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Fish but Not Macroinvertebrates Promote Trophic Cascading Effects in High Density Submersed Plant Experimental Lake Food Webs in Two Contrasting Climate Regions

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Abstract: Predators play a key role in the functioning of shallow lakes. Differences between the response of temperate and subtropical systems to fish predation have been proposed, but experimental evidence is scarce. To elucidate cascading effects produced by predators in contrasting climatic zones, we conducted a mesocosm experiment in three pairs of lakes in Uruguay and Denmark. We used two typical planktivorous-omnivorous fish species (*Jenynsia multidentata* + *Cnesterodon decemmaculatus* and *Gasterosteus aculeatus* + *Perca fluviatilis*) and one littoral omnivorous-predatory macroinvertebrate (*Palaemonetes argentinus* and *Gammarus lacustris*), alone and combined, in numbers resembling natural densities. Fish predation on zooplankton increased phytoplankton biomass in both climate zones, whereas the effects of predatory macroinvertebrates on zooplankton and phytoplankton were not significant in either climate zone. Macroinvertebrates (that freely colonized the sampling devices) were diminished by fish in both climate areas; however, periphyton biomass did not vary among treatments. Our experiments demonstrated that fish affected the structure of both planktonic and littoral herbivorous communities in both climate regions, with a visible positive cascading effect on phytoplankton biomass, but no effects on periphyton. Altogether, fish impacts appeared to be a strong driver of turbid water conditions in shallow lakes regardless of climatic zone by indirectly contributing to increasing phytoplankton biomass.

Keywords: omnivorous fish; littoral macroinvertebrates; zooplankton size structure; cascading effects; mesocosm

1. Introduction

Aquatic communities and food webs are typically structured by either available resources or fish predation [1], classically known as, respectively, bottom-up and top-down control [2]. However, the strength of these drivers in food webs may differ among lake habitats, such as the pelagic and littoral zone. Habitats and local food webs can be connected by diel or seasonal movements of fish, macroinvertebrates and zooplankton [3–5], particularly in shallow lakes.

In temperate lakes, the occurrence of planktivorous fish is known to induce major shifts in size distribution [6–8] and sometimes also in the behavior of zooplankton [9–13], indirectly influencing phytoplankton. By also consuming plant-attached macroinvertebrate grazers (e.g., snails), fish may indirectly enhance periphyton growth as evidenced by experiments [14–16] and field data [17]; however, not all investigations concur on this point [18].

Fish predation is apparently even stronger in warm lakes than in comparable temperate lakes [19–21]; the fish communities in warm lakes typically have higher densities, greater taxonomic and trophic diversity (with predominance of omnivores) and smaller body size [22,23]. Experimental studies have elucidated the effect of planktivorous fish predation on zooplankton communities in subtropical and Mediterranean shallow lakes [24–27]. Other studies including field surveys have focused on the effects of fish predation on zooplankton structure [27,28] and behavioral shifts [25,29–31] or the impact caused by predacious littoral macroinvertebrates [32]. A common feature is that predation on zooplankton in subtropical lakes is high [20,33] and that the zooplankton, therefore, is dominated by small cladocerans, nauplii and rotifers [29,34–39], resulting in a lower grazing pressure on phytoplankton in subtropical lakes than in similar lakes located in colder climates [40]. Moreover, Meerhoff and collaborators [20] found a substantially lower abundance of plant-attached macroinvertebrates and lower biomass of periphyton on artificial plants in subtropical than in similar temperate shallow lakes, arguably because the abundant fish in the former feed on and stir up the periphyton to a larger extent there than in temperate lakes. Hence, fish partly become primary consumers, implying that food webs are more truncated in the subtropics [20,41].

By affecting, among other processes, predator-prey interactions, submerged macrophytes may importantly influence the functioning of the whole lake [42,43]. The typically positive effect of submerged macrophytes on water clarity in temperate shallow lakes [44,45] is substantially reduced in warm lakes [46,47], not least because macrophytes do not act as proper refuges for large zooplankton [29,30]; instead, they host small fish [23,48,49], as well as potential predatory macroinvertebrates like the shrimp *Palaemonetes argentinus* [32], sometimes in extremely high densities [25].

In the present study, we experimentally addressed the role played by fish and predatory macroinvertebrates for the trophic structure in in-lake closed vegetated mesocosms run under different climate conditions. We focused on the trophic interactions among: (i) predators-zooplankton-phytoplankton, hypothesizing that fish and predatory macroinvertebrates indirectly promote phytoplankton growth through trophic cascades in both climatic zones, but more so in the subtropics; (ii) predators-plant-associated macroinvertebrates-periphyton, testing the hypothesis that trophic cascades promoted by fish and predatory macroinvertebrates would strongly promote periphyton growth in temperate systems. By contrast, in the subtropics, fish would not only consume plant-associated macroinvertebrates, but also periphyton, potentially masking the indirect increase in periphyton biomass caused by trophic cascades.

2. Methods

2.1. Study Area

We selected three shallow lakes (Zmax. <3 m in all cases) in Uruguay (30–35° S, Lakes Diario, Blanca and Nutrias) and in Denmark (55–57° N, Lakes Kogleaks, Stigsholm and Bølling), covering a gradient in submerged macrophyte cover (range: 0–70% plant volume inhabited (PVI), sensu [44]), salinity, pH, turbidity, total nutrients and phytoplankton chlorophyll-a (Chl-a) (Table 1). In each lake, we conducted a mesocosm experiment during summer (January–April in Uruguay and August–October in Denmark).

