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Density-dependent positive feedbacks buffer aquatic plants from interactive effects of eutrophication and predator loss

S. DONADI ^{1,2,3,5} Å. N. AUSTIN,¹ E. SVARTGREN,¹ B. K. ERIKSSON,⁴ J. P. HANSEN,³ AND J. S. EKLÖF¹

¹Department of Ecology, Environment and Plant Sciences, Stockholm University, SE-106 91 Stockholm, Sweden

²Department of Aquatic Resources, Institute of Freshwater Research, Swedish University of Agricultural Sciences, Stångholmsvägen 2, SE-178 93 Drottningholm, Sweden

³Groningen Institute for Evolutionary Life-Sciences, University of Groningen, Nijenborgh 7, 9747 AG Groningen, The Netherlands

⁴Baltic Sea Centre, Stockholm University, SE-106 91 Stockholm, Sweden

Abstract. Self-facilitation allows populations to persist under disturbance by ameliorating experienced stress. In coastal ecosystems, eutrophication and declines of large predatory fish are two common disturbances that can synergistically impact habitat-forming plants by benefitting ephemeral algae. In theory, density-dependent intraspecific plant facilitation could weaken such effects by ameliorating the amount of experienced stress. Here, we tested whether and how shoot density of a common aquatic plant (*Myriophyllum spicatum*) alters the response of individual plants to eutrophication and exclusion of large predatory fish, using a 12-week cage experiment in the field. Results showed that high plant density benefitted individual plant performance, but only when the two stressors were combined. Epiphytic algal biomass per plant more than doubled in cages that excluded large predatory fish, indicative of a trophic cascade. Moreover, in this treatment, individual shoot biomass, as well as number of branches, increased with density when nutrients were added, but decreased with density at ambient nutrient levels. In contrast, in open cages that large predatory fish could access, epiphytic algal biomass was low and individual plant biomass and number of branches were unaffected by plant density and eutrophication. Plant performance generally decreased under fertilization, suggesting stressful conditions. Together, these results suggest that intraspecific plant facilitation occurred only when large fish exclusion (causing high epiphyte load) was accompanied by fertilization, and that intraspecific competition instead prevailed when no nutrients were added. As coastal ecosystems are increasingly exposed to multiple and often interacting stressors such as eutrophication and declines of large predatory fish, maintaining high plant density is important for ecosystem-based management.

Key words: aquatic vegetation; Baltic Sea; cage experiment; coastal ecosystem; eutrophication; facilitation; multiple stressors; *Myriophyllum spicatum*; positive feedbacks; predator loss; trophic cascade.

INTRODUCTION

Self-facilitation mediated by density-dependent positive feedbacks (hereafter DPF) occurs when the growth and fitness of individuals increase with the density of the population (Bianchi et al. 1989). DPFs are widespread in organisms that do not show complex social organization (Stone and Weisburd 1992, Maxwell et al. 2017) and can occur through various mechanisms. First, they can manifest when aggregations of individuals, by modifying the abiotic environment, increase resource supply and/or alleviate environmental stress (i.e., ecosystem engineers, sensu Hastings et al. 2007). For example, dense stands of vegetation in arid ecosystems enhance water infiltration into the soil (Lejeune et al. 2002, Rietkerk et al. 2002); invertebrate bioturbators alter geochemical gradients in soft-bottom sediments, ultimately benefitting diatom and microbial growth (Lohrer et al. 2004, Needham et al. 2011); and mussels clumps and cordgrass tussocks provide shelter to conspecifics from wave disturbance (van de Koppel et al. 2005, van Wesenbeeck et al. 2008). Furthermore, DPFs may also occur via direct biotic interactions. High densities of conspecifics can reduce

the growth and fitness of competitors via food and space exploitation (exploitative competition) or via chemical defenses; can reduce susceptibility to predators via cooperative behavior, and can facilitate the survival of beneficiary species in mutualistic relationships (Bertness and Callaway 1994, Stachowicz 2001). Through DPFs, populations can therefore persist at higher densities than otherwise expected (Bianchi et al. 1989, Bertness and Callaway 1994), and increase community resistance and resilience to disturbance (Scheffer et al. 2001).

In shallow coastal ecosystems, both foundation species and associated communities are impacted by multiple and often co-occurring anthropogenic stressors (Lotze et al. 2006). Submerged aquatic vegetation (plants and coarsely structured perennial macroalgae) are important foundation species that influence local abiotic conditions (e.g., light, hydrodynamics, CO₂) and provide complex habitats for diverse invertebrate and fish assemblages (Heck et al. 2003, Hansen et al. 2008). Eutrophication, climate change, extreme weather events, and the anthropogenic modification of coastal landscapes, such as dredging and construction, are among the multiple stressors undermining the survival of aquatic vegetation and coastal communities (Orth et al. 2006, Burkholder et al. 2007, Gustafsson et al. 2012, Fraser et al. 2014). Additionally, declines of large predators can benefit ephemeral algae through trophic cascades that

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⁵E-mail: serena.donadi@slu.se

involve the release of mesopredators and suppression of algal-feeding invertebrates (Eriksson et al. 2009, Baden et al. 2010, Sieben et al. 2011). Recently, these effects have also been shown to affect aquatic vegetation (Donadi et al. 2017). Declines of large predators can also interact with other stressors such as eutrophication (Halpern et al. 2008, Ripple et al. 2014). Theory supported by empirical studies (Oksanen et al. 1981, Moksnes et al. 2008, Eriksson et al. 2012) predicts that the strength of trophic cascades depends on ecosystem productivity, so that the higher the productivity, the stronger the top-down control. Therefore, under eutrophic conditions, the removal of top predators can exacerbate the effects of eutrophication by boosting the growth of ephemeral algae. One such example is shallow coastal areas in the Baltic Sea, where large predatory fish may benefit submerged plants indirectly by suppressing overgrowth of ephemeral algae (Eriksson et al. 2009, Sieben et al. 2011, Donadi et al. 2017). Large predatory fish, however, have declined in many areas (Nilsson et al. 2004, Österblom et al. 2007), raising concern that submerged aquatic vegetation and perennial algae are under increasing stress from algal overgrowth, caused by both eutrophication and food web alterations (Eriksson et al. 2011a).

