

UNIVERSITÉ DU QUÉBEC

LE COUPLAGE D'HABITATS ET LES RELATIONS PRÉDATEUR-PROIE DANS LES LACS BORÉAUX
DANS UN CONTEXTE DE CONTRAINTES THERMIQUES

THE HABITAT COUPLING AND PREDATOR-PREY RELATIONSHIPS IN BOREAL LAKES
IN THE CONTEXT OF THERMAL CONSTRAINTS

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AVANT-PROPOS

Cette thèse rassemble les trois chapitres qui constituent mon doctorat. Ils sont présentés sous la forme de trois articles scientifiques qui sont ou seront soumis à des revues scientifiques avec comités de lectures. Je confirme que ma contribution a été majeure dans l'élaboration de ce projet, dans les échantillonnages, l'analyse des données, la rédaction et la publication des articles. Mes directeurs de recherche Andrea Bertolo et Pierre Magnan ainsi que mes co-auteurs ont pour leur part grandement contribué à bonifier le projet et les articles présentés dans le cadre de la thèse.

TABLE DES MATIÈRES

REMERCIEMENTS	iv
AVANT-PROPOS	vii
LISTE DES FIGURES	xii
RÉSUMÉ	xiii
ABSTRACT	xvi
INTRODUCTION.....	1
Ecology and the predator-prey relationships	1
Habitat coupling by predation	2
Relevance of aquatic systems.....	3
Context and thesis objectives.....	5
CHAPTER I: Within-lake variations in fish predation risk shape the spatio-temporal structure of the zooplankton community	9
Abstract	10
Introduction	11
Methods	16
Study site	16
Spatio-temporal distribution of zooplankton	17
UVP sampling	17
UVP data processing	18
Zooplankton sampling	18
Limnological variables	19
Size spectra metrics	19
Statistical analyses.....	20
Estimation of NBSS parameters.....	20
Spatio-temporal variation in NBSS parameters.....	20
Taxonomic composition and NBSS secondary structures	21
Results	21
Temperature and productivity variations.....	21
NBSS control and demarcation.....	22
Model selection of explanatory variables	22

NBSS spatial variations	22
NBSS temporal variations	23
Taxonomic composition and NBSS secondary structures.....	23
Discussion	24
NBSS spatial pattern	25
NBSS temporal pattern	26
Seasonal variation of zooplankton species composition	28
NBSS: a tool for fine-scale studies	28
Acknowledgments	29
Data availability statement.....	29
References	30
Figures	37
Supplementary	43
Supplementary references	60
CHAPTER II: Integrating connectivity and interindividual variation in fish–zooplankton spatio-temporal coupling.....	61
Abstract	62
Introduction	63
Methods	66
Study site	66
Zooplankton sampling	67
Zooplankton mapping.....	68
Fish tracking.....	69
Habitat selection analysis	70
Generating simulated positions	71
Assigning depth to simulated trajectories.....	71
Assigning physical variables to trajectories	72
Conditional logistic regressions	73
Analysis of HSA results	74
Results	74
Thermal constraint in littoral–pelagic habitat use.....	74

Zooplankton spatial distribution and associations between resource and physical variables	75
Habitat selectivity	75
Fish–zooplankton coupling	76
Discussion	77
Selection of habitat variables	77
Selection of resource variables.....	78
Conclusion	80
Acknowledgments	81
Data availability statement.....	81
Conflict of interest	81
Ethical approval	81
References	82
Tables.....	88
Figures	89
Supplementary	95
CHAPTER III: Avoidance of planktivorous fish in the pelagic zone: expanding the zooplankton diel vertical migration paradigm to boreal lakes	109
Abstract	110
Introduction.....	111
Methods	114
Study site	114
Zooplankton sampling	115
Aggregation of zooplankton data	116
Measurement of environmental variables	116
Statistical analyses.....	117
Diurnal deficit	119
Results	119
Lake environmental variations	119
DVM patterns	120

Fish influence	120
Relationships with temperature, invertebrate predation and food resources	121
Diurnal deficit	122
Discussion	122
DVM patterns and fish influence	122
Relationships with temperature, invertebrate predation and food resources	124
Acknowledgments	127
References	128
Table	133
Figures	134
Supplementary	140
DISCUSSION	154
<i>Synthesis and scientific contribution of the thesis</i>	154
<i>Research perspectives</i>	159
<i>Conclusion</i>	162
BIBLIOGRAPHY	163

LISTE DES FIGURES

INTRODUCTION

Figure 1: Lake Ledoux aerial photo (October 2018) with a superposition of the bathymetry (1 m-isobath). The lake is about 1km long and 150m wide. Dashed lines delimit the three basins of the lake. 5

DISCUSSION

Figure 2: Spatial 3D distribution of the large zooplankton (i.e., > 1mm; in $\mu\text{L/L}$) during the sampling period. The layers depth circled in black represent the two most used layers by fish at each week. 157

RÉSUMÉ

Pour comprendre comment fonctionnent les écosystèmes et leurs réseaux trophiques, les travaux en écologie doivent étudier le couplage des habitats et l'influence de cette connectivité sur les interactions entre les organismes vivants. Les relations prédateur-proie font partie de ces interactions qui peuvent faciliter ce couplage entre habitats qui seraient autrement isolés. Elles sont à l'origine de flux de matière et d'énergie qui renforcent certaines fonctions écosystémiques (p. ex., productivité, recyclage des nutriments). Ce couplage induit par les relations prédateur-proie est dépendant des conditions environnementales de chacun des habitats. Lorsqu'un ou plusieurs habitats deviennent inaccessibles au prédateur, cela produit une rupture de la connectivité entre ces habitats. Une telle rupture modifie les dynamiques au sein des communautés, avec des changements de leur structure en taille, de leur composition spécifique ou des interactions entre les différents niveaux trophiques. Dans les milieux aquatiques, et plus particulièrement dans les lacs, le couplage d'habitats généré par les prédateurs mobiles est principalement assuré par les poissons. Cependant, cette connectivité est interrompue durant l'été dans certains systèmes à cause de la stratification thermique des lacs et l'apparition de l'épilimnion, une couche d'eau de surface trop chaude pour certaines espèces de poissons (sténothermes froids).

L'objectif de cette thèse est de mettre en évidence les conséquences de la rupture estivale de connectivité sur l'écosystème lacustre. Plus particulièrement, je démontre comment, dans un lac boréal (le lac Ledoux) qui subit une stratification thermique estivale, les réseaux trophiques sont modifiés en réponse à la variation de l'intensité de prédation des poissons, qui ne pourront pas accéder à l'épilimnion et aux zones littorales pendant l'été. Une attention particulière est portée sur l'impact de cette rupture de connectivité sur l'utilisation des habitats par le poisson ainsi que sur l'influence que cela peut avoir sur les communautés zooplanctoniques. Des études ont montré que la prédation par le poisson était responsable de la diminution de la taille du zooplancton et pouvait impacter plus fortement certains taxons de zooplancton. Ce phénomène est lié à la sélectivité des proies par le poisson en fonction de leur visibilité (p. ex., taille, pigmentation) et des défenses qu'elles possèdent (p. ex., capsules, comportements de fuite), les rendant plus ou moins vulnérables. La prédation par les poissons est également un des facteurs qui influence la migration verticale journalière du zooplancton, qui reste dans des eaux profondes et sombres le jour pour éviter la prédation visuelle des poissons, et remonte en surface la nuit pour s'alimenter de phytoplancton.

Le lac Ledoux est un petit lac boréal typique du bouclier canadien qui se stratifie pendant l'été. Il est constitué d'un bassin principal profond (profondeur maximale de 18 m) et de deux bassins moins profonds avec des profondeurs moyennes de ~ 1,5 m et ~ 5 m, sur les côtés ouest et est respectivement. La seule espèce de poisson retrouvée dans ce lac est l'omble de fontaine (*Salvelinus fontinalis*), un poisson sténotherme froid qui s'alimente principalement de zooplancton et d'invertébrés benthiques. L'omble de fontaine ne peut pas survivre dans des eaux où la température dépasse les 22°C. Ainsi, lorsque la stratification thermique du lac Ledoux apparaît en été, le poisson devient incapable, non seulement de rester en surface, mais aussi d'aller dans les zones littorales et dans le bassin à l'ouest devenu trop chaud. Cela induit donc une rupture de la connectivité entre le bassin à l'ouest et le reste du lac, entre les zones littorales et pélagiques et entre l'épilimnion et le reste de la colonne d'eau. Je prédis donc que les communautés zooplanctoniques seront distinctes, en termes de structure de taille ou de composition avec une abondance moindre et des individus de plus petite taille, dans le bassin central où le poisson réside. Lorsque la stratification disparaît à l'automne et que le poisson est de nouveau capable de connecter les différents habitats du lac, je prédis une homogénéisation des communautés de zooplancton. De plus, je

prédit que le poisson modifiera son comportement à l'arrivée de l'automne, notamment en termes de sélectivité de proies, dès lors que les invertébrés benthiques qui habitent les zones littorales redeviendront accessibles. Enfin, je prédis que le zooplancton vulnérable au poisson montre un comportement migratoire atypique, en profitant de l'absence de poisson dans l'épilimnion pour y trouver refuge pendant l'été. Pour répondre à ces questions, des échantillonnages hebdomadaires ont eu lieu du 23 juillet au 8 octobre 2018. Le zooplancton a été échantillonné à haute fréquence le jour et la nuit avec un profileur de vision sous-marine (Underwater Vision Profiler : UVP) qui a pris des photos de zooplancton le long de transects verticaux et horizontaux pour obtenir des informations sur la structure de taille et la composition spécifique de la communauté à l'échelle du lac. En parallèle, le suivi de la population d'omble de fontaine a été assuré par télémétrie acoustique qui nous a permis de suivre les déplacements 3D de 21 poissons environ toutes les 20 secondes. En outre, un fluoroprobe a été utilisé pour connaître la distribution verticale du phytoplancton et faire des profils de température. Enfin, des sondes à oxygène ont permis de prendre en compte d'éventuelles conditions d'hypoxie dans la colonne d'eau.