Table 1. Main limnological parameters of the lakes where the experiments were conducted. Shaded = subtropical Uruguayan lakes (30–35° S) and non-shaded = temperate Danish lakes (55–57° N). Shown are mean temperature, dissolved oxygen concentration (DO_2), pH, water turbidity (Nephelometric Turbidity Unit, NTU) and concentration of phytoplankton chlorophyll-a (Chl-a) at the beginning (start) and at the end of the experiments. Distinctive traits of the lakes, including submerged plant percentage of volume inhabited (PVI), total nitrogen (TN) and total phosphorus (TP) concentrations, are also provided.

	Diario		**Blanca**		**Nutrias**		**Kogleaks**		**Stigsholm**		**Bølling**	
	start	**end**	**start**	**end**	**Start**	**end**	**start**	**end**	**start**	**end**	**start**	**End**
Temperature (°C)	28.0	20.5	22.5	21.4	27.0	20.2	16.6	8.3	15.1	10.4	14.7	13.9
DO_2 (mg L^{-1})	9.7	7.0	6.7	10.4	7.4	8.4	5.7	9.1	10.5	9.3	8.7	7.6
pH	8.6	7.5	8.4	7.61	6.14	5.8	6.9	7.5	8.4	7.5	7.1	7.2
Conductivity (mS cm^{-1})	566	617	318	316	75	82	595	542	210	185	116	116
Turbidity (NTU)	6.3	20	14.2	18.2	26.3	39.7	12.4	2.1	3.6	3.9	14.7	17.3
Phyto. Chl-a (μg L^{-1})	15.7	9.6	46.5	56.1	15.7	4.7	8.9	4.7	11.4	8.2	8.4	10.7
Distinctive traits	Slightly brackish		Cyanobacteria blooms		Humic		Slightly brackish		Cyanobacteria blooms		Humic	
PVI (%)	>75%		<25%		0%		ca. 50%		ca. 50%		0%	
TN (μg L^{-1})	970		1391.5		670		2330		2275		1600	
TP (μg L^{-1})	89.2		65.9		122.5		214.2		55		182.8	

2.2. Experimental Design

We used 16 transparent cylindrical PVC enclosures (diameter 1.2 m) in each of the six lakes (Table 2). The water level within the enclosures varied between 0.8 and 1.1 m during the course of the experiment, approximating a total water volume of 1000 L. The mesocosms were kept open to the atmosphere and fixed by a metal ring penetrating ca. 0.30 m into the sediment, thus ensuring isolation from the outside water. A hard plastic ring and several rubber bands mounted to poles secured the top ca. 0.40 m above the water surface. We placed the mesocosms along both sides of a specially-constructed bridge that allowed us to take samples with minimum disturbance (Figure 1).

Figure 1. Eight plastic enclosures were fixed to each side of a bridge, and an artificial plant bed was introduced to all enclosures two weeks before sampling to allow periphyton and macroinvertebrates to colonize the plastic structures.

Prior to the establishment of the mesocosms, we carefully removed all natural vegetation from the area. During the setup, fish were prevented from entering the mesocosms by placing a small-sized mesh net (500 µm) on the bottom of each mesocosm that was removed before initiating the experiment, allowing a direct contact between sediments and water.

To each mesocosm, we introduced an artificial plant bed mimicking submerged plants (120 stems per module, PVI = 75%). The plants were made of "hairy" 1.0 m-long plastic pieces (originally green Christmas' tree garlands) with an architecture similar to that of macrophytes such as *Myriophyllum* sp. or *Cabomba* sp. (as in [29]). The plants were introduced to the mesocosms two weeks before establishing the treatments to allow periphyton and invertebrate colonization.

Assigned treatments were F (only fish); F + INV (fish + predatory macroinvertebrates); INV (only predatory macroinvertebrates) and CON (no predators added); (details of the design are in Table 2). Since we focused on the potential cascading effects of an assemblage of predators, in each country, we used two typically abundant omnivorous/predatory fish species ("Rio de la Plata one-sided live-bearer" *Jenynsia multidentata* Jenyns (1942) and "ten spotted live-bearer" *Cnesterodon decemmaculatus* Jenyns (1942) in the subtropical lakes, and "three-spined stickleback" *Gasterosteus aculeatus* L. and "European perch" *Perca fluviatilis* L. in the temperate lakes) and one omnivorous/predatory macroinvertebrate species (*Palaemonetes argentinus* Nobili (1901) and *Gammarus lacustris* Sars (1863), in subtropical and temperate systems, respectively). Both the species and the abundances to be added resembled natural densities as described in the available literature [23,50,51] (Table 2), representing a rough fish biomass of 110 g of fresh weight in each treatment in both countries and a ca. 5-times higher predatory macroinvertebrate biomass per mesocosm in the subtropical than in the temperate systems (56 and 12 g, according to natural average densities, respectively). We did not select individuals for sex and used individuals smaller than 7.0 cm (standard length) to ensure predominance of zooplanktivory-invertivory feeding habits. In the case of the "live-bearers", we avoided introducing pregnant females.

Table 2. Fish and macroinvertebrate predator species and numbers added to the mesocosms in the treatments: F (fish); F + INV (fish + predatory macroinvertebrates) and INV (predatory macroinvertebrates). F and F + INV treatments had individuals of both fish species (fish densities at the beginning, expressed as ind. m^{-2}, are given in parenthesis). For each species, biomass was estimated by multiplying introduced densities by average weight and expressed in grams of fresh weight. The selected densities of predators were based on information in the literature: * [23]; + [50]; ¤ [51].