Several density-dependent positive feedbacks can, alone or in concert, benefit aquatic vegetation (Maxwell et al. 2017). Dense (vs. sparse) vegetation can increase individual plant performance by (1) increasing direct competition for light and dissolved nutrients with ephemeral epiphytic algae and phytoplankton (Kemp et al. 1983, Krause-Jensen et al. 2008, Baden et al. 2010, Werner et al. 2016), (2) facilitating invertebrate grazers that in turn feed on epiphytes (mutualistic mesograzer model; Duffy et al. 2013, Donadi et al. 2017), (3) baffling mechanical disturbance from waves and water currents (Bouma et al. 2005), and (4) reducing sediment resuspension and enhancing particle sedimentation inside of macrophyte beds, which increases light penetration (Vermaat et al. 2000, Gacia and Duarte 2001, Moore 2004, Le Bagousse-Pinguet et al. 2012, Austin et al. 2017). However, high plant density can also *reduce* individual plant performance, by increasing intraspecific competition for light and nutrients (Antonovics and Levin 1980), and/or by facilitating mesopredators, which in turn benefit epiphytic algae by controlling grazer abundance or feeding rates (Warfe and Barmuta 2006).

Here, we experimentally tested whether high plant density can buffer effects of two common and often interacting stressors on individual plants: eutrophication and loss of large predatory fish. In short, we planted Eurasian water-milfoil (*Myriophyllum spicatum* L.), a common aquatic plant in many freshwater and coastal areas, in three different densities and tested the single and joint effects of nutrient enrichment and exclusion of large predatory fish (using cages) in three coastal bays in the Baltic Sea. We hypothesized that (1) nutrient enrichment and exclusion of large predatory fish increases biomass of epiphytic algae and reduces the performance of plants (Östman et al. 2016) and that (2) these effects are weakened by high plant density, through one or several of the positive feedback mechanisms outlined above. Furthermore, in line with the stress gradient hypothesis (Bertness and Callaway 1994, Bruno et al. 2003), we suggest that (3) the effect of high plant density should

shift from negative to positive with increasing stress, and therefore be most beneficial when eutrophication and loss of large predators co-occur.

METHODS

Study area

The Baltic Sea is a large brackish marginal sea situated in northern Europe. Vast archipelagos create a highly heterogeneous coastal seascape, that host diverse assemblages of aquatic vegetation (plants and perennial macroalgae) and invertebrates (Hansen et al. 2008), and function as spawning and recruitment ground for many coastal fish species (Snickars et al. 2009). One of the most common plants is the Eurasian watermilfoil (*Myriophyllum spicatum*, hereafter “*Myriophyllum*”); a native rooted angiosperm with a long slender and branched stem, leaves in pinnate whorls of four, and numerous needle-like leaflets. The complex leaves form a formidable substrate for epiphytic macroalgae such as *Cladophora* spp., *Pilayella littoralis*, and *Ectocarpus siliculosus*, but also a habitat for macroinvertebrate grazer assemblages dominated by amphipods (*Gammarus* spp.), isopods (*Idothea* spp.), gastropods (*Hydrobia* spp., *Theodoxus fluviatilis*), and insect larvae (e.g., Chironomidae, Limnephilidae). Amphipods and isopods feed mainly on epiphytic macroalgae, while gastropods mainly consume microalgae, such as diatoms and dinoflagellates (MacNeil et al. 1997, Råberg and Kautsky 2007). Insect larvae include carnivores, detritus and filter feeders, and grazers (Wallace and Merritt 1980). The most abundant mesopredator in the system is the three-spined stickleback, *Gasterosteus aculeatus* (Sieben et al. 2011, Bergström et al. 2015), which in turn is predated by large piscivorous fish, such as Eurasian perch (*Perca fluviatilis*) and Northern pike (*Esox lucius*) (Beaudoin et al. 1999, Donadi et al. 2017).

Field experiment

The field experiment was conducted between 4 June and 26 August 2015 (12 weeks) in three shallow coastal bays (maximum depth 3 m) around Askö Island (Sweden) in the western Baltic proper (58°49' N, 17°39' E). The area is suffering from eutrophication, as showed by the classification in the Water Framework Directive and the regional monitoring data (WISS 2015). The experiment was a full factorial combination of three treatments: (1) plant density (three levels: low, medium, and high), (2) exclusion of large predatory fish using cages (two levels: open vs. closed cages), and (3) nutrient enrichment (two levels: ambient and enriched). Each of the 12 treatment combinations was replicated once per bay, resulting in 36 plots in total.

Large predatory fish were excluded using stainless-steel cages (120 × 55 × 100 cm [length × width × height]), covered with a plastic net (mesh size 1.4 cm) and firmly attached to the bottom at ~1 m depth (Fig. 1A). Plots were positioned at least 3 m apart to avoid cross-fertilization of treatments. One-half of the cages had openings on two non-opposite sides along the diagonals, which allowed large fish to enter the cages. In each bay, two no-cage plots with medium plant density served as a control for potential cage artifacts.