Dans le premier chapitre, l'objectif a été de mettre en évidence les différences de communautés de zooplancton dans des habitats parfois distants de quelques dizaines de mètres seulement, en relation avec les variations spatio-temporelles du risque de prédation par le poisson. La structure en taille de la communauté zooplanctonique a été étudiée en utilisant le cadre analytique des spectres de taille normalisés (Normalized Size Spectra : NSS). Les résultats ont montré que la structure de taille du zooplancton et la composition des espèces sont distinctes dans les bassins les plus et moins profonds. De façon inattendue, nous avons observé une plus grande proportion de petits individus dans le bassin peu profond par rapport au bassin profond. En revanche, la plus grande abondance de zooplancton dans le bassin peu profond est conforme à l'hypothèse que l'impact de la prédation par le poisson est plus faible que dans le bassin central. Dans le bassin profond, une proportion inattendue de zooplancton de grande taille a été trouvée. Puisque les poissons sont davantage susceptibles de se nourrir de grands individus, cela suggère que les poissons pourraient être moins importants que les *Chaoborus* sp. (prédateurs invertébrés pélagiques se nourrissant de zooplanctons plus petits) dans la pression de prédation réelle. Cependant, la proportion du zooplancton de grande taille a diminué dans les deux bassins, ouest et central, en automne avec la levée de la barrière thermique. Cela suggère un impact accru de la prédation des poissons sur les communautés de zooplancton en automne, démontrant ainsi la restauration du couplage d'habitats par les poissons entre les zones pélagiques et littorales et entre l'épilimnion et le reste de la colonne d'eau à cette période de l'année. Nos résultats montrent que les variations spatiales et temporelles de la structure de la communauté zooplanctonique dans les écosystèmes de lacs boréaux sont probablement dues à une interaction complexe entre la prédation sélective des poissons, les contraintes physiques et d'autres prédateurs potentiels.

Dans le deuxième chapitre, l'objectif a été de comprendre (i) comment les poissons adaptent leurs tactiques d'alimentation pour faire face à leur besoin de thermorégulation ; (ii) si la sélectivité des proies par l'omble de fontaine dépend des variations saisonnières de connectivité des habitats en lien avec la barrière thermique ; et (iii) s'il y a des différences interindividuelles dans la sélection des ressources au sein de la population de poisson dû à un polymorphisme associé aux ressources. Des analyses de sélection d'habitat ont été utilisées pour comprendre comment l'omble de fontaine sélectionne son habitat en fonction des variables abiotiques comme la température ou biotiques comme l'abondance de zooplancton. Les résultats ont d'abord montré très clairement que le poisson était absent des zones peu profondes et de l'épilimnion en été et se retrouvait beaucoup plus fréquemment dans ces habitats en automne. Cela confirme bien la rupture de la connectivité des différents habitats du lac Ledoux en été. De plus, bien que certains individus ne sélectionnent jamais le zooplancton, que ce soit en été ou à l'automne, la plupart des

individus suivis ont sélectionné leur habitat en fonction de la densité du zooplancton, plus ou moins fréquemment. Malgré la prédiction d'une plus grande sélectivité du zooplancton en été lorsque les invertébrés benthiques étaient inaccessibles, c'est en automne qu'elle était la plus forte. Cela pourrait confirmer l'hypothèse qu'en été, le zooplancton est difficile d'accès pour le poisson car il exploiterait l'épilimnion comme refuge. En revanche, les poissons ont sélectionné de manière égale les taxons vulnérables et ceux plus petits ou avec des défenses ce qui entre en contradiction avec les résultats du Chapitre I où les individus de grandes tailles étaient les plus impactés par la prédation par le poisson.

Dans le Chapitre III, l'objectif a été de démontrer que le poisson ne change pas seulement la structure de la communauté de zooplancton mais aussi le comportement de certains taxa, avec des impacts sur le fonctionnement de l'écosystème. Je prédis une migration verticale journalière du zooplancton différente de ce qui a été observé généralement dans la littérature en présence de poissons planctivores. Le zooplancton vulnérable devrait utiliser l'épilimnion comme un refuge au lieu de l'éviter pendant le jour. Les prédateurs sténothermes froids qui n'ont pas accès à la surface des lacs boréaux pendant l'été devraient en effet exercer une pression de prédation plus faible dans l'épilimnion. Une sélection de modèles a été utilisée pour déterminer l'existence ou non de patrons de migrations chez différents groupes de zooplancton ainsi que leurs variations saisonnières. De plus, les données de distribution verticale des poissons, de la température et du phytoplancton ont été ajoutées aux modèles pour déterminer l'influence de celles-ci sur le comportement migratoire des différents groupes de zooplancton. Les résultats suggèrent que les taxons vulnérables comme *Daphnia* spp. effectuent une migration inverse (en profondeur la nuit et en surface le jour) ou restent même dans l'épilimnion pendant tout le cycle de 24h lorsqu'il s'agit des grands individus plus vulnérables. La migration verticale du zooplancton moins vulnérable aux poissons a été au contraire davantage influencée par la température ou la distribution du phytoplancton. En automne, le zooplancton vulnérable a changé son comportement lorsque l'épilimnion s'est refroidi et est devenu disponible pour les poissons. Les résultats suggèrent aussi une influence de la prédation par *Chaoborus* sp. sur le patron de migration du zooplancton de petite taille. Ces résultats montrent bien les interactions qui peuvent exister entre les différents types de prédation, la distribution des ressources ou la température dans la dynamique des réseaux trophiques.

Cette thèse a mis en évidence l'impact que peut avoir une rupture de la connectivité à l'intérieur d'un écosystème. Les relations prédateur-proie peuvent être complexes et changer de nature et d'intensité sur de courtes distances, contrairement aux conditions homogènes auxquelles on pourrait s'attendre dans un si petit écosystème. En modifiant le comportement des poissons, la barrière thermique estivale a un impact indirect sur l'ensemble du réseau trophique. Le couplage d'habitats effectué par le poisson est interrompu en été, créant différents niveaux de risques de prédation pour le zooplancton. Cela provoque non seulement un changement de la structure en taille et de la composition de la communauté de zooplancton, mais aussi un changement du comportement migratoire de certains taxons avec des impacts potentiels sur le fonctionnement des écosystèmes comme la séquestration de carbone ou l'efficacité de transfert de matière au sein du réseau trophique. Ce travail démontre l'importance de considérer à la fois les changements de comportements d'un prédateur mais aussi les variations de la dynamique de ses proies pour obtenir une compréhension plus fine du fonctionnement des relations prédateur-proie et les conséquences qui en découlent. Cela démontre aussi la nécessité d'une approche globale qui intègre plusieurs compartiments trophiques, la structure des communautés mais aussi leur comportement et ses variations interindividuelles pour comprendre l'effet de rupture de couplage dans un ou plusieurs écosystèmes.

Mots clés : zooplancton, omble de fontaine, barrière thermique, sélectivité, migration, structure des communautés.

ABSTRACT

To understand the functioning of ecosystems and their trophic networks, ecological studies must consider the coupling of habitats and the influence of this connectivity on the interactions between living organisms. Predator-prey relationships are part of these interactions and can facilitate this coupling between habitats that would otherwise be isolated. They are at the origin of organic matter and energy flows that reinforce ecosystem functions (e.g., productivity, nutrient cycling). This coupling induced by predator-prey relationships is related to the environmental conditions of each habitat. When one or more habitats become inaccessible to the predator, this produces a break in connectivity between habitats. Such a disruption alters the dynamics within communities, with changes in their size structure, species composition or interactions between different trophic levels. In aquatic environments and more particularly in lakes, habitat coupling generated by mobile predators is mainly provided by fish. However, this connectivity is interrupted during summer in some systems due to the thermal stratification and the appearance of the epilimnion, a surface layer of water too warm for some fish species (cold-stenotherm).

The objective of this thesis is to highlight the consequences of disruption in summer connectivity on the lake ecosystem. Specifically, I seek to demonstrate how trophic network of a boreal lake (Lake Ledoux), which undergoes summer thermal stratification, will be altered in response to the variation in predation intensity by fish, which will not be able to access the epilimnion and littoral areas during summer. I will focus specifically on the impact of disruption in connectivity on habitat use by fish and the influence that may have on zooplankton communities. Studies have shown that predation by fish is responsible for a decrease in the size of zooplankton and can impact some zooplankton taxa more strongly. This phenomenon is related to prey selectivity by fish based on the prey conspicuousness (e.g., size, pigmentation) and defenses (e.g., capsules, escape behavior), making them more or less vulnerable. Fish predation is also a factor that influences the diel vertical migration of zooplankton, which will stay in deep, dark water during the day to avoid visual predation by fish, and come to the surface at night to feed on phytoplankton.

Lake Ledoux is typical of small Boreal Shield lakes and is thermally stratified during summer. The lake consists of a deep main basin (maximum depth 18 m) and two shallow basins with average depths of ~ 1.5 m and ~ 5 m, on the west and east sides, respectively. The only fish species in this lake is brook charr (*Salvelinus fontinalis*), a cold-stenothermic fish that feeds primarily on zooplankton and benthic invertebrates. Brook charr cannot survive at water temperatures above 22°C. Thus, when thermal stratification of Lake Ledoux occurs during summer, the fish are neither able to stay at the surface nor can they move into littoral areas or to the western basin which are too warm. This leads to a break in connectivity between the western basin and the rest of the lake, between the littoral and pelagic areas and between the epilimnion and the rest of the water column. Therefore, I predict distinct zooplankton communities in terms of size structure or composition with lower abundance and smaller individuals in the central basin where the fish are. When the stratification disappears during fall and fish are again able to connect the different habitats of the lake, I expect to have an homogenization of zooplankton communities. Furthermore, I expect that fish will change their behavior during fall, especially their prey selectivity, as benthic invertebrates inhabiting the nearshore areas become accessible. Finally, fish-vulnerable zooplankton should show atypical migratory behavior, taking advantage of the absence of fish in the epilimnion to find refuge during summer. To answer these questions, weekly samplings were conducted from July 23 to October 8, 2018. Zooplankton was sampled at high frequency during day and night with an Underwater Vision Profiler (UVP) that took pictures of zooplankton along vertical and

horizontal transects to get information on the size structure and specific composition of the community at the lake scale. Monitoring of the brook charr population was done by acoustic telemetry, to follow 3D positions and movements of 21 fish at approximately every 20 sec. Furthermore, a fluoroprobe was used to know the vertical distribution of phytoplankton and to get temperature profiles. Finally, oxygen probes were installed to take into account possible hypoxic conditions in the water column.

The objective of Chapter I was to highlight the differences in zooplankton communities in habitats sometimes only few meters apart in relation to the spatio-temporal variations of the fish predation risk. The size structure of the zooplankton community was studied using the normalized size spectra (NSS) analytical framework. Results showed that zooplankton size structure and species composition were distinct between the deepest and the shallowest basins. There was an unexpected higher proportion of small individuals in the shallow basin compared to the deeper one. In contrast, the greater abundance of zooplankton observed in the shallow basin is consistent with the hypothesis that the impact of fish predation is lower than in the deeper basin. In the latter, an unexpected proportion of large zooplankton was found. Since fish are more likely to feed on large individuals, this suggests that fish may be less important than *Chaoborus* sp. (pelagic invertebrate predators feeding on small zooplankton) in the actual predation pressure. Nonetheless, the proportion of large zooplankton decreased in both the western and deep basins during fall with the disappearance of the thermal barrier caused by the stratification. This suggests a higher impact of fish predation on zooplankton communities in autumn, demonstrating the restoration of habitat coupling by fish between pelagic and littoral areas and between the epilimnion and the rest of the water column at this time of the year. Our results show that spatial and temporal variation in zooplankton community structure in boreal lake ecosystems is likely due to a complex interaction between selective fish predation, physical constraints, and other potential predators.