		Fish Species 1	**Fish Species 2**	**Macroinvertebrates**
Subtropical	Name	*Cnesterodon decemmaculatus*	*Jenynsia multidentata*	*Palaemonetes argentinus*
	Density	50 (42) *	40 (33) *	120 (100) *
	Biomass	26.5	80.8	56
Temperate	Name	*Gasterosteus aculeatus*	*Perca fluviatilis*	*Gammarus lacustris*
	Density	12 (10) +	6 (5) *	240 (200) ¤
	Biomass	29.2	81.8	12

2.3. Sampling and Sample Analysis

We took water samples for physical and chemical analyses and measured in situ parameters (see Table 1) immediately prior to the allocation of treatments (T0) and seven weeks (49 days) later (TF) in all lakes, except for one where some mesocosms had been damaged during a storm (Lake Bølling, Denmark). Since we conducted an extra sampling campaign in Lake Bølling four weeks after T0, those results were considered adequate to represent the final conditions in the lake. We collected depth-integrated water samples for analyses of phytoplankton, zooplankton and nutrients, using a pump that integrated different depths and zones inside each mesocosm.

For quantitative analysis of zooplankton, we filtered 6–8 L water through a 50-µm mesh net and fixed the filtrate with acid Lugol. We performed counts according to [52] and classified the identified cladocerans as pelagic/free-swimming and benthic/plant-associated (following [29]); other zooplankters were categorized as rotifers, calanoid and cyclopoid copepods (both juvenile and adults) and nauplii.

We measured 20 individuals of all of the crustaceans in each sample from the initial and final sampling campaigns and determined the biomass of total zooplankton (dry weight (DW)) using the length/weight relationships of [53]. As an indirect measure of the zooplankton grazing impact on phytoplankton, we estimated the zooplankton to phytoplankton biomass ratio (zoo:phyt; sensu [54]). Phytoplankton Chl-a was measured spectrophotometrically following cold ethanol extraction [55]. For determination of the zoo:phyt ratio, we converted the Chl-a concentration to phytoplankton DW using a Chl-a:C ratio of 30 and a DW:C ratio of 2.2 [54].

We removed one "plant" from each mesocosm (avoiding the outer part for potential wall effects) and shook it inside a 250-µm mesh-sized net to collect the plant-associated macroinvertebrates. Another plant was used to determine the biomass of attached periphyton in the laboratory (as Chl-a [55]). The animals were preserved with alcohol (70%) for taxonomic identification and later classified according to their feeding habits in accordance with the literature [56].

Total macroinvertebrate biomass and biomasses of feeding groups were calculated using length/weight relationships from the literature when available (e.g., [56]) or were directly weighed and expressed in mg. Every individual present was measured to the nearest mm. Based on the literature, the feeding groups were defined as gatherers (some Odonata and some Chironomidae), shredders (Oligochaeta, some Chironomidae and Ephemeroptera), scrapers (Gastropoda), filterers (some Chironomidae and some Trichoptera), piercers (some Trichoptera and Coleoptera) and predators (some Odonata and some Chironomidae). The first four groups were considered potential periphyton consumers, and their biomasses (InvBiom) were used for further calculations of biomass ratios.

The invertebrate to periphyton biomass ratio (InvBiom:periphyt) was also calculated by dividing the sum of gatherers, shredders and scrapers invertebrate biomasses (mg/g plant) by periphyton biomass (µg/g plant) measured as Chl-a (Chl-a concentration converted to periphyton DW, using a Chl-a:C ratio of 30 and a DW:C ratio of 2.2 [54]). We recaptured the added fish and predatory macroinvertebrates (*P. argentinus* and *G. lacustris*) after the final sampling (TF) by electro-fishing and by vigorously sweeping a hand net inside the mesocosms.

2.4. Data Analysis

We performed one-way ANOVA analyses to test for initial (after 14 days' incubation without any treatment) and final between-treatment differences (i.e., F, F + INV, INV and CON) in each lake, in the biomass and abundance of zooplankton, biomass of phytoplankton and periphyton and plant-associated macroinvertebrate abundance. When significant differences emerged, we used Tukey's HSD post hoc test. Prior to the analyses, we tested for homoscedasticity and the normal distribution of residuals using Cochran's C-test and visual inspection of fitted values, respectively. When needed, we transformed the data by square-root or 4th root to homogenize variances. For response variables for which analysis assumptions could not be made (i.e., invertebrate biomass and the InvBiom:periphyt ratio), we used the Kruskal–Wallis H-test.

3. Results

3.1. Pelagic Trophic Interactions

After the 49-day experimental period, we observed strong fish effects on zooplankton abundance (Figure 2) and phytoplankton biomass (Figure 3A,B) in both climatic zones. Fish, both alone and together with predatory macroinvertebrates (i.e., similar responses in the F and F + INV treatments), generated strong negative effects on zooplankton, whereas predatory macroinvertebrates alone (INV treatment) generally had no significant effects on any of the response variables (Figure 2).

In all of the Uruguayan lakes, we found lower cladoceran abundances in the presence of fish (one-way ANOVA tests, $F_{3,12} = 4.7$, $p < 0.05$; $F_{3,12} = 4.4$, $p < 0.05$ and $F_{3,12} = 9.8$, $p < 0.01$ for Lakes

Blanca, Diario and Nutrias, respectively, Figure 2A,C,E) than in their absence. *Daphnia* spp. were absent from the fish mesocosms (F and F + INV), but occurred in some of the fishless treatments (INV and CON); however, their abundance fell at the end of the experiment from 43.0 ± 20.0 to 5.1 ± 1.1 and from 5.2 ± 1.3 to 2.1 ± 0.2 ind. L^{-1} + SE in Lakes Diario and Nutrias, respectively. The abundances of calanoid copepods were also significantly lower in the F and F + INV treatments in Lake Blanca ($F_{3,12}$ = 8.4, $p < 0.01$) and Lake Diario ($F_{3,12}$ = 11.9, $p < 0.01$). Copepod nauplii were negatively affected by fish only in Lake Blanca ($F_{3,12}$ = 5.5, $p < 0.05$), whereas cyclopoid copepods never seemed to be affected by fish or predatory macroinvertebrates in the subtropical lakes.