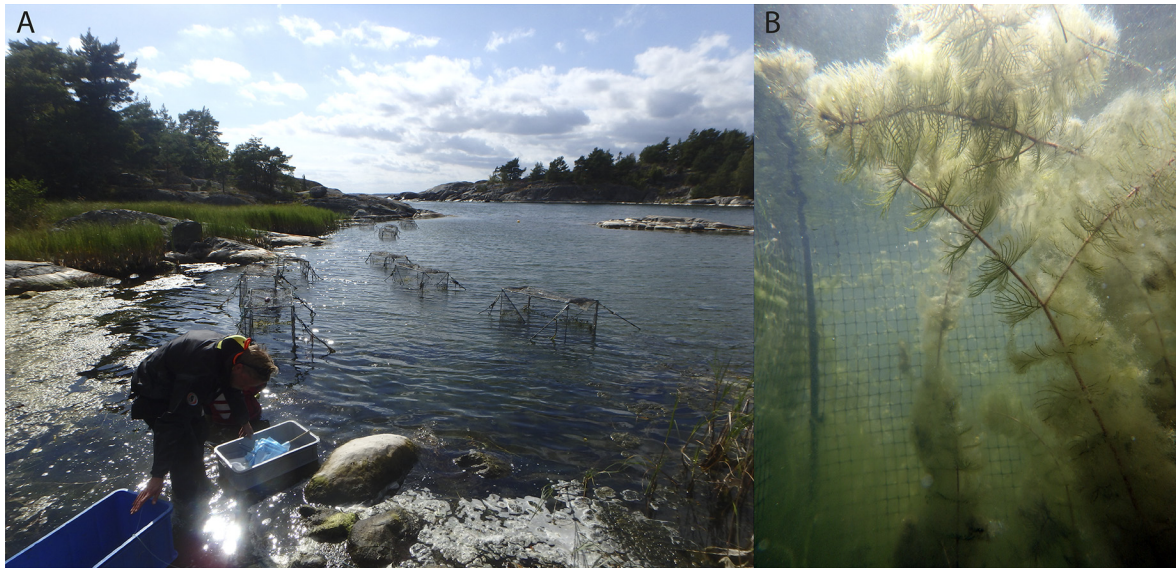


FIG. 1. (A) One block of 12 experimental cages in one of the bays. (B) Shoots of *Myriophyllum spicatum* partly covered by epiphytic algae in one of the experimental cages.

At the bottom of each cage (0.66 m^2), we placed a fine mesh and attached apical, unbranched, *Myriophyllum* shoots in one of three densities: 3, 10, or 24 shoots per cage (4.5, 15, and 36 shoots/ m^2 , respectively). These are hereafter referred to as “low,” “medium,” and “high” density, respectively. Experimental densities were in the range of natural densities in the study area. The shoots (average wet mass \pm SD: $0.07 \pm 0.02 \text{ g}$, $N = 20$ shoots) were collected in a nearby area, carefully cleaned from epiphytes in the laboratory, and standardized to 10 cm length prior to attachment (see, e.g., Strand and Weisner 2001). Shoot densities decreased during the course of the experiment, but remained significantly higher in the high-density treatment compared to the medium- and low-density treatment (Appendix S1).

To simulate eutrophication, nutrients were added to one-half of the cages using four 1-mm mesh bags per cage ($20 \times 10 \text{ cm}$), each containing 120 g of coated slow-release fertilizer (N:P:K = 14:14:14, Plantacote Depot 6 M, Urania Agrochem, Hamburg, Germany). The fertilizer bags were attached to the bottom of the cages at two opposite corners and at two opposite sides and were replaced after 6 weeks. On 15–17 June (approximately two weeks after the start), water samples were collected $\sim 15 \text{ cm}$ from the bottom of each cage using airtight 50-mL clean syringes, filtered through a 0.45 mm glass-fiber filter (Whatman, Sarstedt, Germany), and frozen. Concentrations of dissolved nitrogen and phosphorus were later measured in duplicates per cage, using segmented flow colorimetric analysis (Koroleff 1983) with the Alpkem FlowSolution IV system from OI Analytical. Nutrient enrichment increased dissolved inorganic nitrogen by 125% (compared to plots with ambient nutrient concentrations of $2.68 \pm 0.593 \text{ } \mu\text{g/L}$ [mean \pm SD]; ANOVA, $N = 42$, $F_{1,40} = 29.46$, $P < 0.001$), but did not affect dissolved phosphorus, whose ambient concentrations were already relatively high (ambient nutrient level = $9.53 \pm 0.995 \text{ } \mu\text{g/L}$; mean \pm SD).

The same fish exclusion cages and nutrient enrichment treatments have been successfully used in the same study

area in several previous experiments (Eriksson et al. 2009, Sieben et al. 2011). To reduce the influence of cage artifacts such as fouling, we once per week brushed off periphyton growing on the cage net, and removed all new macroscopic vegetation that was not part of the treatments. To assess potential differences in the hydrodynamic regime inside open and closed cages, we assessed the mass loss of the bags of coated fertilizers after 6 weeks in cages with medium plant density (as a proxy for hydrodynamics). We did not detect any differences between open and closed cages, suggesting that differences in the water circulation, if any, were negligible (ANOVA, $N = 10$, $F_{1,9} = 2.55$, $P = 0.145$).