The objective of Chapter II was to understand (i) how fish adapt their feeding tactics to meet their thermoregulatory needs; (ii) whether prey selectivity by brook charr depends on seasonal variations in habitat connectivity related to the thermal barrier; and (iii) whether there are inter-individual differences in resource selection within the fish population due to a resource polymorphism. Habitat selection analyses were used to understand how brook charr selected their habitat based on abiotic variables such as temperature and biotic variables such as zooplankton abundance. The results showed very clearly that fish were absent from shallow areas and epilimnion during summer and were mainly found in these habitats during autumn. This confirms the break in connectivity of the different habitats of Lake Ledoux in summer. Furthermore, although some individuals never selected zooplankton, neither in summer nor fall, most individuals selected their habitat based on zooplankton density, more or less frequently. While greater zooplankton selectivity was expected during summer when benthic invertebrates were inaccessible, it was highest during fall. This may suggest that in summer, zooplankton is not easily accessible to fish because it exploits the epilimnion as a refuge. On the other hand, fish selected equally vulnerable taxa and smaller or individuals with defenses, which contradicts the results of Chapter 1 where larger individuals were most impacted by fish predation.

The objective of Chapter 3 was to demonstrate that fish not only change the structure of the zooplankton community but also the behavior of some taxa, with impacts on ecosystem functioning. I predict a daily vertical migration of zooplankton differing from what is observed in the presence of planktivorous fish. Vulnerable zooplankton are expected to use the epilimnion as a refuge rather than avoiding it during the day. Cold stenothermic predators that cannot go to the surface of boreal lakes during summer should exert abnormally low predation pressure in the epilimnion. A selection of models was used to investigate migration patterns in different groups of zooplankton and their seasonal variations.

Furthermore, data on vertical distribution of fish, temperature, and phytoplankton were added to the models to determine the influence of these variables on the migratory behavior of different zooplankton groups. The results suggest that vulnerable taxa such as *Daphnia* spp. performed a reverse migration (in the deep strata at night and at the surface during the day) or remain in the epilimnion for the entire 24-hour cycle when considering larger, more vulnerable individuals. In contrast, the vertical migration of less vulnerable zooplankton was more influenced by temperature or phytoplankton distribution. In autumn, vulnerable zooplankton changed their behavior when the epilimnion cooled and became available to fish. Results also suggest an influence of predation by *Chaoborus* sp. on the migration pattern of small zooplankton. Together, these results demonstrate the interactions that can exist between the different types of predation, the distribution of resources or the temperature in the dynamics of food webs.

This thesis has highlighted the impact of an interruption in connectivity can have within an ecosystem. Predator-prey relationships can be complex and change in nature and intensity over small distances, in contrast to the homogeneous conditions one might expect in such a small ecosystem. By altering fish behavior, the summer thermal barrier have an indirect impact on the entire food web. Habitat coupling by fish is interrupted in summer, creating different levels of predation risk for zooplankton. This not only causes a change in the size structure and composition of the zooplankton community but also in the migratory behavior of certain taxa with potential impacts on ecosystem functioning like carbon sequestration or efficiency of matter transfer within the trophic network. This work has shown the importance of considering both changes in predator behavior and variation in prey dynamics for a detailed understanding of the functioning of predator-prey relationships and their consequences. This also demonstrates the need of a global approach integrating several trophic compartments, community structure but also their behavior and its inter-individual variations to understand the effect of coupling disruptions in one or more ecosystems.

Keywords : zooplankton, brook charr, thermal barrier, selectivity, migration, structure of communities.

INTRODUCTION

Ecology and the predator-prey relationships

The quintessential question in ecology is the understanding of the relationships connecting living organisms together and to their environment. These connections are at the core of an ecosystem but are also responsible for the inter-connectivity between distant ecosystems (Polis et al. 1997, Gounand et al. 2018a). The connectivity is at the foundation of many crucial functions in ecosystems from production to remineralization. While very challenging, an holistic understanding of these relationships is crucial to apprehend the functioning of an ecosystem, its status and trajectory in the context of global changes (Harvey et al. 2017), which is arguably paramount in the current climate situation.

Predator-prey relationships are one of the first connections that ecological studies have sought to explain and predict. Given their easy conceptualization, these relationships have been theorized/modelized early (e.g., Lotka Volterra models in the mid 20s, Wangersky 1978, Abrams 2000). One of the most obvious consequences that predation has on ecosystems is the reduction of prey population abundances (Abrams 2000). However, predators can also have more subtle implications on prey populations, affecting for example their size and age structure in aquatic systems (Magnan 1988, Quintana et al. 2015), their competitiveness and the diversity of communities (Sih et al. 1985, Abrams 2000). Predation can also eradicate diseases by prey selection (Mukhopadhyay & Bhattacharyya 2009) and create subsidies between two ecosystems due to predators moving across them (Polis et al. 1997, Gounand et al. 2018b). These findings highlighted that predators can have a strong influence on ecosystem. When its impact is modified (e.g., variation of predator density, distribution or behavior), this can cause substantial consequences on the trophic network and the ecosystem equilibrium, with potential cascading effects (Schmitz et al. 1997).

Predator-prey relationships have been studied for a long time and contemporary ecological studies are now integrating much more information to assess the impact of predation on prey populations. For instance, analyses of prey selection by predators have deepened by integrating the body-size of both preys and predators, taxonomy, phenology and density-dependent responses (e.g., Becker et al. 2021, Gicquel et al. 2022). The advance of technology and knowledge enabled to reach a higher complexity in our conceptualization of predator-prey relationships (Suraci et al. 2022). Moreover, this allowed to further

consider behavioral responses of the entire trophic networks and consider ecosystems as a whole instead of considering only a prey compartment related to a predator compartment.

Habitat coupling by predation

Predators are crucial to make connections between distant ecosystems, but also between habitats within an heterogeneous environment (Polis et al. 1997, Schindler & Sheuerell 2002, Guzzo et al. 2017). By their movements, highly mobile predators are able to use different habitats to feed, rest and reproduce, and will therefore connect different systems and their trophic networks, creating transfers of matter or energy among habitats (Polis et al. 1997, Gounand et al. 2018b). This predator-induced habitat coupling has major consequences on the functioning of ecosystems, connecting trophic networks and enhancing nutrient cycling (Wetzel et al. 2005, Williams et al. 2018). However, this coupling is directly affected by the possibility for the predator to move across habitats. Although predation can connect two or more heterogeneous habitats, it implies that all these systems have to remain accessible for the predator. Thus, when a barrier (physical, chemical, biological, etc.) prevents a predator from moving through one of the habitats, it disrupts the habitat coupling (e.g., Thurfjell et al. 2014, Guzzo et al. 2017). The newly inaccessible habitat is not the only one to be affected by the modification of the predator access. Suitable habitats can also be impacted by an increased predation pressure, disrupting the previous trophic balance (Abrams 2000).

Affordable remote tracking technologies popularized the studies of connectivity on animal movements and hence habitat coupling (e.g., Thurfjell et al. 2014, Guzzo et al. 2017). Many studies looked at the impact of anthropogenic physical barriers, such as dams or roads, which altered movements of consumers and therefore the habitat coupling (e.g., Raymond 1979, Thurfjell et al. 2014). Furthermore, recent developments in the numerical analyzes of the large datasets on animal movements enabled better identification of processes of habitat selection and decision making (Thurfjell et al. 2014, Fieberg et al. 2021). While these studies focused on large terrestrial mammals which could carry heavy GPS collars (e.g., Thurfjell et al. 2014), their attention extended more recently to smaller animals and to aquatic systems thanks to miniaturization and acoustic telemetry (Williams et al. 2018). In this analytical framework, the accuracy of habitat or resource selection measurements allowed to disentangle the preferences of the tracked animals among the different features of an ecosystem like food abundance, forest cover, temperature or distance to a road (Thurfjell et al. 2014, Fieberg et al. 2021, Munden et al. 2021). A direct consequence of this should be a better assesement of the strength of the habitat coupling generated by

the movement of an animal, by considering their selectivity. However, studying habitat coupling induced by consumers or predators solely with their movements is insufficient to make inferences on their specific impacts on prey populations. Predator-prey relationships and habitat selection were often considered separately and deducted from one another. This limitation is often due to the large investments in time and money this kind of integrative approach implies. Nonetheless, considering both points of view together is central to correctly apprehend predator-prey relationships. Furthermore, it is important to understand how a predator will select a prey in different habitats, making a tradeoff between physical habitat selection (e.g., temperature, water supply, substrate or vegetation) and prey selection (e.g., based on taxonomy, size, health condition or energy involved in searching and handling prey). Then, it is necessary to assess the impact of prey selectivity by predators on ecosystems, especially on prey population structure and composition. Assessing the causes and consequences of predation does not only helps to better understand the functioning of an ecosystem but also to predict the changes that might occur when an ecosystem is altered.

Relevance of aquatic systems

In freshwater and marine environments, predation is not only regulating prey abundances, but is also responsible for the largest migration on earth. Zooplankton Diel Vertical Migration (DVM, Pearre 2003, Hays 2003, Bandara et al. 2021) is indeed the greatest movement of biomass among ecosystems on earth and is mainly triggered by fish predation (Hays 2003, Bandara et al. 2021). The most common DVM pattern consist of a movement of zooplankters from deep dark waters during the day, to avoid predation by visual predators (i.e., fish) to the surface during the night (i.e., when fish are not able to see zooplankton) to feed on phytoplankton (Hays 2003, Bandara et al. 2021). In this context, predation has an impact on the behavior of the prey community. DVM has multiple consequences on ecosystem functioning, beyond the interaction between fish and zooplankton (e.g., the “biological pump”, which described the enhanced export of organic particulate matter to deep waters by zooplankton migration, Steinberg et al. 2000, 2002). In freshwater and marine environments, most ecological and physiological processes are size-dependent and aquatic ecosystems often exhibit size-structured trophic interactions (Dickie et al. 1987, Brown et al. 2004). Body size explains a large part of the metabolic rates and is positively related to the relative abundance of organisms (mainly in ectotherms, Brown et al. 2004, Trebilco et al. 2013). Furthermore, body size in aquatic ecosystems is usually directly correlated to the trophic position of organisms (Brown et al. 2004, Gómez-Canchong et al. 2013, Trebilco et al. 2013). Given these characteristics, many aquatic ecosystem functions (e.g., productivity, trophic transfer efficiency, etc.) can be estimated from the size

structure of the community and a strong analytical framework was developed for this purpose (Normalized Size Spectra analysis, NSS, Sprules & Munawar 1986, Sprules & Barth, 2016).