In contrast, phytoplankton biomass (measured as Chl-a) was higher in the treatments with fish ($F_{3,12}$ = 3.3, $p < 0.05$; $F_{3,12}$ = 10.34, $p < 0.01$ and $F_{3,12}$ = 4.3, $p < 0.01$ in Lakes Blanca, Diario and Nutrias, respectively, Figure 3C). Only in Lake Nutrias did predatory macroinvertebrates alone (INV) affect phytoplankton biomass, with similar effects as in the treatments with fish (F and F + INV; Figure 3C).

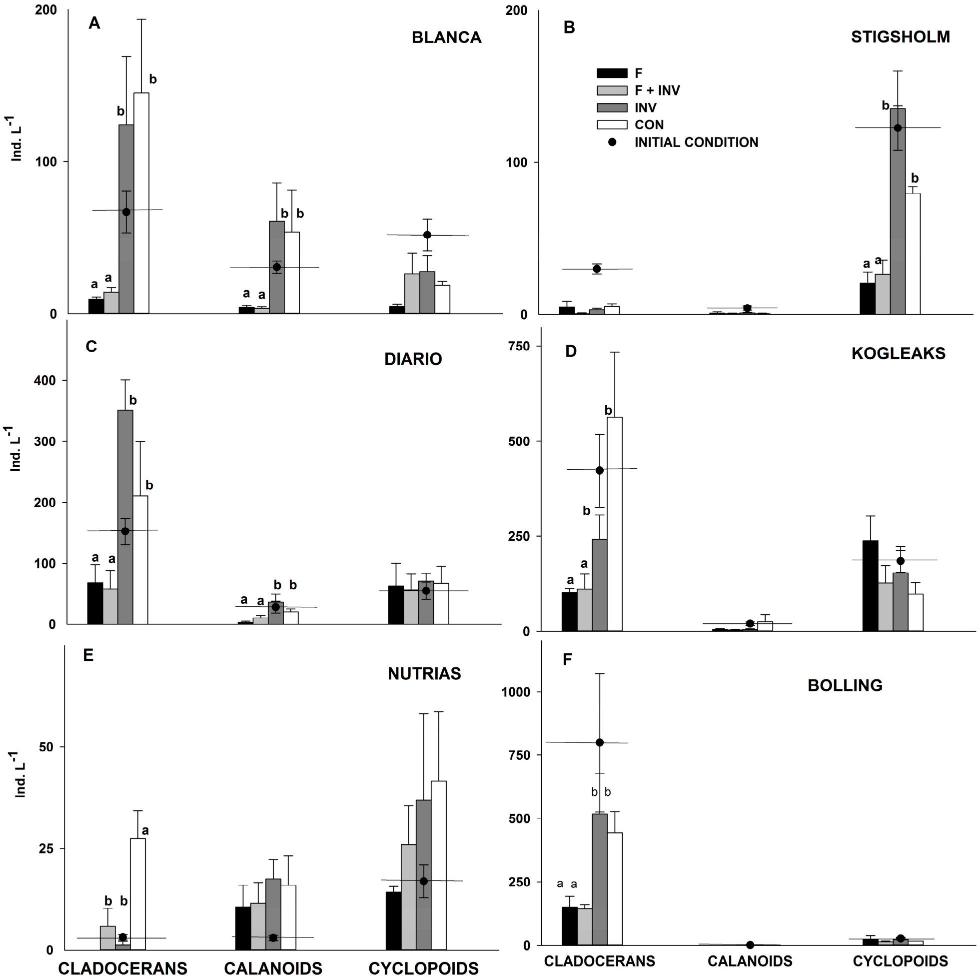

Figure 2. Effects of experimental treatments on the abundance of zooplankton in subtropical (pannels **A**, **C** and **E**) and temperate (panels **B**, **D** and **F**)) lakes. Dotted and horizontal lines represent the initial conditions (just before treatment assignment and 15 days after fish exclusion), while columns show the final conditions (49 days after fish and macroinvertebrate addition). F (fish); F + INV (fish + predatory macroinvertebrates) and INV (predatory macroinvertebrates). Error bars represent ±1 standard error. Please note the different scales on the *y*-axes. Letters indicate groups based on Tukey's post hoc tests when ANOVA tests were significant.

