily cascade through other trophic levels due to complex direct and indirect effects (Wootton 1994).

Trophic properties such as high species diversity, a high degree of omnivory, competition and intra-guild predation make systems resilient to cascading effects (Williams & Martinez 2004, Duffy 2006). Certainly, a large number of juvenile fishes with omnivorous diets (*Girella tricuspidata* and Monacanthid species) inhabit seagrass beds in southeastern Australia and were at relatively high abundances during this study

(Burchmore et al. 1984, Wressnig & Booth 2008). Another possible explanation is that juvenile fish diets are subsidised with invertebrates from the detrital food web at reference sites where epiphyte grazers are scarce (Attayde & Ripa 2008). Also, non-trophic factors, such as open population structures, ontogenetic habitat shifts of juvenile fish (Duffy 2006) or movement of piscivores to alternative habitats in search of food (Hindell et al. 2000), may also play a role in the decoupling of higher trophic levels from the bottom-up forcing seen at lower trophic levels.

Although no significant differences in nutrient concentrations between developed and undeveloped catchments were detected during this study, a large body of evidence has demonstrated that instantaneous measures of water column nutrients are not always indicative of nutrient loads, due to the fact that nitrogen is rapidly taken up by algae, seagrass and sediments (Lee et al. 2004, Scanes et al. 2007). This means that over-enrichment of coastal ecosystems can rarely be detected by direct measurements of water column nitrogen concentrations. Typically, urbanised catchments have more than double the nutrient loads of forested watersheds (Wahl et al. 1997) and more thorough, long-term analyses have confirmed significantly higher nutrient levels at the enriched sites used in this study (Paterson et al. 2003).

There is a paucity of studies that test the long-term effects of nutrient enrichment through food chains and allow the comparison of the predictions of both resource- and ratio-dependent hypotheses. One such study on the west coast of North America (Ware & Thomson 2005) found a strong bottom-up link, with increased fisheries yields strongly correlated with high phytoplankton and zooplankton abundances in areas enriched by coastal upwelling and river discharge. Another synthesis of studies of eutrophication in the North Sea (Colijn et al. 2002) established a link between increases in phytoplankton production and greater biomass of zooplankton and, more tentatively, of species such as shrimp and fish in higher trophic levels. These findings support the predictions of the ratio-dependent hypothesis. Despite this, more work is required in diverse ecosystems with a range of nutrient loads and sources to establish the generality of this concept.

In our study, the predictions of neither ratio- nor resource-dependent hypotheses completely fitted the seagrass epiphyte food chain investigated. The system does, however, appear to show ratio dependence at lower trophic levels with an attenuation of trophic interactions higher in the food chain. Further

studies are required to determine if this pattern of ratio-dependence at lower trophic levels holds elsewhere. Being able to predict ecosystem-level impacts of moderate nutrient enrichment is critical to enable successful estuarine management that is preventative rather than reactionary.

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