Given that many fish species are visual predators, they exert different predation pressure depending on light intensity and conspicuousness of prey, based on body size, color, etc. (Confer et al. 1978, Magnan 1988). Thus, selective predation by fish can affect the size structure and species composition of the zooplankton community (Vanni 1986, Magnan 1988, Lacasse & Magnan 1992, Rodríguez et al. 1993). This predator selectivity coupled with preys' behavioral and morphological defenses lead to greater impacts of predation on some taxa and/or size classes of prey compared to less vulnerable zooplankters (Ohman 1988, Lacasse & Magnan 1992, Rodríguez et al. 1993). Therefore, considering the influence of predation on an imaginary large and homogeneous zooplankton trophic compartment is too simplistic to really understand how predators affect zooplankton demography or their behavior and hence ecosystem functions. Instead, variations of zooplankton community size structure and composition should be studied to understand the subtle effect of predation on ecosystem functioning. Integrating the other parts of the trophic networks such as information on invertebrate predators or phytoplankton (i.e., resources) is also required to have a holistic approach of predator-prey relationships as part of the functioning of aquatic ecosystems (Ohman et al. 1983).

Due to their high mobility, fish are strong candidates for habitat coupling between different ecosystems (Moore et al. 2007) and among heterogeneous habitats within an ecosystem (Guzzo et al. 2017). For instance, the salmon spawning migration brings a tremendous amount of matter and energy from the ocean to rivers upstream (Moore et al. 2007). Without these subsidies, many living organisms would be deprived of food, altering the existing trophic network (Hilderbrand et al. 1999, Moore et al. 2007). In lakes, fish also play a critical role in coupling pelagic and littoral areas, binding both trophic networks (Guzzo et al. 2017). However, the fish-induced habitat coupling can be affected by barriers preventing the free movements of predators. In the case of the migrating salmon, dams cut access to the river upstream, disrupting the energy fluxes between downstream and upstream. Furthermore, in oceans, seas and lakes, strong barriers occur naturally such as sudden changes in temperature or oxygen levels (Boehrer & Schultze 2008). Seasonally, the water column becomes stratified when atmosphere temperature increases, occasionally decreasing the habitat coupling according to fish thermal tolerances for example (Guzzo et al. 2017). Studying such variations of habitat coupling by fish and the resulting impacts on the trophic networks is necessary to understand anthropogenic impacts on the functioning of

ecosystems, especially in the context of global change and climate warming which will increase the frequency and duration of thermal barriers.

Context and objectives of the thesis

In order to improve our understanding of the habitat coupling operated by predators and its consequences on trophic networks, this thesis focuses on the aquatic communities of a single model system: Lake Ledoux (Figure 1). It is a small oligotrophic boreal lake of the Mastigouche Wildlife Reserve, Quebec, Canada (46.802381, -73.277136). The lake is typical of Canadian Shield lakes and is located in a relatively protected area, where logging is limited and sport fishing is strictly controlled by the Quebec Government (fishing was not allowed during the study period). Although it is a natural system, for which a holistic ecological approach could be time-consuming and costly, large amount of data on physical-chemistry (e.g., stratification, oxygenation, bathymetry), zooplankton and fish populations are already available (e.g., Bourke et al. 1996, Bertolo et al. 2001, Goyer et al. 2014, Pepino et al. 2015, Gignac-Brassard et al. 2022). The trophic network of this lake is composed of phytoplankton, zooplankton, benthic invertebrates and fish.



Figure 1: Lake Ledoux aerial photo (October 2018) with a superposition of the bathymetry (1 m-isobath). The lake is about 1km long and 150m wide. Dashed lines delimit the three basins of the lake.

Fluxes of energy between the lake and other systems exist but are negligible since the water input at the inlets are low making the lake an almost closed system. Zooplankton is mainly predated by glassworms (*Chaoborus sp.*) and brook charr (*Salvelinus fontinalis*), a cold-stenothermic salmonid fish. Brook charr is the only fish species present in the lake and its main prey are zooplankton in pelagic areas and zoobenthos in littoral areas (Magnan 1988, Bourke et al. 1999). Thus, brook charr should play an important role in the coupling of pelagic and littoral areas in Lake Ledoux.

The lake consists in a main deep basin (max. depth 18 m) and two shallower basins with mean depths of ~ 1.5 m and ~5 m, at the west and east sides respectively (Figure 1). Previous studies suggested that brook charr cannot survive in waters when the temperature is exceeding a threshold temperature of 22°C (Bourke et al. 1996, Bertolo et al. 2011, Goyer et al. 2014, Smith & Ridgway 2019). This means that brook charr cannot access the warm surface layer during summer (i.e., epilimnion), thus almost entirely preventing the access of the large - and warm - shallow area of the lake due to a thermal barrier (see Figure VII in appendices of Chapter I). In summer, there should then be a disruption of the habitat coupling made by fish between littoral and pelagic areas, which would be restored when surface temperature decreases in the fall (e.g., Guzzo et al. 2017). This results in a separation between benthic and pelagic trophic networks, forcing brook charr to mainly feed on zooplankton during summer.

This thesis aims to test the hypothesis that predator-prey relationships will fluctuate in boreal lakes, in their nature (i.e., prey or habitat targeted) and intensity, due to the variations in accessibility to the different habitat for a cold-stenothermic fish. To test this hypothesis, we monitored phytoplankton, zooplankton and fish populations in addition to physico-chemical conditions. To overcome the challenge of detecting such fine scale fluctuations in a small lake (e.g., few meters between littoral and pelagic habitats), recent state-of-the-art technologies were used. Specifically, an Underwater Vision Profiler (UVP) took in-situ pictures of zooplankton at high frequency for high-resolution samplings (Picheral et al. 2010) generating information on both taxon and size of zooplankton (i.e., its vulnerability). Primary producers (i.e., phytoplankton) were also measured at high resolution, using a Fluoroprobe (bbe Moldaenke). Finally, acoustic telemetry was used to accurately track the predators (i.e., brook charr) by measuring the 3D position of 21 fish every 20 sec throughout the study period.

The objective of Chapter I was to test if different levels of predation risk by fish would impact the community structure of zooplankton (i.e., taxon and size), among close but contrasted habitat. I sought to

emphasize the seasonal variations linked to the presence and absence of the thermal barrier. I expected that during summer, the shallow basin and the littoral area of the deeper basin would contain more abundant and larger zooplankters than the pelagic area, where conspicuous zooplankters should be predated by fish. I also expected that fish predation risk, concentrated in the deeper basin, should give an advantage to some zooplankton taxa that possessed defenses in this basin compared to the shallow one (e.g., jelly capsule of *Holopedium spp.*). Finally, I expected a homogenization of zooplankton communities across the different habitats in autumn since the disappearance of the thermal barrier allows the fish to freely access to the entire lake.

The first objective of Chapter II was to understand how fish adapt their food acquisition tactics to cope with their thermoregulation needs. I tested the hypothesis that fish selected areas where zooplankton is more abundant, when it is in a suitable habitat (e.g., depth, temperature). I expected stronger selection of large and more conspicuous zooplankton taxa. Furthermore, I expected that fish would make a tradeoff between a thermal refuge and the proximity of littoral areas or epilimnion to make short feeding excursions in these habitats (Goyer et al. 2014, Pepino et al. 2015). The second objective was to test if food acquisition tactics change from summer to autumn since the requirement of a thermal refuge would no longer be needed when surface temperature decreases in the fall. Finally the third objective was to test for individual variations of behaviors in brook charr population. I expected that the presence of resource polymorphism in brook charr population of this system (Bourke et al. 1999, Dynes et al. 1999, Rainville et al. 2021a) should bring inter-individual variations in these tactics and thus complexify the predator-prey relationships. To avoid thermal stress, fish exhibit behavioral thermoregulation which varies according to individuals (Goyer et al. 2014). These individual variations could thus have cascading effects on brook charr feeding strategy at the within-population level.

The objective of Chapter III was to demonstrate an impact of fish in the behavior of some zooplankton taxa. I wanted to highlight what we called the “boreal anomaly” of zooplankton DVM (i.e., epilimnion as a refuge instead of a risky area) in relation to cold-stenotherm predators, which are unable to go to the surface during summer. I tested the hypothesis that zooplankton migration patterns were linked to the interplay between brook charr and *Chaoborus spp.* depth distributions, the prey’s vulnerability to predation and the distribution of their resources (i.e., phytoplankton and temperature). I expected that large vulnerable taxa would remain in the epilimnion during summer, mostly during the day, while small taxa would perform a reverse diel migration to avoid *Chaoborus spp.* which migrate to the

surface at night (Bandara et al. 2021, Gignac-Brassard et al. 2022). Finally, I expected that zooplankters vulnerable to the fish would change their migration behavior in autumn, when the thermal barrier disappeared, resuming a normal DVM.

In this thesis, I therefore investigated if predator-prey relationships can be complex and change in nature and intensity across small distances in contrast to the homogeneous conditions that would be expected in such a small ecosystem. The impact of the thermal barrier occurring in summer on the trophic network and on the habitat coupling will also be investigated in a comprehensive approach. I will simultaneously focus on the predator behavior (Chapter II), the prey community structure (Chapter I) and the prey behavior (Chapter III). This will bring the necessary information to better understand the functioning of such ecosystems which is crucial to better assess the potential impact of warmer and longer summers, especially in boreal regions.

CHAPTER I: Within-lake variations in fish predation risk shape the spatio-temporal structure of the zooplankton community