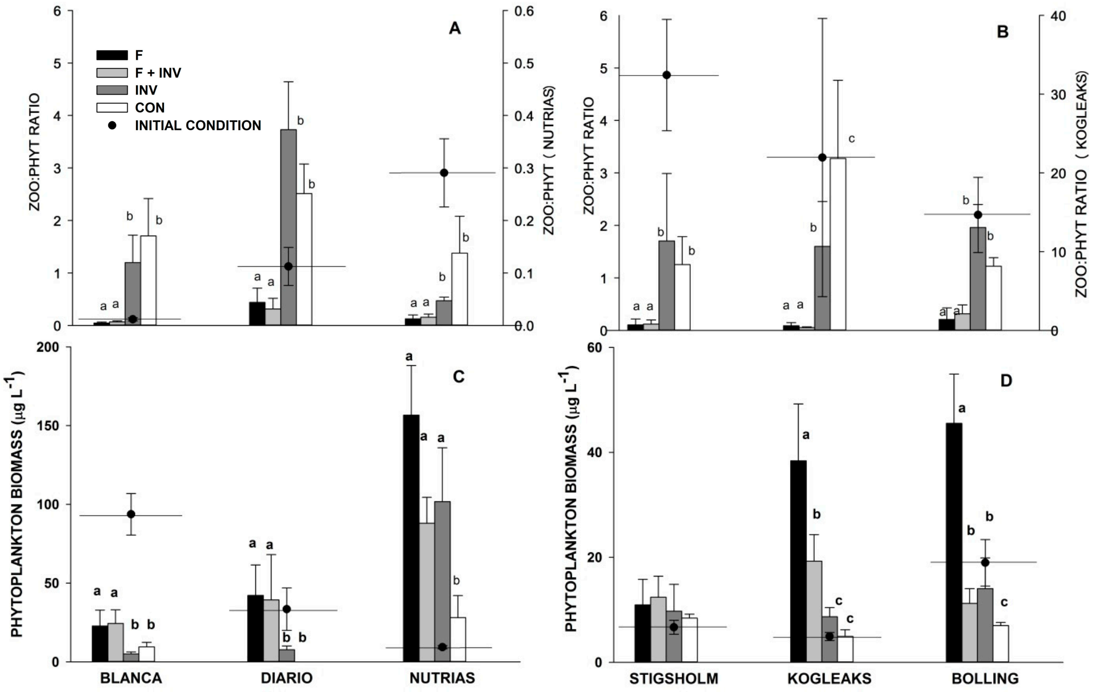

Figure 3. Effects of treatments on potential zooplankton grazing pressure (**A**,**B**) and phytoplankton biomass (**C**,**D**) in subtropical (**left**) and temperate (**right**) lakes. Dotted and horizontal lines represent initial values (just before treatment assignment and 15 days after fish exclusion), while columns depict final values (49 days after fish and invertebrate addition). F (fish); F + INV (fish + predatory macroinvertebrates) and INV (predatory macroinvertebrates). Error bars represent ±1 standard error. Please note the different scales on the *y*-axis. Letters indicate groups according to Tukey's post hoc tests when ANOVA tests were significant.

Similarly, in two Danish lakes, Lake Kogleaks and Lake Bølling, we found statistically significant effects of fish (both alone, F, and together with *Gammarus lacustris*, F + INV) on cladoceran abundances ($F_{3,12} = 6.6$, $p < 0.01$ and $F_{3,12} = 8.2$, $p < 0.01$ in Lake Kogleaks and Lake Bølling, respectively). In Lake Stigsholm, in contrast, cladoceran abundances decreased in all treatments. Contrary to the patterns observed in subtropical lakes, here, *Daphnia* followed the same patterns as total cladocerans. In the three Danish lakes, calanoid copepods occurred in abundances so low that no statistical analysis could be performed. Cyclopoid copepods and nauplii decreased significantly only in the treatments including fish and only in Lake Stigsholm (F and F + INV, $F_{3,12} = 15.7$, $p < 0.001$ and $F_{3,12} = 4.3$, $p < 0.05$) (Figure 2). The predatory macroinvertebrates alone did not affect the zooplankton here either.

Phytoplankton biomass was also higher in the treatments including fish in those lakes where zooplankton was affected (i.e., in Lake Kogleaks and Lake Bølling, $F_{3,12} = 6.1$, $p < 0.001$; $F_{3,12} = 9.3$, $p < 0.01$, respectively). In Lake Stigsholm, however, phytoplankton biomass did not differ among treatments (Figure 3D), despite presumed differences in grazing pressure (Figure 3B). In all lakes in both climatic zones, the potential grazing pressure of zooplankton (estimated as the zoo:phyt biomass ratio) decreased significantly in treatments where fish were present (F and F + INV), but not in the treatment with only predatory macroinvertebrates (Figure 3B). Despite similar trends among the treatments, the estimated grazing pressure varied greatly among lakes, being extremely low in all of the treatments in Lake Nutrias (Uruguay, Figure 3A) and relatively high in all of the treatments in Lake Kogleaks (Denmark, Figure 3B).

3.2. Littoral Trophic Interactions

The interaction patterns observed between predators, plant-associated macroinvertebrates and periphyton were different than those described above between predators, zooplankton and phytoplankton. Here, predators affected the plant-associated macroinvertebrate, but there was not a clear effect on periphyton. Fish (in both the F and F + INV treatments), but sometimes also predatory macroinvertebrates alone (the INV treatments) reduced the abundances of plant-associated macroinvertebrates in both climate zones (Figure 4A,B).

There were only a few differences in the presence and composition of the trophic groups of plant-associated macroinvertebrates between the regions; piercers were not registered in the temperate lakes. In terms of biomass, in both regions, gatherers dominated the community, followed by shredders (Table 3), and they exhibited responses to fish and invertebrate predation throughout the experiment.

The most frequent taxa occurring in the subtropical lakes were Oligochaeta, Chironomidae and Ostracoda, but also Hirudinea and Gasteropoda appeared. Lake Nutrias had a very low abundance of plant-associated macroinvertebrates during the entire study period, preventing meaningful statistical analyses. The density of plant-associated macroinvertebrates was, however, significantly higher in the absence of fish predators in the other two subtropical lakes ($F_{3,12} = 14.0$, $p < 0.001$ and $F_{3,12} = 6.4$, $p < 0.01$ in Lakes Blanca and Diario, respectively, Figure 4A). Fish effects were stronger than those of shrimp (as evidenced in the post hoc tests), which were only noticeable in Lake Blanca (Figure 4A).

In terms of abundance, Oligochaeta and Chironomidae dominated the plant-associated macroinvertebrate communities of the temperate lakes, but also Ostracoda were important in Lakes Kogleaks and Bølling. In Lake Stigsholm, also Trichoptera and Isopoda (e.g., *Asellus* sp.) occurred. In the three temperate lakes, the negative effects of fish on the density of plant-associated macroinvertebrates were significant ($F_{3,12} = 4.3$, $p < 0.05$; $F_{3,8} = 4.3$, $p < 0.05$ and $F_{3,12} = 5.6$, $p < 0.05$ in Lakes Kogleaks, Bølling and Stigsholm, respectively, Figure 4B). Moreover, in two of the three temperate systems, the effects of the predatory macroinvertebrates were similar to those of fish (as evidenced in the post hoc tests, Figure 4B).