Laboratory analyses

After 12 weeks (end of August), a plastic bag was gently pulled over each individual *Myriophyllum* shoot (enclosing all of the associated fauna and epiphytes, Fig. 1B), and all shoots were harvested. In the laboratory, epifauna and epiphytes were separated and identified. For each *Myriophyllum* shoot, we measured the main stem length (mm), the number of branches, and the total branch length (the sum of the length of all branches). The biomass of individual plants and associated epiphytes species (g DM) was determined separately after drying (60°C for 48 h). In the statistical analyses, we used the pooled biomass of truly epiphytic algal species (Appendix S2), and excluded drifting algae that may have got temporarily entangled on the experimental plants. Epifaunal macroalgal grazers (hereafter “grazers”) were identified (Appendix S3), measured (body length, mm), and their biomass estimated as g ash-free dry mass (AFDM) using taxon-specific length:AFDM correlations (Eklöf et al. 2017). As the *Myriophyllum* plants constitute the substrate that the epiphytic algae grow on, epiphyte biomass was standardized on plant biomass. Similarly, grazer biomass and abundance were standardized on the pooled biomass of plants and epiphytic

algae, as both provide habitat for macrofauna (Eriksson et al. 2011b).

The water retention time and wave exposure of coastal bays are well known to affect aquatic vegetation and epifaunal communities (Hansen et al. 2008). To account for the potential influence of different retention time and wave exposure between the three bays, bay retention time ($\ln(Ty)$, measured in d) was calculated as $\ln(Ty) = -4.33 \times \sqrt{100 At/a} + 3.49$, where At is the smallest cross-sectional area of each bay connected to the sea, calculated from field measurements of inlet depth and width, a is the bay surface area, and $\sqrt{}$ is square root (Persson et al. 1994), estimated from Google Earth satellite images. We then estimated wave exposure as effective fetch (EF; km) for each experimental unit (cage), as $EF = \sum x_i \cos y_i / \sum \cos y_i$, where x_i is the distance to land along direction i and y_i is the respective angle to the perpendicular to the shore (Håkanson and Jansson 1983). The distance from each experimental unit to land was estimated from Google Earth satellite images in nine directions (every 10° to both sides of the perpendicular to the shore). Water temperature was recorded every 10 min throughout the experiment using Hobo loggers (Onset Computing Corporation, Bourne, Massachusetts, USA) attached to the bottom of three cages per site ($N_{\text{total}} = 9$).

Statistical analyses

Single and interactive effects of treatments on all response variables (1, plant biomass; 2, number of branches; 3, total branch length; 4, main stem length; 5, biomass of epiphytic algae; 6 grazer biomass; and 7, grazer abundance) were estimated using general linear mixed models including the fixed factors cage treatment, plant density, nutrient enrichment, and their interactions. Since the responses occurred at a shoot level, we included two random factors: cage (36 levels) nested within site (three levels). All response variables except individual plant biomass were square-root transformed to meet assumptions of normality and homoscedasticity. The grazers are mobile and likely to move around between shoots; therefore, we chose to analyze the single and interactive effects of treatments on grazer biomass and abundance at the cage (not shoot) level, and included site as a random variable. Finally, wind fetch and retention time were included as two continuous covariates, after ensuring that they were not collinear (variance inflation factor ≤ 2). Water temperature (averages per site over the full course of the experiment) was collinear with retention time and therefore not included. As the number of individual plants differed per cage, type-III sum of square ANOVA was used when analyzing effects on plant performance and epiphytic algae biomass (Fox 2016). Maximum likelihood estimation methods were instead used for grazer biomass and abundance, as advised for linear mixed models with equal replication, and significant effects of explanatory factors and interactive terms were assessed through a stepwise backward elimination method, where nested models were compared through a likelihood ratio test (LRT, which approximately follows a chi-square distribution; Zuur et al. 2009). Since we were not interested in all potential contrasts, we used Fisher's least-significance difference (LSD) test to avoid excessive Type II error when testing post hoc differences between

specific treatment combinations. Model validation was performed by plotting residuals vs. fitted values and observed vs. theoretical quantiles.

To assess potential cage artifacts, all response variables were also compared between open cage vs. no-cage plots using general linear mixed-models, including nutrient enrichment, cage treatment and their interaction as fixed factors, and cage nested within site as a random factor. No significant differences were found between open cages and no-cage plots for any of the response variables (Appendix S4). All statistical calculations were performed using R version 3.2.3 (R Core Team 2015) and the R package nlme (Pinheiro et al. 2016).

RESULTS

After 12 weeks, epiphytic algae biomass was 2.4 times higher in the large predatory fish enclosures (closed cages) than the fish access (open) cages but was unaffected by nutrient enrichment and plant density (main effect of cage treatment, $F_{1,19} = 6.12$, $P = 0.020$, Appendix S5, Fig. 2A).

Plant density interacted with nutrient enrichment and exclusion of large fish (three-way interaction, Appendix S5) to affect plant performance: in closed cages (where algal biomass was generally higher, see above) and under nutrient-enriched conditions, high plant density increased individual plant biomass 4.7 times compared to medium plant density (Fisher's LSD test, $F_{2,19} = 6.19$, $P = 0.009$, Fig. 3A), and number of branches per plant 2.3 and 2.8 times (Fisher's LSD test, $F_{2,19} = 3.74$, $P = 0.043$, Fig. 3B), compared to medium and low plant density, respectively. However, in closed cages with ambient nutrient levels, high plant density instead decreased individual plant performance (2.2 and 2.9 times lower plant biomass than medium and low plant density treatments, Fig. 3A). The addition of nutrients reduced total branch length, albeit only at medium plant densities (interaction nutrient enrichment \times density, Appendix S5, $F_{2,19} = 4.24$, $P = 0.030$, Fig. 4A). Main stem length was unaffected by the treatments (Appendix S5, Fig. 4B).