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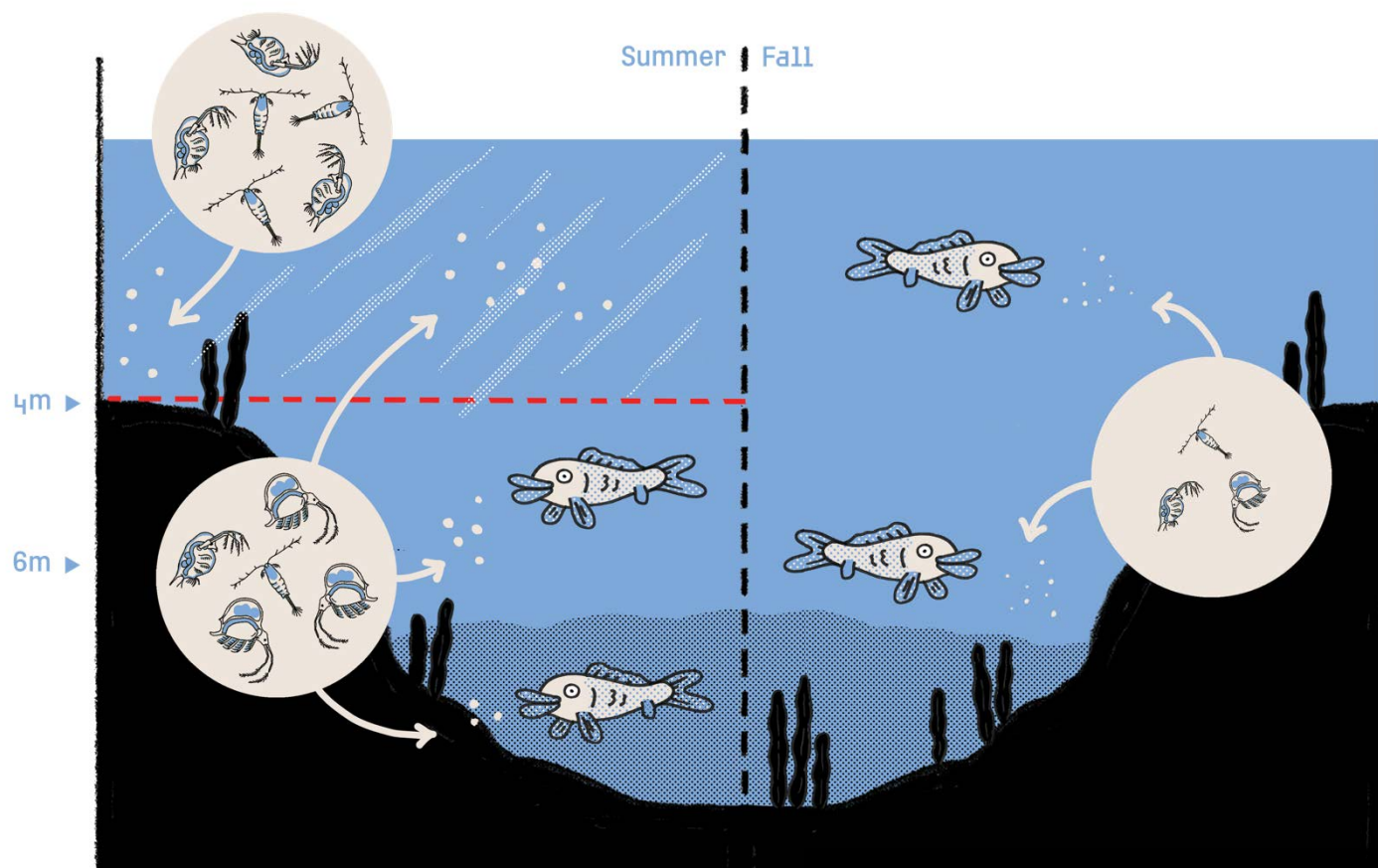
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Abstract

While it is well accepted that predation plays a key role in shaping both size structure and species composition in natural ecosystems, the fine-scale variation in predation risk within ecosystems has been largely neglected. The objective of this study was to use normalized size spectra (NSS) to investigate how small-scale spatial variations in fish predation risk in a small boreal lake can modulate zooplankton community structure at the within-lake level. Using in situ imagery sampling, we compared zooplankton NSS from two hydrologically well-connected basins of the lake exhibiting contrasting thermal regimes. The first is a well-mixed shallow basin (max. depth 2.5 m) that is inaccessible during summer to brook charr (*Salvelinus fontinalis*), a cold stenothermic species, because temperatures are too warm. The second is a deeper basin (max. depth 18 m) offering a thermal refuge during summer because of lake stratification. Our results show that both the zooplankton size structure and species dominance are clearly distinct in the two basins, with a higher proportion of small individuals and a higher abundance of zooplankton in the shallow basin compared to the deep one. Furthermore, following cooling of the epilimnion in late summer, the zooplankton communities of both basins exhibited a clear shift in size structure and species composition: in autumn, there were smaller individuals and larger proportions of bosminidae and daphnidae compared to colonial rotifers than in summer, suggesting a potential effect of the change in the spatial distribution of brook charr. Our results show that within-lake spatial and temporal variations in zooplankton community structure in these small boreal lake ecosystems are likely due to a complex interplay of selective fish predation, physical constraints, and other potential predators. This study improves our understanding of how within-lake ecological variations must be taken into account to disentangle ecological processes and predict their future changes.

Keywords: brook charr, size structure, Normalized Size Spectra (NSS), spatial heterogeneity, Underwater Vision Profiler (UVP)



Introduction

Variations in community size structure provide valuable information on ecosystem processes like productivity and energy transfers within trophic networks (Petchey & Belgrano 2010, Gómez-Canchong et al. 2013a, Trebilco et al. 2013). Indeed, most ecological and physiological processes are size-dependent, and aquatic systems often exhibit size-structured trophic interactions (Dickie et al. 1987, Brown et al. 2004). Whereas it is clearly accepted that predation can explain differences in the size structure of prey populations among systems, fine-scale spatial heterogeneity in predator distribution could be critical in shaping the prey community structure even within a given system (Pinel-Alloul 1995, Mehner et al. 2005). This phenomenon is probably more strongly linked to biotic than to abiotic processes, suggesting that it is essential to consider such a heterogeneity to understand the functioning of an ecosystem as a whole (Pinel-Alloul & Ghadouani 2007, Potts et al. 2020). Furthermore, fine-scale heterogeneity in community size structure could possibly be of the same magnitude as broad-scale heterogeneity (Mehner et al. 2005) and understanding the factors regulating these variations will be important to understand how ecosystems may be altered by continued global change.

During summer, deep and cold waters in stratified temperate zone lakes offer a thermal refuge to organisms that do not tolerate long exposure to warm temperatures (Littlefair et al. 2021). Shallow areas situated far from thermal refuges could thus become poorly accessible to cold-water stenothermic fish, resulting in a heterogeneous distribution of predation risk within the lake, with potential consequences for the trophic dynamics of the entire system. Such a spatial heterogeneity in the distribution of predation risk should be reduced at the end of the summer, when cooling surface waters allow cold-water stenothermic fish to access shallow areas that were too warm during summer (Guzzo et al. 2017). Such restored connectivity could enhance habitat coupling between littoral and pelagic areas (Schindler & Scheuerell 2002, Vadeboncoeur et al. 2002) and dramatically change the distribution of predation risk within the lake, with potential consequences on the size-structure of the lower trophic levels.

A great deal of research has shown that predation can strongly affect zooplankton community size structure, either through a reduction of body size in more abundant species or by shifting the dominance from larger to smaller taxa (Magnan 1988, Quintana et al. 2015). However, while much research has focussed on how predation affects zooplankton community structure at the among-lake level (Vanni 1986,

Magnan 1988, Lacasse & Magnan 1992, Rodríguez et al. 1993), only a few studies have looked at within-lake differences in community structure (e.g., Lake Superior, 82103 km²; Yurista et al. 2014).

The normalized size spectra (NSS) approach, proposed by Sprules & Munawar (1986), could help to address the comparison of inter- and intra-system differences in community structure since it is not focussed on taxonomic differences that could exist at both levels. The conceptual framework behind NSS provides a tool to incorporate all community size-structure data (i.e., from different trophic levels) into a single metric to effectively analyze their patterns. More specifically, NSS is a representation of the biomass or biovolume of organisms found in log-spaced size classes forming a straight line (Sprules & Munawar 1986). Both body size and the relative abundance of organisms are negatively correlated with their trophic position in aquatic ecosystems, allowing NSS to be directly used to explore the organism's trophic structure and eventually its dynamics (Brown et al. 2004, Gómez-Canchong et al. 2013a, Trebilco et al. 2013). The slope coefficient of NSS encompassing multiple trophic levels represents the amount of biomass exchanged along the size spectrum, forming a trophic pyramid (Trebilco et al. 2013). It has been used as a proxy of both growth and metabolic processes (Platt & Denman 1977). Furthermore, the relationship between NSS slope and trophic interactions has been used as proxy of trophic transfer efficiency (TTE) and the predator-to-prey mass ratio (PPMR) (Mehner et al. 2018), showing that ecological processes are also considered by this approach (Kerr and Dickie 2001). TTE is the ratio of production rates from one trophic level to the one above while PPMR is simply the mass ratio of one predator and its prey (Brown et al. 2004). However, when only one trophic level is represented by NSS in a system with more than one trophic level, it is somewhat complicated to make assumptions on TTE from NSS slopes. In this case, NSS slopes can simply be interpreted as variations in the size structure of the studied community. In this context, a steeper NSS slope of a given trophic level will be associated with higher proportions of small organisms in this trophic level. On the other hand, NSS intercepts are linked to overall organism abundance and to system productivity, whether we consider one trophic level or the entire network (Sprules & Barth 2016). A high NSS intercept is thus expected to be associated with a highly productive system with high biomass (Yurista et al. 2014, Sprules & Barth 2016). NSS deviations from theoretical linearity (sometimes called "secondary structures"; e.g., Arranz et al. 2019) could also provide information on changes in productivity and trophic relationships (Thiebaut & Dickie 1992, Yurista et al. 2014, Sprules & Barth 2016). Predators tend to linearize NSS by controlling prey populations so that secondary structures in NSS could reflect predator avoidance or a trophic bottleneck (Mehner et al. 2018, Arranz et al. 2019).

In this study, we used the zooplankton community of a small boreal lake to analyze within-lake variations in prey size structure in relation to both among-basin differences in predation risk as well as thermal regime and system productivity. Thermal regime is expected to change the size of zooplankters, with potential effects on NSS slope (Moore et al. 1996, Evans et al. 2020) whereas primary productivity could play a role in NSS intercept variations, with higher productivity leading to increased zooplankton biomass (McCauley & Kalff 1981, Yurista et al. 2014, Sprules & Barth 2016).

We used individual body size data to build NSS and taxonomic information to interpret any secondary structures in the data from zooplankton samples collected in the main (deep) and a shallow basin of the lake. The only fish species in the system is the brook charr (*Salvelinus fontinalis*), a cold stenothermic species. It has two main sources of prey (i.e., zoobenthos and zooplankton) but it has been found to preferentially feed on zooplankton in these small boreal lakes (Magnan 1988, Lacasse & Magnan 1992, Rodríguez et al. 1993, Bourke et al. 1999). These conditions make this lake an ideal model to study the top-down effect on zooplankton within the same system. Furthermore, previous radiotelemetry studies have suggested that the shallow basin is not accessible to brook charr during summer because of elevated temperatures (Bertolo et al. 2011, Goyer et al. 2014). More recent data from brook charr equipped with 3D acoustic tags confirmed that they spend more than 95% of their time in the deep basin of Lake Ledoux during the summer (P. Magnan, unpub. data). Zooplankton are thus expected to be free of or experience lower brook charr predation risk in the shallow compared to the deeper basin. We performed weekly samplings using an *in situ* imagery system, the Underwater Vision Profiler (UVP), of zooplankton abundance, size, and composition at the whole-lake scale to achieve three objectives:

The first objective of the study focussed on the spatial pattern of zooplankton community size structure, which is expected to differ not only between the two lake basins, but also between the littoral and pelagic habitats of the deeper basin. We expected that the shallow basin and the littoral area of the deeper basin would contain more abundant and larger zooplankters. We made this prediction because 1) shallow habitats are warmer and less light limited, thus more productive (Petersen et al. 1997), but also 2) zooplankters in shallow habitats are exposed only to invertebrate predators (both benthic, like odonates, and free-swimming, like chaoborids) compared to the deeper central area, where they are exposed to planktivorous fish and to a lesser extent chaoborids, whose population should be controlled by fish (Vanni 1986, Burks et al. 2002, Quintana et al. 2015). Based on these expected differences in productivity and predation risk between the two basins, we expected, respectively, a higher NSS intercept (higher

productivity) and a less negative NSS slope in the shallow basin compared to the deep basin. For the same reasons, within the deep basin, the pelagic area should exhibit a lower intercept and more negative slope compared to the littoral area.