Periphyton biomass increased at the end of the experiment. However, the treatments effects were not significant (Figure 4E,F). Moreover, the potential grazing pressure of plant-associated macroinvertebrates (estimated as the InvBiom:periphyt ratio) decreased, particularly so in Lakes Blanca (Figure 4G) and Bølling (Figure 4H), in the treatments where fish were present (F and F + INV), but not in the treatment with only predatory macroinvertebrates. Despite the similar trends experienced by treatments, the estimated potential grazing pressure varied greatly among the lakes, being extremely low in all the treatments in Lake Nutrias (Uruguay, Figure 4G) and very high in all the treatments in Lake Kogleaks (Denmark, Figure 4H).

Table 3. Trophic group biomass (mg g^{-1} plant) of plant-attached invertebrates at the start (To) and at the end of the experiment. (CON (Control); F (fish); F + INV (fish + predatory macroinvertebrates) and INV (predatory macroinvertebrates). Upper panel shows the subtropical Uruguayan lakes (30–35° S) and the lower panel the temperate Danish lakes (55–57° N). Standard errors are in brackets.

	Diario					**Blanca**					**Nutrias**				
	To	**CON**	**F**	**INV**	**F + INV**	**To**	**CON**	**F**	**INV**	**F + INV**	**To**	**CON**	**F**	**INV**	**F + INV**
Predator	0,01 (0,0)	0,0 (-)	0,0 (-)	0,04 (0,1)	0,0 (-)	0,0 (-)	0,0 (-)	0,0 (-)	0,0 (-)	0,0 (-)	0,0 (-)	0,0 (-)	0,0 (-)	0,0 (-)	0,0 (-)
Gatherer	14,8 (5,8)	90,5 (41,4)	1,3 (0,4)	2,9 (0,7)	3,2 (0,1)	17,2 (6,3)	19,3 (9,2)	10,1 (2,0)	21,2 (12,5)	2,3 (1,0)	0,0 (-)	0,0 (-)	0,0 (-)	0,6 (0,2)	0,0 (-)
Shredder	1,9 (1)	0,0 (-)	0,0 (-)	0,23 (0,3)	0,0 (-)	0,8 (0,5)	0,0 (-)	3,8 (0,6)	0,0 (-)	0,0 (-)	0,1 (0,0)	0,0 (-)	0,0 (-)	0,4 (0,1)	0,2 (0,1)
Filterer	0,09 (0,09)	0,03 (0,01)	0,04 (0,02)	0,0 (-)	0,0 (-)	1,9 (1,9)	0,06 (0,06)	0,0 (-)	0,05 (0,03)	0,0 (-)	0,0 (-)	0,01 (0,0)	0,0 (-)	0,0 (-)	0,0 (-)
Scrapers	0,14 (0,14)	0,0 (-)	0,0 (-)	37,8 (37,8)	40,9 (40,9)	0,0 (-)	0,0 (-)	0,0 (-)	0,0 (-)	0,0 (-)	0,0 (-)	0,0 (-)	0,0 (-)	0,0 (-)	0,0 (-)
Piercer	0,02 (0,02)	0,0 (-)	0,0 (-)	0,0 (-)	0,0 (-)	0,0 (-)	0,0 (-)	0,0 (-)	0,0 (-)	0,0 (-)	0,0 (-)	0,0 (-)	0,0 (-)	0,0 (-)	0,0 (-)
Total Biomass	15,3 (5,8)	90,5(41,4)	1,3 (0,4)	40,7 (37,8)	44,0 (40,9)	19,1 (6,2)	19,4 (9,1)	10,5 (2,1)	21,2 (12,5)	22,3 (0,9)	0,01 (0,0)	0,01 (0,0)	0,0 (-)	0,7 (0,2)	0,02 (0,01)
	Stigsholm					**Kogleaks**					**Bøllingso**				
	To	**CON**	**F**	**INV**	**F + INV**	**To**	**CON**	**F**	**INV**	**F + INV**	**To**	**CON**	**F**	**INV**	**F + INV**
Predator	0,3 (0,2)	0,6 (0,6)	0,1 (0,02)	0,5 (0,2)	0,09 (0,09)	0,01 (0,0)	0,0 (-)	0,0 (-)	0,0 (-)	0,0 (-)	0,02 (0,01)	0,0 (-)	0,0 (-)	0,0 (-)	0,02 (0,02)
Gatherer	1,5 (0,9)	3,4 (0,3)	0,02 (0,0)	0,8 (0,3)	0,1 (0,08)	6,4 (2,3)	36,7 (19,4)	0,9 (1,7)	0,01 (0,01)	0,4 (0,2)	4,5 (1,4)	12,8 (8,2)	4,2 (1,4)	6,2 (2,5)	2,9 (1,6)
Shredder	3,7 (2,7)	7,6 (6,0)	1,4 (0,8)	4,9 (3,95)	0,01 (0,01)	0,02 (0,01)	0,05 (0,0)	0,01 (0,01)	0,0 (-)	0,01 (0,01)	1,0 (0,9)	0,3 (0,3)	8,4 (8,4)	12,5 (12,5)	0,02 (0,01)
Filterer	0,0 (-)	0,0 (-)	0,0 (-)	0,0 (-)	2,2 (2,2)	0,0 (-)	0,0 (-)	0,0(-)	0,0 (-)	0,0 (-)	0,5 (0,2)	0,0 (-)	2,1 (2,1)	2,2 (2,2)	0,8 (0,8)
Scrapers	0,0 (-)	0,0 (-)	0,0 (-)	0,0 (-)	0,0 (-)	0,0 (-)	0,0 (-)	0,0 (-)	0,0 (-)	0,0 (-)	0,0 (-)	0,0 (-)	0,0 (-)	0,0 (-)	0,0 (-)
Piercer	0,0 (-)	0,0 (-)	0,0 (-)	0,0 (-)	0,0 (-)	0,0 (-)	0,0 (-)	0,0 (-)	0,0 (-)	0,0 (-)	0,0 (-)	0,0 (-)	0,0 (-)	0,0 (-)	0,0 (-)
Total Biomass	5,5 (3,7)	11,6 (9,3)	1,6 (0,8)	6,2 (3,3)	0,4 (0,1)	6,4 (2,3)	36,7 (1,9)	0,9(1,7)	0,01 (0,0)	0,4 (0,2)	6,1 (1,2)	13,1 (8,2)	12,8 (9,8)	18,9 (1,3)	3,0 (1,6)