Grazer abundance (measured at the cage level) was 2.3 times higher in nutrient-enriched than ambient cages (main effect of nutrient treatment, LRT = 3.93, $df = 1$, $P = 0.047$) and 2.6 times higher in closed than open cages (main effect of cage treatment, LRT = 5.24, $df = 1$, $P = 0.022$, Appendix S5, Fig. 2B). Meanwhile, grazer biomass was not affected by the treatments (Appendix S5, Fig. 2C).

Wind fetch reduced plant biomass ($F_{1,19} = 24.87$, $P < 0.001$), number of branches ($F_{1,19} = 14.28$, $P = 0.001$), total branch length ($F_{1,19} = 13.84$, $P = 0.002$), and main stem length ($F_{1,19} = 12.49$, $P = 0.002$), while retention time did not affect any of the response variables (Appendix S5).

DISCUSSION

Using a three-month factorial field experiment across three coastal bays, we find that density-dependent positive feedbacks can buffer aquatic plants from indirect and interactive effects of eutrophication and loss of large predators. In cages accessible to large predators, epiphytic algal biomass was low, and plant density did not affect individual plant performance. In cages that instead excluded large

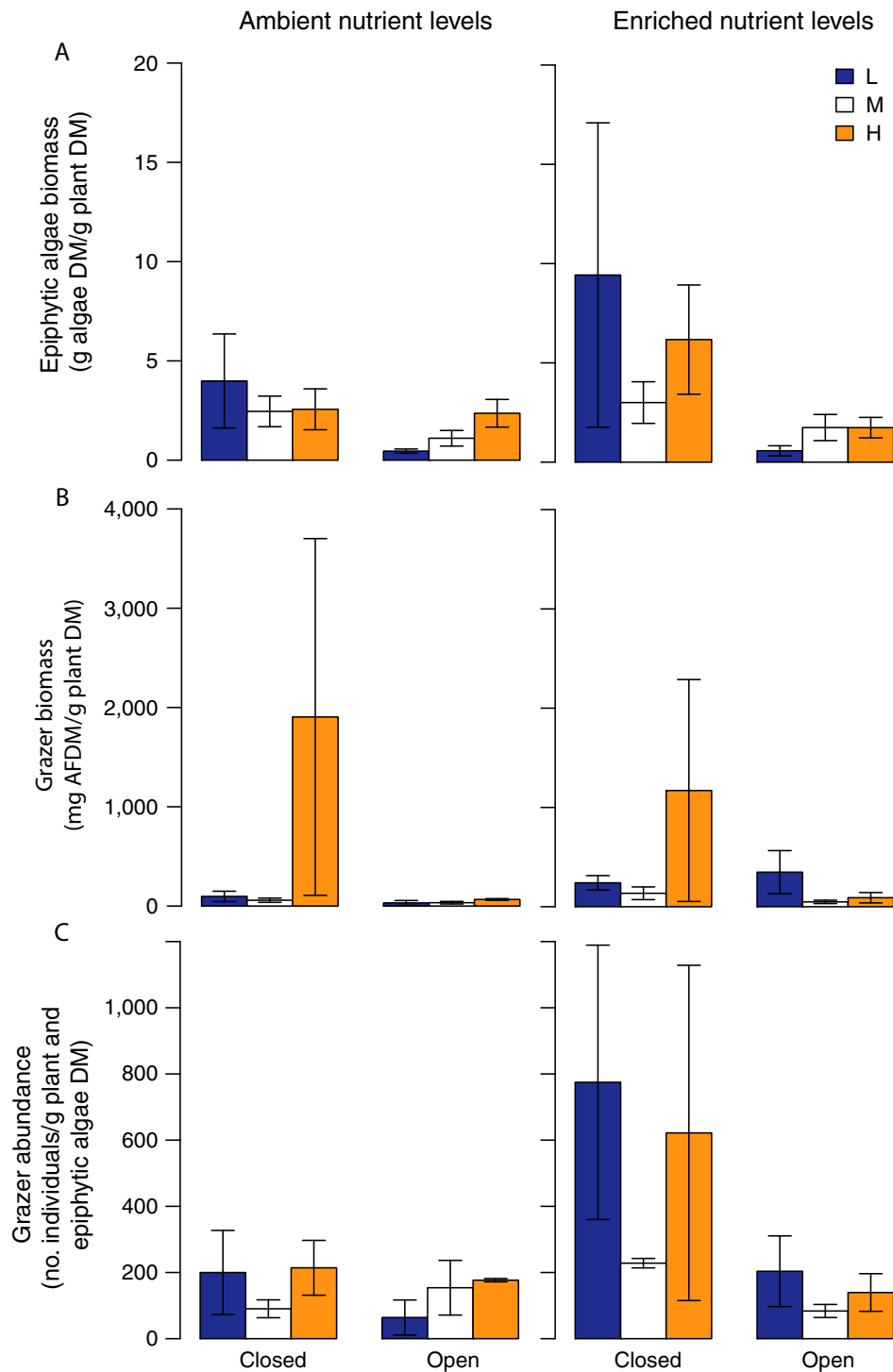


FIG. 2. (A) Epiphytic algae biomass (DM, dry mass), (B) grazer biomass (AFDM, ash-free dry mass), and (C) grazer abundance (number of individuals per g DM plant + epiphytic algae), at low (L), medium (M), and high (H) experimental plant densities, in open and closed cages, under ambient (left) and enriched (right) nutrient conditions. Values shown are mean \pm SE. (A) $N_{\text{total}} = 149$ shoots; ambient closed $N_L = 9$, $N_M = 21$, $N_H = 13$; ambient open $N_L = 6$, $N_M = 16$, $N_H = 16$; enriched closed $N_L = 4$, $N_M = 12$, $N_H = 10$; enriched open $N_L = 8$, $N_M = 19$, $N_H = 15$. (B and C) $N_{\text{total}} = 36$ cages, $N = 3$ replicates per treatment combination.

predators (closed cages), epiphytic algal biomass per *Myriophyllum* shoot more than doubled, and plant performance (i.e., biomass and number of branches) increased at high plant density, indicating a density-dependent positive

feedback (Fig. 5). This effect, however, occurred only in nutrient-enriched cages, while in cages with ambient nutrient levels, individual plant performance decreased with plant density, suggesting intra- and interspecific competition

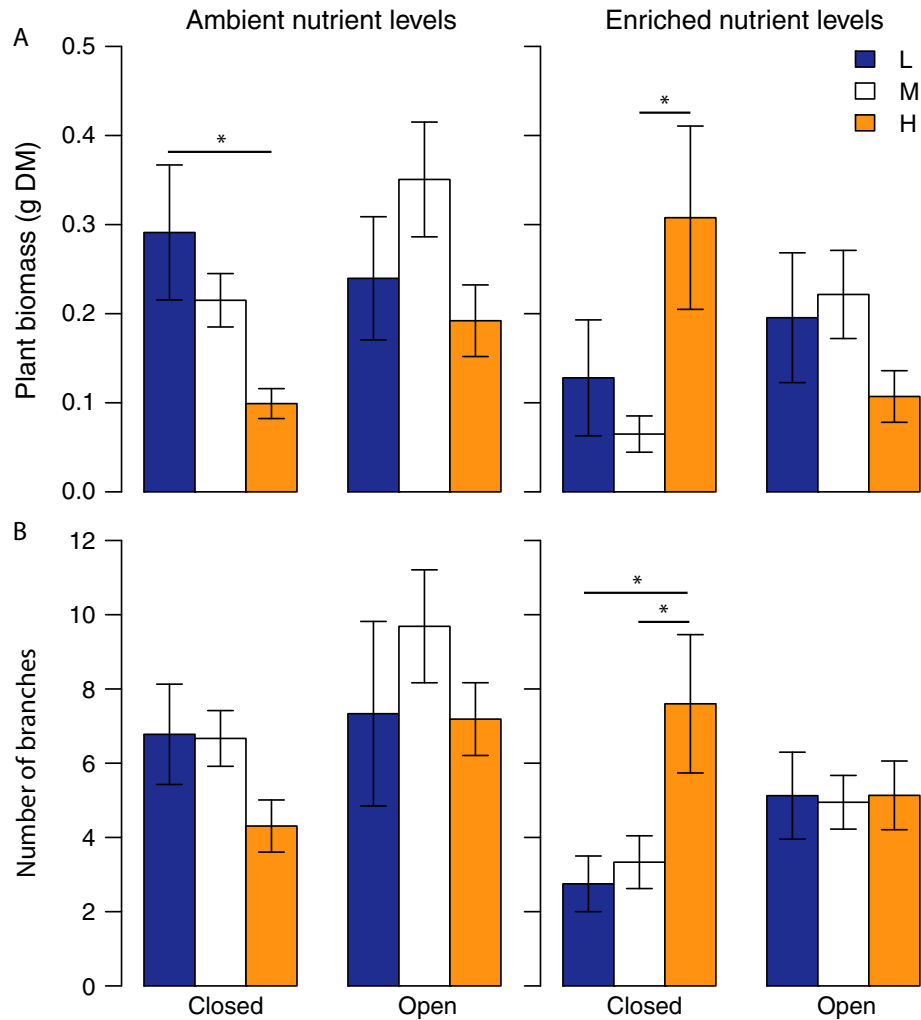


FIG. 3. (A) Plant biomass and (B) number of branches per plant, at low (L), medium (M), and high (H) experimental plant densities, in open and closed cages, under ambient (left) and enriched (right) nutrient conditions. Values shown are mean \pm SE. $N_{\text{total}} = 149$ shoots; ambient closed $N_L = 9$, $N_M = 21$, $N_H = 13$; ambient open $N_L = 6$, $N_M = 16$, $N_H = 16$; enriched closed $N_L = 4$, $N_M = 12$, $N_H = 10$; enriched open $N_L = 8$, $N_M = 19$, $N_H = 15$. Significant pairwise contrasts are indicated by asterisks over lines (Fisher's LSD test, $P \leq 0.05$).

(Fig. 5). In line with the stress gradient hypothesis, the observed shift from negative to positive effects of plant density with increasing stress highlights the importance of intraspecific facilitation also for submerged vegetation (Bertness and Callaway 1994, Bruno et al. 2003).