The second objective focussed on the temporal pattern of the zooplankton community's size structure, which is expected to change seasonally in the different basins. Monitoring the zooplankton community structure from summer to autumn should reveal a stronger shift of the zooplankton community structure in the shallow basin, with the disappearance of the thermal barrier in late summer and unlimited access to all parts of the lake by fish. Based on the current knowledge of the effects of fish predation on zooplankton (Vanni 1986, Rodriguez et al. 1993, Brucet et al. 2010), this should lead to an eventual convergence of the zooplankton communities and therefore of NSS slopes and intercepts in the two basins. We thus expected a more negative slope, especially in the shallow basin, from the moment the thermal barrier to fish predators disappeared. In contrast, the temperature decrease in autumn may have the opposite effect on NSS slopes: warm temperatures are linked to a reduced average body size in zooplankton (Moore et al. 1996, Evans et al. 2020), and this should similarly affect NSS slopes in both basins. Furthermore, we expected a lower intercept with the disappearance of the thermal barrier, especially in the shallow basin, and the assumed resumption of consumption by brook trout. The temporal variations in phytoplankton productivity should also have a cascading impact on zooplankton biomass and thus on the NSS intercept (McCauley & Kalff 1981, Yurista et al. 2014, Sprules & Barth 2016).

The third objective focussed on addressing potential determinants of NSS secondary structures. Here, we analyzed zooplankton community composition to see whether zooplankton size structure variations are linked to a shift in individual size distributions within a given taxon or to a change in community composition. Thus, we should be able to assess whether potential secondary structures in the NSS are generated by a particular taxon dynamic. Vulnerable taxa like large *Daphnia* spp. should be more impacted by differences in predation risk while taxa such as *Holopedium* sp., which are embedded in a gelatinous capsule, should show less marked relationships with variations in predation risk (Detmer et al. 2017), eventually leading to a trophic bottleneck. Thus, we expect to find more large-bodied zooplankters in the shallow basin during summer and secondary structures in the NSS associated with relatively high abundances of *Holopedium* sp. in the deep basin.

To achieve these objectives, it was necessary to disentangle the effects of predation risk, temperature, and productivity on NSS. Even though variations in predation between basins are confounded with basin morphometry, thermal regime, and productivity in our study system, we did not expect that predation would cause the same effects on NSS characteristics compared to the other confounding variables.

This study aimed to show that within-lake heterogeneity at fine spatial and temporal scales could be critical to the understanding of trophic relationships in heterogeneous ecosystems. Given that the main driver here is temperature, we anticipate that global warming will lead to dramatic changes in predator habitat use in boreal lakes, with potential consequences for the functioning of the whole ecosystem.

Methods

Study site

We used Lake Ledoux (Mastigouche Wildlife Reserve, Quebec, Canada; 46.802381°N, 73.277136°W) as a model system because its morphology allowed us to test of our working hypotheses. It is a small oligotrophic temperate lake for which a large amount of data on brook charr habitat use are available (e.g., Bourke et al. 1997, Bertolo et al. 2011, Goyer et al. 2014). Lake Ledoux has a main deep basin (max. depth 18 m) and two shallower basins with mean depths of ~ 1.5 m and ~5 m at the west and east sides, respectively (Fig. 1). The lake is typical of Canadian Shield lakes and is located in a relatively protected area, where logging is limited and sport fishing is strictly controlled by the Quebec Government. During the whole study period, Lake Ledoux was closed to fishing. Despite its relatively small size (1 km fetch), this lake is a good system to explore fine spatial scale variations in productivity and trophic dynamics at the lake level because of the contrasting habitats offered by the above-mentioned basins. This study focussed on the large shallow western basin and the deep basin.

The shallow basin is inaccessible to brook charr during summer because it is too warm (Bertolo et al. 2011, Goyer et al. 2014). The deep basin offers a thermal refuge in deep cold waters, allowing fish to thermoregulate in the metalimnion and thus continue to feed on zooplankton during the whole year. The littoral area of the deep basin was included in the study to compare it to the shallow basin: this allowed us to compare two locations with similar depths but with sharp differences in connectivity with the thermal refuge in the central pelagic area. The eastern basin was not included so that we could focus our sampling

effort on the most contrasted areas in terms of predation risk. Sampling occurred from 23 July to 8 October 2018. Submerged aquatic vegetation was relatively abundant in the shallow western basin (hereafter “shallow basin”) and only patchily distributed in the littoral areas of the deep basin. Brook charr individuals make excursions to the epilimnion but often stay in the 5–6 m depth layer, which corresponds to their preferential temperature in the metalimnion (Bertolo et al. 2011, Goyer et al. 2014). Brook charr avoid temperatures above 22°C (Goyer et al. 2014), so individuals encounter strong thermal constraints to feeding in the shallow basin during the stratified period (May to September) (based on recent high-frequency hydroacoustic telemetry surveys; P. Magnan, unpub. data).

Spatio-temporal distribution of zooplankton

We used weekly sampling with an Underwater Vision Profiler (UVP 5, Hydroptic, L'Isle-Jourdain, France) to obtain an appropriate spatio-temporal resolution of the zooplankton community. On a few occasions (see zooplankton sampling section for details), UVP sampling was coupled with Schindler-Patalas sampling to get qualitative validation of the UVP.

UVP sampling

Weekly UVP sampling took place from 23 July to 8 October. To account for potential diel variations in the vertical distribution of zooplankton and thus ensure that the whole zooplankton community was included in the NSS, UVP sampling was performed each week at noon and midnight (solar time). This reduced potential biases from zooplankton moving into the sediment during the day. Night sampling on 6 August and 1 October was not done because of weather constraints.

Vertical UVP profiles were performed at four different stations in the deep basin to assess the vertical distribution of the zooplankton community as well as the shore-to-centre gradient ($D_{z1} - D_{z4}$; Fig. 1). The uppermost 0.4 m of the water column was avoided due to the height of the UVP, whereas the deepest 2 m was avoided to reduce the risk of interference by bottom substrate and/or sediment resuspension. Thus, the deep basin sampling extended from 0.4 m to ~2 m (D_{z1}), ~4 m (D_{z2}), ~8 m (D_{z3}), and ~11 m (D_{z4}). The vertical spatial resolution was given by the accuracy of the UVP depth sensor (0.1 m). Since zooplankton diel vertical migration generally occurs at dawn and dusk, the four stations were sampled within an hour to ensure that no zooplankton migration began during sampling (Lampert 1989). Vertical sampling was used to get information on the zooplankton community from stations D_{z1} to D_{z4} , so each profile was integrated from the surface to the bottom. If zooplankton were concentrated in the water

column rather than in the sediments, the noon and midnight integrated profiles should be similar for a given week and station. Since the depth of the shallow basin did not allow vertical profiles with the UVP, we sampled a 150 m horizontal transect parallel to the shore at its centre (Fig. 1).

UVP data processing

The UVP counts and measures particles with an equivalent spherical diameter (ESD) greater than 68 μm , but it can also take high frequency pictures of larger particles (including living zooplankton) directly in the water (Picheral et al. 2010). Originally designed for sampling relatively large particles (greater than 1 or 2 mm, e.g., zooplankton found in marine habitats), the UVP used here was calibrated to optimize the capture of relatively small freshwater zooplankton (see below) by zooming the camera optics and reducing the focal length between the lens and the lit area (M. Picheral, LOV oceanographic observatory, Villefranche sur Mer, France, pers. comm.). The sampling volume of each image was 0.18 L and the sampling frequency was 11 images per second (see Appendix I for UVP processing configuration).

To minimize the incertitude linked to image resolution, UVP pictures (hereafter called vignettes) were not identified at the lowest taxonomic level possible but rather grouped into the following coarse categories: copepods, Holopediidae (*Holopedium glacialis*), other cladocerans, Chaoboridae, rotifer colonies (*Conochilus* sp.), and several rarer taxa that made up fewer than 0.8% of the particles (e.g., Volvocales, Arachnidae, Notonectidae; Fig. 2). “Other cladocerans” included mainly Bosminidae and Daphnidae, the latter being largely dominant in large size classes. Non-living particles were classified into the “detritus” and “badfocus/artefact” categories. Submerged aquatic vegetation were placed into the “SAV” category whereas the “Others” category was created for living-particle vignettes that could not be identified (too small or blurry) (Fig. 2). Details about the size distribution of the main zooplankton categories listed above are given in Appendix II.

Zooplankton sampling

Zooplankton was collected at both noon and midnight with a 30 L Schindler-Patalas box on three occasions during the season, on the day following UVP sampling (25 July, 15 August, and 6 September). Sampling took place at the same first three stations as the vertical UVP sampling: at 1.5 m depth for D_{z1} and D_{z2} and at 1.5, 3, 5, and 8 m depth for D_{z3} . There were no night samples on 6 September because of a storm event. Samples were stored in a glucose-formaldehyde solution (4%) prior to analysis (Prepas 1978). Zooplankters were identified with the Zooscan processing system (Gorsky et al. 2010), which uses

platforms similar to our UVP (Zooprocess and Ecotaxa) and provides the same kind of measurements. Identification protocols were identical, even though resolution of the Zooscan is sixfold higher and thus allows visual validation of samples at the genus level for some taxa. All 64,359 Zooscan vignette predictions were checked manually. These data were compared to UVP taxonomy data to provide some clues about unidentified individuals in UVP vignettes that were sorted into the “Others” category. It was difficult to identify small zooplankters because UVP resolution was not high enough to detect discriminating attributes like appendages. This explains why half of the individuals smaller than 600 μm were classified into the “Others” category. However, the comparison with Zooscan data provided some insight on these undefined individuals, which are likely mostly copepods and to a lesser extent *Daphnia* spp. or Bosminidae (Appendix III). We notice some bias from UVP imaging, but it is constant through time as was the methodology used. Thus, this did not influence the zooplankton variations described in this study.

Limnological variables

Vertical profiles of fluorescence and temperature (fluoroprobe, bbe Moldaenke) were recorded weekly at station D₂₄ during all sampling periods and at station S₂₁ from 13 August to 08 October. The fluorescence profile reflected the vertical distribution of phytoplankton concentration. Thus, the phytoplankton concentration (PC; proxy of primary production) of each station was estimated per surface unit by multiplying the average phytoplankton concentration by the depth of the water. The epilimnion temperature of the lake was considered to be homogeneous (both in the horizontal and vertical axes; Bertolo et al. 2011, Goyer et al. 2014) and was estimated by the temperature measured at 1 m depth at D₂₄. Spatio-temporal variations of temperature and phytoplankton concentration were graphically visualized to assess them with respect to NSS differences. Spatial differences in PC were assessed with Wilcoxon tests while temporal variations in PC were tested for each station using generalized additive models (GAM).