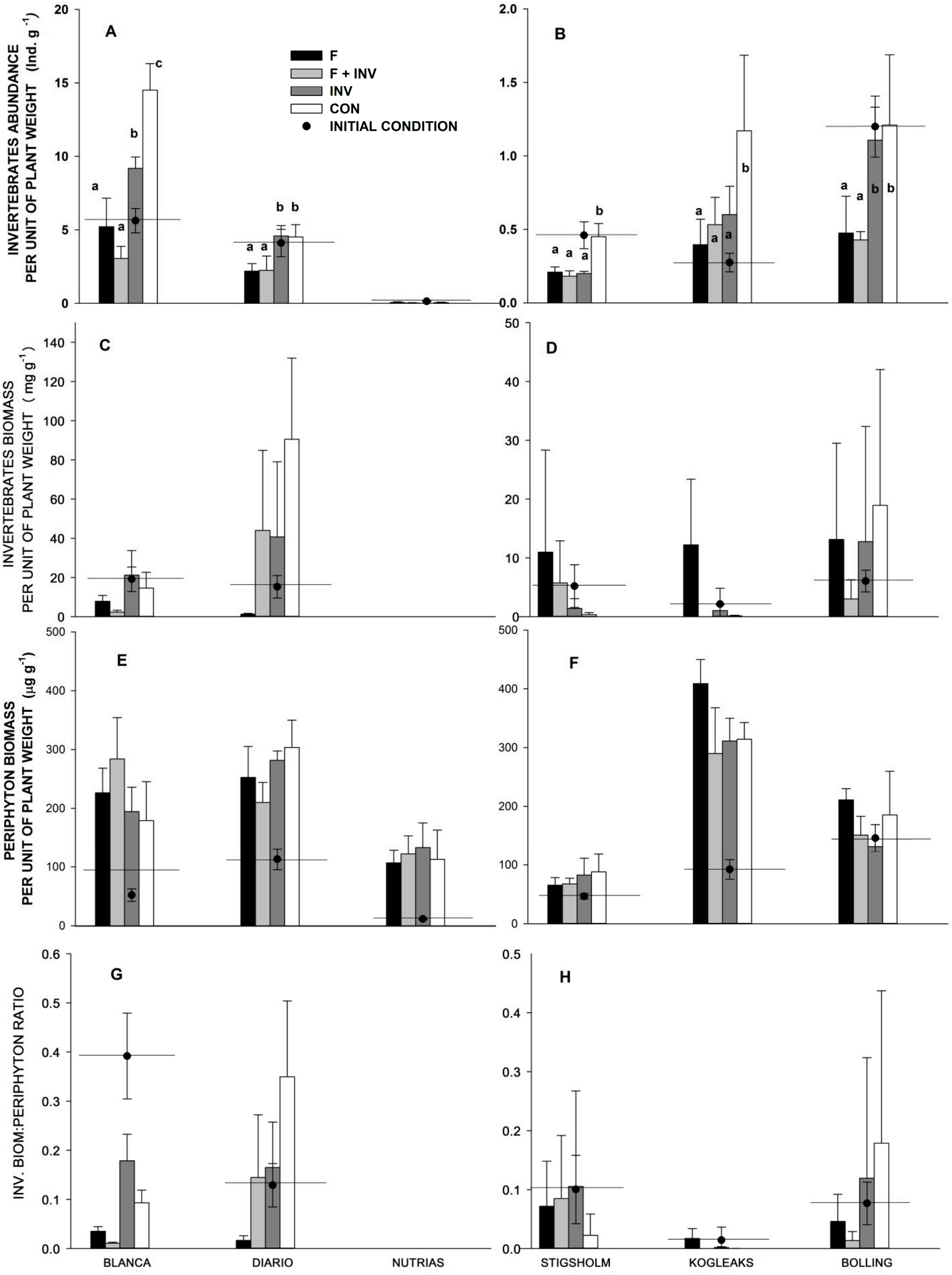

Figure 4. Density (**A**,**B**) and biomass (**C**,**D**) of plant-associated macroinvertebrates, periphyton biomass (**E**,**F**) and potential macroinvertebrates grazing pressure (**G**,**H**) in subtropical (**left**) and temperate (**right**) lakes. Dotted and horizontal lines represent initial values (15 days after fish exclusion and just before treatment assignment), while columns represent final conditions (49 days after treatment additions). F (fish); F + INV (fish + predatory macroinvertebrates) and INV (predatory macroinvertebrates). Error bars represent ±1 standard error. Please note the different scales on the *y*-axes. Letters indicate groups according to Tukey's post hoc tests when ANOVA tests were significant.

4. Discussion

Our experiment highlights the role of predators (mainly fish) as a strong structuring factor in the food webs in shallow lakes, promoting phytoplankton development in both temperate and subtropical climates. During the course of the experiment, we found a significant decrease in phytoplankton biomass and an increase in zooplankton abundance and biomass only when fish were absent. By contrast, in the fish treatments, a cascading effect was revealed by a major reduction of the zooplankton grazing potential and a significant increase in phytoplankton biomass in both climatic zones. Our findings confirm the occurrence of a fish predation-driven structure of zooplankton [2], as suggested also in previous works in subtropical, e.g., [19,26,32,56–60], temperate, e.g., [61–64], and even sub-polar systems [65,66].