Density-dependent positive feedbacks occur in both terrestrial and aquatic plants (e.g., Suding et al. 2004, van der Heide et al. 2008, Kéfi et al. 2016). In the marine and freshwater realm, aquatic plants typically compete for light and dissolved nutrients with epiphytic algae and phytoplankton (Sand-Jensen 1977, Baden et al. 2010); therefore, the ability of plants to outcompete algae can increase with plant density (Le Bagousse-Pinguet et al. 2012). Moreover, facilitation of algal-feeding grazers by dense plant assemblages (through provision of habitat, food, and shelter) could enhance plant growth and survival (Scheinin et al. 2012, Duffy et al. 2013, Donadi et al. 2017). Both of these feedbacks should result in that epiphytic algae biomass decreases with increased plant density. In our study, however,

epiphytic algae biomass per plant was unaffected by plant density. We therefore propose that the density-dependent positive feedbacks acting in closed and nutrient-enriched cages (where plant density had positive effects on per capita plant performance) were most likely caused by amelioration of light conditions via nutrient uptake and reduction of phytoplankton growth and particle trapping (Agawin and Duarte 2002, Moore 2004, van der Heide et al. 2011, Le Bagousse-Pinguet et al. 2012); a mechanism also identified in a large-scale field survey including the study area (Austin et al. 2017). Moreover, high plant density can attenuate wave and current energy (Bouma et al. 2005), thus preventing the plants from breaking. Such an “ecosystem engineering” effect may be particularly important when plants are overgrown by epiphytic algae and/or grow in turbid or deep water, and typically invest more resources in shoot elongation (Strand and Weisner 2001). Indeed, in the predatory fish exclusion (closed cages) and nutrient-enriched conditions, the number of branches per plant and, possibly the

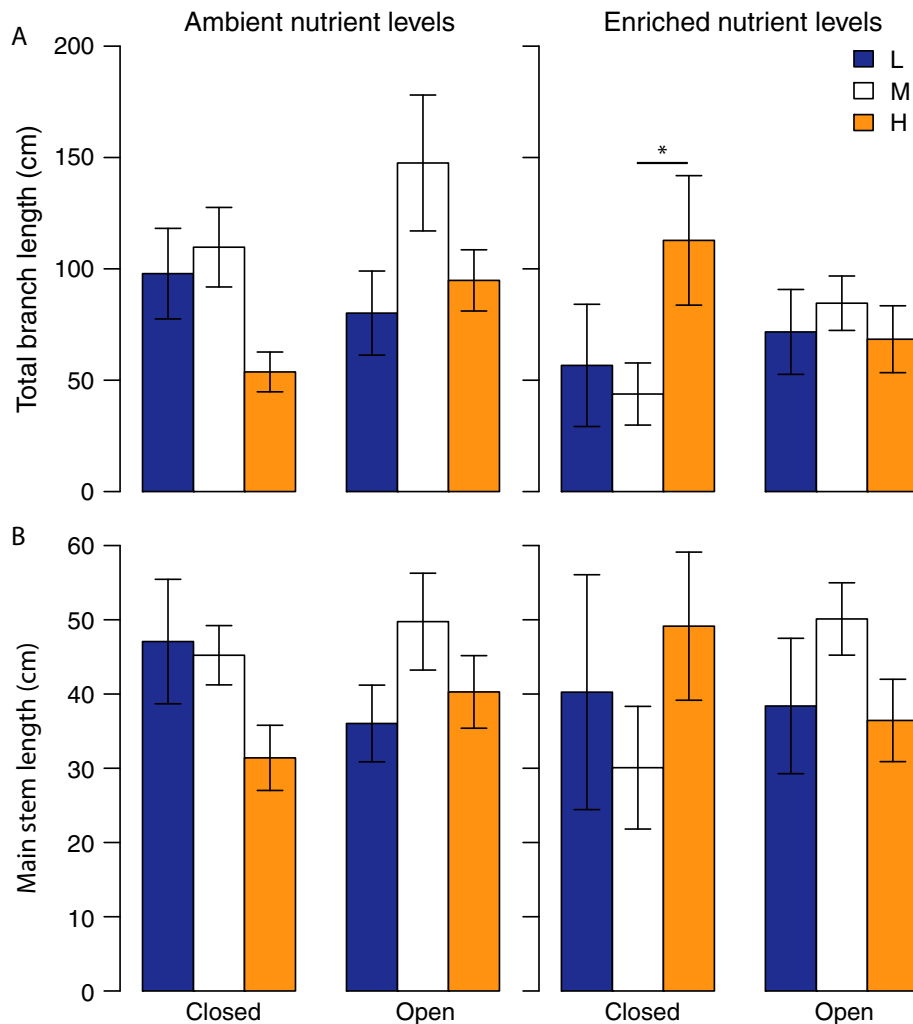


FIG. 4. (A) Total branch length (cm) and (B) main stem length (cm), at low (L), medium (M), and high (H) experimental plant densities, in open and closed cages, under ambient (left) and enriched (right) nutrient conditions. Values shown are mean \pm SE. $N_{\text{total}} = 149$ shoots; ambient closed $N_L = 9$, $N_M = 21$, $N_H = 13$; ambient open $N_L = 6$, $N_M = 16$, $N_H = 16$; enriched closed $N_L = 4$, $N_M = 12$, $N_H = 10$; enriched open $N_L = 8$, $N_M = 19$, $N_H = 15$.

total branch length (the three-way interaction was close to significant, Appendix S5), increased with plant density. This indicates that the high-density plants could better utilize the added resources (nutrients) to increase their biomass (see also Svanfeldt et al. 2017).