Size spectra metrics

Equivalent spherical diameter (ESD) was calculated from area measurements for each zooplankton picture and transformed into biovolume. These biovolumes were integrated and normalized into log-spaced size classes to build the normalized biovolume size spectrum (NBSS) (Sprules & Munawar 1986, Sprules & Barth, 2016; Appendix IV).

Statistical analyses

Estimation of NBSS parameters

To analyze the relative contributions of location and seasonality on zooplankton size structure, NBSS values were calculated from zooplankton biovolumes sampled weekly at each station. Noon and midnight samplings were also distinguished to account for sampling design. The NBSS is expected to follow a power law distribution whose slopes and intercepts can be estimated by linear regression on a log–log plot (White et al. 2008, Edwards et al. 2017). Since we sampled five stations for 11 weeks at two periods of the day (noon and midnight), we built a total of 110 NBSS for which we derived both the slope and the intercept. The intercept can be strongly correlated with the slope (Gómez-Canchong et al. 2013b). To avoid this correlation, and given the fact that the 0 point is far to the right of the considered size spectrum, the midpoint height was used instead of the intercept (Gómez-Canchong et al. 2013a, Sprules & Barth 2016). The ordinate of the point at the middle of the size spectrum (hereafter “height”) was calculated from the corresponding linear regression equation. NBSS heights were strongly correlated with the total biovolume of zooplankton (Pearson $R^2 = 0.72$, $p < 0.001$).

Spatio-temporal variation in NBSS parameters

The 110 estimates of NBSS parameters were used to build different linear models to analyze the effects of the station, week, and period of the day to test the spatial and temporal hypotheses and verify that potential zooplankton vertical migration did not bias the results. The most complex model includes equations such as [Slope/height ~ Station + Week + Period + Interaction terms], and the equation of the simplest model is [Slope/height ~1]. The Akaike Information Criteria (AIC), the Δ_{AIC} ($AIC_{\text{model}} - AIC_{\text{min}}$), and degrees of freedom (as an index of the number of variables used in a model) were used to select the best and most parsimonious model (package *aod*; Lesnoff & Lancelot 2012). Models were ranked using Δ_{AIC} , i.e., the difference in AIC between a candidate model and the model with the lowest (best) AIC. The larger the Δ_{AIC} , the less plausible the model: models with $\Delta_{AIC} < 2$ have substantial support, those where $4 < \Delta_{AIC} < 7$ have considerably less support, those with $\Delta_{AIC} < 10$ are “plausible,” while those with $\Delta_{AIC} > 10$ have essentially no support (Burnham & Anderson 2004). Once the best model was retained, a Tukey post-hoc test was used to identify which groups were different in terms of slopes and heights. Correlations between temperature and NBSS slopes or heights were tested using a Spearman rank correlation test to assess if

there was a relationship between temperature and community structure. This could reflect an indirect effect of predation risk when fish are limited in their movements by temperature. All analyses were performed with R software (R Core Team 2021).

Taxonomic composition and NBSS secondary structures

Taxonomic information gathered with the UVP were used to discriminate the contributions of major taxa to the NBSS. Differences in zooplankton composition among weeks and stations were also explored graphically by visualizing the biovolume of each category per week for each station. We fit a polynomial regression model to these points to see if it could adequately describe the observed variations of zooplankton biovolume. This information was also valuable to better understand the possible deviations from linearity in the NBSS. Non-linearities of the NBSS line were determined visually on the log–log plot of each station. The linear model was compared to a second-order polynomial model to test for the presence of non-linearity. Only NBSS size class ranges that clearly visually deviated from linearity were tested with this approach (Appendix V). When a polynomial model was retained based on its AIC, the NBSS was recalculated by removing zooplankton taxa one at a time to assess graphically whether linearity was restored (Appendix VI). This allowed us to assess if the non-linearity of the NBSS was caused by a particular taxon, thus pinpointing the position of this taxon that stood out in the trophic network.

Results

Temperature and productivity variations

Temperatures profiles showed no differences for the common depth of both basins (i.e., epilimnion; Appendix VII). Thermal stratification and the thermocline remained stable at station D_{z4} from the beginning of sampling period, 23 July, to 6 September, with a surface temperature above 20°C. The epilimnion temperature then gradually decreased, with the near disappearance of stratification in October.

The phytoplankton concentration (PC) was significantly different between D_{z4} and S_{z1} (p.value < 0.001), with the deep basin being more productive because of a deep chlorophyll maximum (Appendix VIII). However, S_{z1} had a higher PC than D_{z4} when considering only the common 0–2 m layer (p.value < 0.001). We were able to graphically discern a decrease in PC in the final two weeks of sampling in the deep basin, but no temporal pattern was detected by the GAM for D_{z4} or S_{z1} (p.values of 0.12 and 0.18, respectfully).

NBSS control and demarcation

When all zooplankton data were pooled into a single NBSS, an almost perfectly straight line connected points from the fourth to the fourteenth class, whereas the first three classes and the last two clearly deviated from the line (Appendix IX). The three smallest size classes were removed from the analysis because they were probably underestimated due to the UVP's optical detection limit (Forest et al. 2012). The two larger size classes were also removed because we considered these outliers to be artifacts (e.g., avoidance behaviour by large organisms).

Model selection of explanatory variables

Based on AIC and the degrees of freedom, the best model to explain NBSS slope variations included both station and date, and both terms were significant (Appendix X; $p < 0.01$ and < 0.001 , respectively). The best model to explain NBSS height variations included the effects of station and period of the day. While station had a significant effect ($p < 0.001$), period of the day did not ($p = 0.06$). Models with interaction terms between explanatory variables were not selected, meaning that spatial differences in NBSS were constant through time (Appendix X).

NBSS spatial variations

Based on post-hoc tests, we graphically represented NBSS variations according to station and date variables to visualize them. The NBSS slope at station D_{z1} (NBSS slope median = -1.31) differed significantly from the other three stations in the deep basin (NBSS slope medians = -1.05, -1.05, and -1.00 at station D_{z2} , D_{z3} , and D_{z4} , respectively), but not from the NBSS slope in the shallow basin (S_{z1} ; NBSS slope median = -1.18; Fig. 3 and 4A). The slope at S_{z1} (shallow basin) was also significantly steeper than the three stations in the deep basin (D_{z2} , D_{z3} , and D_{z4} ; Fig. 3 and 4A), which were not significantly different from one another. This result indicates that the shallow basin and to some extent the littoral zone of the deep basin (D_{z1}) exhibited higher proportions of small individuals than deep-basin stations D_{z2} , D_{z3} , and D_{z4} .

While results for the NBSS slopes showed some similarities between the two shallow stations (S_{z1} and D_{z1} ; Fig. 4A) and among the three deeper stations of the deep basin, the results of NBSS height showed a clear pattern separating the two basins (Fig. 4B). Post-hoc tests revealed that NBSS height was greater at station S_{z1} compared to all deep-basin stations (D_{z1-z4} ; Fig. 4B), indicating that zooplankton biovolumes were significantly higher in the shallow basin (S_{z1}) compared to the four deep-basin samples (D_{z1-z4}). This

difference was clear for the smaller half of the size classes but decreased for the larger half at stations D_{z2-z4} (Fig. 3). Furthermore, NBSS height was lower at D_{z1} than at D_{z2} and D_{z3} but did not differ significantly from D_{z4}. Stations D_{z2-z4} did not differ significantly from each other. This means that there was a lower overall abundance of zooplankton (biovolume) with a higher proportion of small individuals in the littoral (D_{z1}) than in the pelagic (D_{z2-z4}) areas of the deep basin (Fig. 3 and 4).

NBSS temporal variations

NBSS slopes tended to flatten during the first five weeks (the median of NBSS slopes from 23 July to 20 August varied from -0.97 to -0.67, respectively) before dropping during the next two weeks and reaching a lower plateau during the last four weeks (late September and October; median of NBSS slopes between -1.22 and -1.28; Fig. 5A). From this, we defined three groups: “summer” from 23 July to 20 August, “transition summer–autumn” from 27 August to 04 September, and “autumn” from 10 September to 08 October. Based on post-hoc tests, NBSS slopes did not differ within the summer or the autumn periods. However, they were significantly different between summer and autumn except for 23 July, which did not differ significantly from three of the autumn weeks. The observed temporal patterns were apparently related to changes in temperature, since we found a positive relationship between the lake’s epilimnion temperature and NBSS slopes ($p < 0.001$, $r = 0.63$). Temporal variations in NBSS slopes matched the weekly changes in epilimnion temperature (Fig. 5A). NBSS slopes began to decline when the epilimnion temperature dropped below 22°C (i.e., in late August). Beyond this threshold, slopes remained stable even though the epilimnion temperature decreased further. These results indicate a shift from larger to smaller individuals through the sampling period (summer to autumn).

Since the variable “week” was retained in some plausible models to explain NBSS height, graphical representation can reveal tendencies. As expected, NBSS heights did not show any temporal pattern but tended to decrease until 9 September and then increased during the last four weeks of sampling (Fig. 5B). However, post-hoc tests did not show any differences between weeks, as expected with the previous model selection. Heights were also not correlated with temperature ($p\text{-value} = 0.36$, $r = 0.09$).

Taxonomic composition and NBSS secondary structures

The structure of the zooplankton community clearly differed between the shallow and deep basins of the lake (Fig. 6). First, the abundance of *H. glacialis* was more than sixfold higher in the deep basin (D_{z4}) than in the shallow one. Second, colonies of the rotifer *Conochilus* sp. were 2.1 times more abundant at

S_{z1} compared to D_{z4} . All stations of the deep basin had similar taxonomic compositions, although D_{z1} and D_{z2} had fewer *H. glacialis*. D_{z1} also had low abundances of large-sized individuals, with individuals larger than 1 mm being completely absent in some samples. Finally, there were few Chaoboridae in the shallow basin whereas they were always abundant in the deep basin. One can clearly see the contrast in zooplankton community compositions before and after the late August threshold (Figs. 6A and 6B; July and September, respectively). While there were variations between these two sampling dates, *H. glacialis* remained more abundant in the deep basin whereas colonies of *Conochilus* sp. were more abundant in the shallow basin.

Populations of both copepods and “other cladocerans” had different size structures between the two basins, with the larger individuals of both groups being more abundant in the shallow basin (Appendix VI). Later in the season, “other cladocerans” increased their proportions compared to *Conochilus* sp. colonies or *H. glacialis* (i.e., jelly taxa) in both the shallow and deep basins. Copepods showed no clear spatial or temporal patterns.

Visual inspection of the residuals of the different NBSS slopes showed an apparent deviation from linearity for stations D_{z2-z4} (Fig. 3, Appendix V). More specifically, we observed an overabundance of individuals with equivalent spherical diameters from 0.84 to 2.02 mm. Taxonomic data showed that the dome-shaped NBSS in the lake’s deep basin was directly associated with the contribution of *H. glacialis*. When this taxon is removed from the NBSS calculation, NBSS becomes linear (Appendix VI).