Moreover, our highly structured environment (high artificial PVI of ca. 75%, potentially offering refuge, as shown in [67]) and the availability of alternative food items (such as macroinvertebrates and periphyton attached to the artificial plants) did not counteract the negative effects of fish predation on zooplankton, as could have been expected *a priori*, but rather concurs with the hypothesis that alternative food sources may enhance fish predation on zooplankton [5]. We found that large-bodied zooplankton like *Daphnia* spp. were depleted by fish predation, suggesting that the fish effects were strong even at this high artificial plant PVI, regardless of climatic zone.

Positive cascading effects on water transparency at the whole-lake level following changes in the fish community are often observed under mesotrophic or eutrophic conditions, particularly in temperate climates [61,68,69]. Conversely, long-term effective control of phytoplankton biomass by zooplankton is rarely seen in similar lakes in the subtropics in whole-lake studies with the presence of natural fish communities [39,70,71] and has mainly been reported when fish disappear (e.g., with summer fish kills [33,60]). However, the three-level food web tested displayed the typical trophic cascade effects produced by planktivorous fish, resulting in enhanced phytoplankton biomass. This is a typical pattern in temperate eutrophic lake systems [43,72] and also represents the most commonly-observed response in subtropical lakes already in mesotrophic conditions [46]. The main reasons proposed for the observed zooplankton biomass and body size distribution in the subtropics are the typically high densities and preferential use of macrophytes as habitats by fish [19]. Moreover, omnivory seems to be the most common feeding habit of fish there [23,73,74]. Physiological constraints of cladocerans with increased temperatures have also been suggested as causes for the observed patterns in warm systems [75]. The evidence provided by our experimental study confirms these previous empirical suggestions, but in contrast to expectations, there was no clear indication of the proposed differences between temperate and subtropical food web responses [20].

Unexpectedly, in our experiment, neither the shrimp *P. argentinus* in the subtropics, nor the amphipod *G. lacustris* in the temperate lakes (unless together with fish) triggered a change in zooplankton abundance and biomass and on phytoplankton biomass, contradicting our expectations based on previous field studies (shrimp [76]; amphipod [51]). In a field and laboratory experiment, González-Sagrario and collaborators identified *P. argentinus* as a strong predator on zooplankton [32], but their experiment involved a seven-times higher predator density, a much lower plant PVI in their field mesocosms (ca. 10% vs. our 75%) and a much lower experimental volume in the laboratory than our experiments (0.6 L vs. 1000 L), and any or all of these three factors have likely influenced the hunting and feeding efficiency of the shrimp. Besides, in temperate systems, the lack of effects promoted by *G. lacustris* can also be explained by differences between experimental designs, as we used bottomless mesocosms, allowing the presence of alternative food resources. As an opportunistic feeder, *G. lacustris* tend to use the most easily available food resources [76–78], which was probably not the case for zooplankton in our design where other food items could become available.

Our second hypothesis focused on the behavior of the littoral pathway [4,79]. We did find a clear effect of fish predation on plant-associated macroinvertebrates in all lakes in both countries, as previously suggested for both temperate [15,80] and subtropical shallow lakes [20]. In two out of the three temperate lakes, macroinvertebrate predators (*G. lacustris*) negatively affected the abundance of plant-associated macroinvertebrates, while in the subtropics, the plant-associated macroinvertebrates were not affected by the added shrimps. These differential effects of macroinvertebrate predation revealed in our experiment constitute the strongest contrast between the food webs in the two regions, and they contradict the idea that shrimp may exert a stronger predation pressure than *Gammarus* (or other invertebrate predators like *Chaoborus* larvae [25,81,82]) as suggested from earlier field evidence [20,32].

Contrary to our expectations, however, we found no cascading effects of any of the predators on the biomass of periphyton, suggesting the absence of indirect cascading effects, as well as of direct (i.e., feeding) effects of the used fish or macroinvertebrate predators under our experimental conditions. In the temperate region, the lack of direct effects of fish on periphyton is relatively common in lake studies [83–86] and in mesocosm experiments [17,85]. In contrast, in the subtropics, we expected a direct negative effect of fish on periphyton, as has previously been found [17,25,86] due to the omnivorous feeding behavior and habitat preferences of most subtropical fish [23]. The absence of (negative) effects may reflect an oversimplification of our predator assemblages, particularly with a lower richness of fish under our experimental than under natural conditions, leading to greater availability of alternative, more nutritious food sources, such as zooplankton and macroinvertebrates. Moreover, the low mean densities of plant-associated macroinvertebrates in our study might be an effect of the short colonization phase and low accessibility to the mesocosms, notably of snails, which may have prevented indirect positive effects of fish on periphyton, as previously reported for temperate systems [17,85,87,88]. Besides, the extremely high PVI used (ca. 75%) would have provided a large habitat surface for periphyton development, potentially high enough to counteract and/or mask any fish feeding effect. Our results on the effects of macroinvertebrates alone and interactive effects with fish must therefore be interpreted with caution. Moreover, if intraguild predation is important [89], more complex experiments resembling natural taxonomic and functional diversity besides densities are needed before firm conclusions can be drawn on the littoral food webs under different climate zones.

Altogether, the experimental evidence presented here concurs with the idea that at whole-system level, planktivorous-omnivorous fish [20,86], but not predatory macroinvertebrates [20,32], might constitute a switch forward mechanism to establish and maintain turbid water conditions in shallow lakes regardless of climatic zone by significantly contributing to phytoplankton biomass development.

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