In treatments where algal biomass increased (closed cages excluding predatory fish) but no nutrients were added, individual plant performance decreased with plant density. This negative density dependence was most likely caused by intraspecific competition for nutrients, which was exacerbated by the high algal biomass (Svanfeldt et al. 2017). Hence, the addition of nutrients possibly resulted in stressful conditions that lowered plant performance in all but one treatment combination: when the plant occurred at high density and with high epiphyte load (closed cages). In this case, fertilization appears to have provided additional resources that increased individual shoot biomass compared to ambient cages. Our experiment showed no clear effects of plant density at lower algal biomass (in open cages), regardless of nutrient addition, suggesting that plants were then

not as stressed by light or nutrient limitation or mechanical drag from the epiphytes.

Fertilization did not increase the standing biomass of epiphytic algae, but instead increased grazer abundance. Also, grazer abundance was higher in closed than open cages. The taxa that mostly contributed to the overall grazer abundance and biomass were *Idothea* spp. (40% of total biomass and abundance, respectively) and *Gammarus* spp. (26% of biomass, 39% of abundance). High biomass in high-density, no-nutrients, closed cages was not mirrored by a peak in abundance, due to the occurrence of few large individuals of *Idotea* spp. and *Limnephilidae* spp. larvae. These treatment effects on grazer biomass and abundance do not match theoretical expectations of a simple trophic cascade (where one would expect grazer abundance and biomass to decrease in closed cages). This is most likely because in reality, trophic cascades play out over time in temporally dynamic ecosystems, where top-down and bottom-up control can change seasonally (for recent review, see Piovio-Scott et al. 2017). Importantly, previous cage experiments in our study

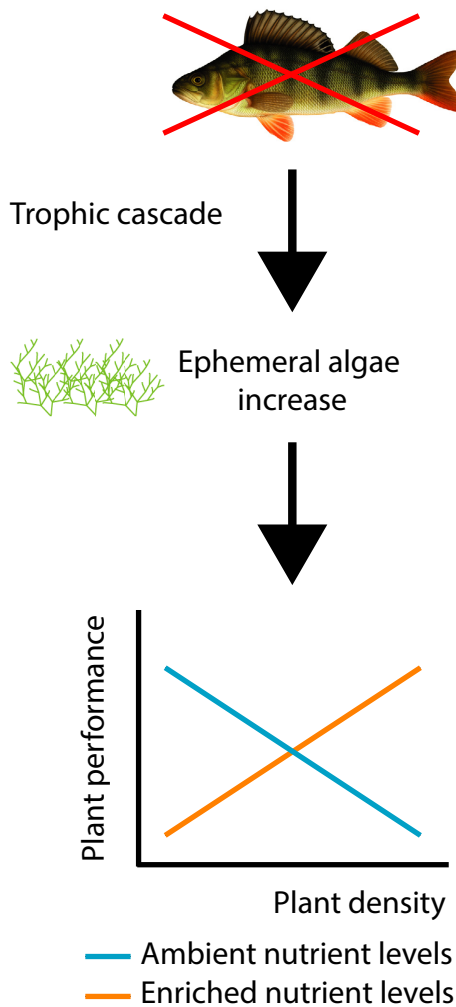


FIG. 5. Conceptual model summarizing the main results. The exclusion of large predatory fish in closed cages resulted in the increase of epiphyte biomass. Under these conditions, plant performance showed positive density dependence in nutrient-enriched cages, and negative density dependence at ambient nutrient levels (perch illustration by Linda Nyman, originally published in The Encyclopedia of the Swedish Flora and Fauna, The Swedish Species Information Centre, SLU. Epiphytes illustration by Joakim Hansen and Pierre Drackner).

system have demonstrated a temporal mismatch between patterns and processes (Sieben et al. 2011). The main mesopredator three-spined sticklebacks move away from the coastal zone at the end of July/early August, which gives time for grazer species with fast reproduction to recover (e.g., *Gammarus* spp.), and for other grazers to move to high-food environments (areas with high epiphyte biomass, for example the closed cages). Meanwhile, the ecological effect of grazing on epiphytic algae can lag behind and become (or still be) conspicuous at the end of the summer (see Sieben et al. 2011). In this study area, as in others, top-down and bottom-up control likely alternates with seasonality (Whalen et al. 2013) and system productivity (Oksanen and Oksanen 2000). In fact, in the absence of strong top-down control (at the end of the summer), grazer biomass increases following fertilization, as predicted by theory

(Oksanen et al. 1981) and observed in previous field experiments on a global scale (Duffy et al. 2015). This is likely caused by nutrients stimulating algal production, which is channeled into grazer biomass production through herbivory. We again, however, emphasize that our sampling is a snapshot in time, which limits our ability to capture the processes (algal growth, grazing, predation, etc.) that played out during the 3-month experiment.

In summary, our study suggests that plant density can buffer the interactive effects of multiple stressors, such as eutrophication and declines in large predatory fish, on the individual performance of aquatic, habitat-forming plants. However, if density-dependent positive feedbacks can increase community resilience to disturbance, the same feedbacks could also increase the risk for sudden declines (collapses) and the formation of societally undesirable but persistent alternative ecosystem regimes (Kéfi et al. 2016, Maxwell et al. 2017). We therefore suggest it is crucial to study the mechanisms underlying both intra- and interspecific positive feedbacks in greater detail, identify potential threshold values in plant density needed to uphold the feedbacks, and maintain plant density above those thresholds, to improve ecosystem-based approaches to the conservation and management of habitat-forming submerged aquatic plants.

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DATA AVAILABILITY

Data are available from the Dryad Digital Repository: <https://doi.org/10.5061/dryad.m1fb658>