Discussion

Our study revealed that the size structure of the zooplankton community can vary sharply even within a relatively small lake, with clear differences observed not only between basins but also within small ranges (40 m) of a given basin. Our results also suggest that the presence of a thermal barrier limiting movements of the top predator can shape the whole trophic network, even though the effects of predation on zooplankton are not as straightforward as expected. The NBSS approach showed that the range of the spatio-temporal variations in the size structure of the zooplankton community is similar to ranges observed among different systems (Dai et al. 2017). This approach also revealed the key role of seasonal temperature changes in shaping the trophic network by modulating the degree of functional coupling between habitats by the top predator. Moreover, linking NBSS with taxonomic data allowed us

to demonstrate that the strong spatial pattern observed in zooplankton body size was associated with a clear pattern in community composition.

NBSS spatial pattern

Ecological theory predicts a slope of -1 for the NBSS of aquatic multi-trophic communities (Brown et al. 2004, Mehner et al. 2018). Lake Ledoux, with an average NBSS slope of -0.81, is within the range observed for freshwater ecosystems (-0.8 to -1.2; Yurista et al. 2014, Sprules & Barth 2016, Dai et al. 2017, Mehner et al. 2018). However, the value observed in Lake Ledoux for zooplankton is closer to -0.75, which is expected for communities composed of a single trophic level (Brown et al. 2004, Trebilco et al. 2013), suggesting that this trophic network is mainly composed of herbivorous organisms exposed to little predation and thus potentially forming secondary NBSS structures (Mehner et al. 2018). On the other hand, the within-lake differences in NBSS observed in this study were relatively large, showing that variations in zooplankton community structure can differ sharply at a very small spatial scale (about 40 m between D_{z1} and D_{z2}, with median slopes of -1.31 and -1.05, respectively), likely due to differences at higher trophic levels.

The overall zooplankton biovolume concentration was higher in the shallow basin, and the higher absolute biovolume of small individuals increased the NBSS slope, suggesting higher community productivity (Trebilco et al. 2013, Yurista et al. 2014, Sprules & Barth 2016). However, the unexpectedly low proportion of large individuals and the large proportion of colonial rotifers in the zooplankton community of the shallow basin may suggest that—contrary to fish—invertebrate littoral predators (e.g., notonectid and odonates) were abundant, thus potentially consuming large numbers of large-sized zooplankton. The steeper slope in the shallow basin compared to the deep one could reflect a more complex trophic network than expected (Brown et al. 2004, Mehner et al. 2016, 2018). This result is somewhat puzzling since a visual predator like brook charr would have been expected to select large individuals in the zooplankton community in the deep basin, so the opposite pattern should have been observed (Blumenshine et al. 2000). One possible explanation could be that zooplankton face more predation risk by gape-limited *Chaoborus* sp., which is relatively abundant in Lake Ledoux compared to other lakes in the same region (Drouin et al. 2009, Gignac-Brassard et al. 2022), and thus the largest zooplankton could be in a size refugium (Braun et al. 2021; this has been shown by Mehner et al. 2016, Arranz et al. 2019 for prey fish). Thus, *Chaoborus* sp. most likely played an important role in the dynamics of small size classes of zooplankton, as confirmed by the significant negative correlation between

Chaoborus sp. abundance and the abundance of both small and middle size classes (ESD < 1 mm; Spearman rank correlation test with $\rho = -0.38$; $p < 0.01$), but not of large zooplankters.

The flat NBSS slope in the deep basin might also be due to differences in zooplankton species composition (i.e., higher abundances of *H. glacialis* in the deep basin). Even though Holopediidae have been shown to avoid the littoral zone when fish are present (Lauridsen et al. 2001), it is not clear why they did not proliferate in the shallow basin where another jelly organism, *Conochilus* sp., dominated. Their large abundance in the deep basin formed a bump in the NBSS that can be interpreted as a trophic bottleneck in the presence of gape-limited predators (here *Chaoborus* sp.). Brook charr could also feed on medium-sized zooplankton like *Daphnia* spp. and copepods rather than larger zooplankton, but its impact should be much lower (e.g., Magnan 1988). This could explain the relatively smaller size of daphnids in the deep basin compared to the shallow one. The flatter NBSS slope in the deep basin could also reflect the observed high concentrations of large zooplankton in the epilimnion during summer, where warm temperature reduced fish predation risk (Appendix XI).

Our study also revealed a certain degree of heterogeneity at the within-basin level. In the deep basin, stations close to the shore had higher zooplankton concentrations, which could be the result of a higher zooplankton concentration in the epilimnion. However, the most littoral station showed relatively low abundances of zooplankton, especially in the large size classes (Appendix XII). This could result from the vertical distribution of large zooplankton like *H. glacialis*, which avoid shallow waters. *Conochilus* sp. colonies, *H. glacialis*, and other cladocerans were sparse in D_{z1} compared to D_{z2-z4} (Appendix XIII). This could suggest zooplankton avoidance of the shore when no predation refugia like aquatic plants are present (Gliwicz & Rykowska 1992).

NBSS temporal pattern

No temporal pattern was related to NBSS height (i.e., zooplankton overall biovolume) across stations, which is in accordance with the relative stability of phytoplankton concentration that we found during the sampling period. However, zooplankton size structure changed abruptly over time, as shown by the dramatic drop of NBSS slopes starting in late August, a period corresponding to a decrease in epilimnion temperature below 22°C. Zooplankton size structure did not further change (i.e., NBSS slopes did not steepen further) after the temperature drop even though surface temperature continued to decrease from 22°C to 11°C. This suggests a threshold effect linked to the drop in the thermal barrier for

fish (Goyer et al. 2014). Therefore, this result clearly does not support a physiological effect on zooplankton growth driven by temperature. Indeed, both zooplankton growth rate and age at maturity should respectively decrease and increase with the temperature drop (Brown et al. 2004), leading to an overall larger average body size (Moore et al. 1996, Evans et al. 2020) and therefore a flatter slope than in warm waters.

Unexpectedly, the observed steeper slopes at the end of the summer suggested lower trophic transfer efficiencies (TTE) (considering a multi-trophic zooplankton community and fixed predator-to-prey mass ratio) when fish were able to access virtually all basins/depths in Lake Ledoux. Considering that we found higher TTE (i.e., less steep slopes) in the deep basin, we expected to find the opposite pattern. Unfortunately, we cannot explain this puzzling result by calculating the NBSS for the whole aquatic community—from bacteria to fish—and thus, we cannot know the real TTE value in this system with the data at hand. Integrating benthic invertebrates in the size spectrum could be valuable to better evaluate the effect of fish on the trophic network.

The flatter NBSS slope in the deep basin could suggest that brook charr did not have a strong influence on the zooplankton community but rather an indirect effect by eating *Chaoborus* sp. during summer. In contrast, brook charr might have a stronger effect on zooplankton community structure in autumn since the NBSS slope suddenly became steeper. Contrary to our predictions, the weakening of the thermal barrier separating the two basins did not coincide with a convergence of the zooplankton community structures from these basins. The steeper NBSS slope across all stations in autumn suggests a more complex trophic network with stronger coupling of benthic and pelagic resources due to an increased access to littoral benthic resources by fish. When fish were able to access habitats that were previously too warm (i.e., epilimnion and littoral area), they could feed on newly accessible zooplankton, thus resulting in increasingly steep NBSS slopes (Brown et al. 2004, Mehner et al. 2018, Zhang et al. 2019). This could explain the increase of zooplankton biovolume in both the small and middle size classes, when fish are able to feed on benthic invertebrates in littoral areas, thus releasing predation pressure by invertebrates on zooplankton (e.g., small “other cladocerans”; Appendix XIV). The *Chaoborus* sp. population supports this hypothesis: it increased or remained stable at all stations during summer while it decreased during the autumn except at D₂₄, where the population remained stable (Appendix XIV). These results suggest that when the thermal barrier was lifted, brook charr left the deep area (D₂₄) for the littoral area and the shallow basin, preying upon *Chaoborus* sp. A similar effect of the thermal barrier on the

strength of the littoral–pelagic coupling was observed by Guzzo et al. (2017). The earlier the onset of summer temperatures, the earlier the fish-related coupling between pelagic and littoral habitats was interrupted, with potential cascading effects on both trophic networks (Schindler & Scheuerell 2002) and fish condition (Guzzo et al. 2017). The average swimming speed of brook charr increased from summer to autumn, corroborating this interpretation (Appendix XV), as did previous studies showing that juveniles brook charr decreased their feeding activity above 22°C in this system (e.g., Marchand et al. 2002). This suggests that brook charr has a lower influence on the prey community when surface temperatures exceed the 22°C threshold.

Seasonal variation of zooplankton species composition

While the NBSS showed differences between basins in terms of size/trophic structure, the taxonomic data helped to determine the specificity of each trophic network and to interpret their secondary structures. Some taxa were clearly under-represented in the isolated shallow basin (e.g., *H. glacialis* and *Chaoborus* sp.) whereas others (e.g., *Conochilus* sp. and daphnids) were relatively more abundant than in the deep basin. When the thermal barrier for the top predator was lifted, zooplankton size structure and composition rapidly changed in both basins (see Appendices XIII & XIV). Whereas brook charr is known to feed on *H. glacialis*, they can also efficiently suppress competitors of *H. glacialis* by eating large *Daphnia* spp. and copepods as well as the invertebrate predator of *H. glacialis*—*Chaoborus* sp.—during autumn, thus indirectly favouring the *H. glacialis* population (Detmer et al. 2017). Therefore, the zooplankton community shift is not only shaped directly by fish predation, but also by its indirect effects on invertebrate predators and competition dynamics, together with changes in environmental factors such as light period and temperature (Bürgi et al. 1985).

NBSS: a tool for fine-scale studies

Our results clearly show that fine-scale variations in zooplankton distribution can be of the same magnitude as reported inter-lake variations (Brucet et al. 2010, Dai et al. 2017). We also showed how habitat coupling can dramatically increase when thermal barriers for top predators become permeable. Global warming has an increasingly strong impact on lake temperature, especially on boreal lakes (O'Reilly et al. 2015, Dokulil et al. 2021), and should increase the spatial and seasonal differences of thermal regimes in the future, with potential consequences on the trophic coupling between the littoral and pelagic habitats. In this context, such habitat decoupling could last longer in the summer (Dokulil et al. 2021) and

even occur in previously coupled ecosystems. Consequently, global warming could disrupt trophic dynamics and, in turn, the functioning of the whole ecosystem (Sharma et al. 2007, Dokulil et al. 2021).

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Data availability statement

A common repository dedicated to RIVE studies is being built to gather database, metadata and R scripts from the research group. In the meantime, all data used to produce this article are available from the authors upon reasonable request.

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Figures

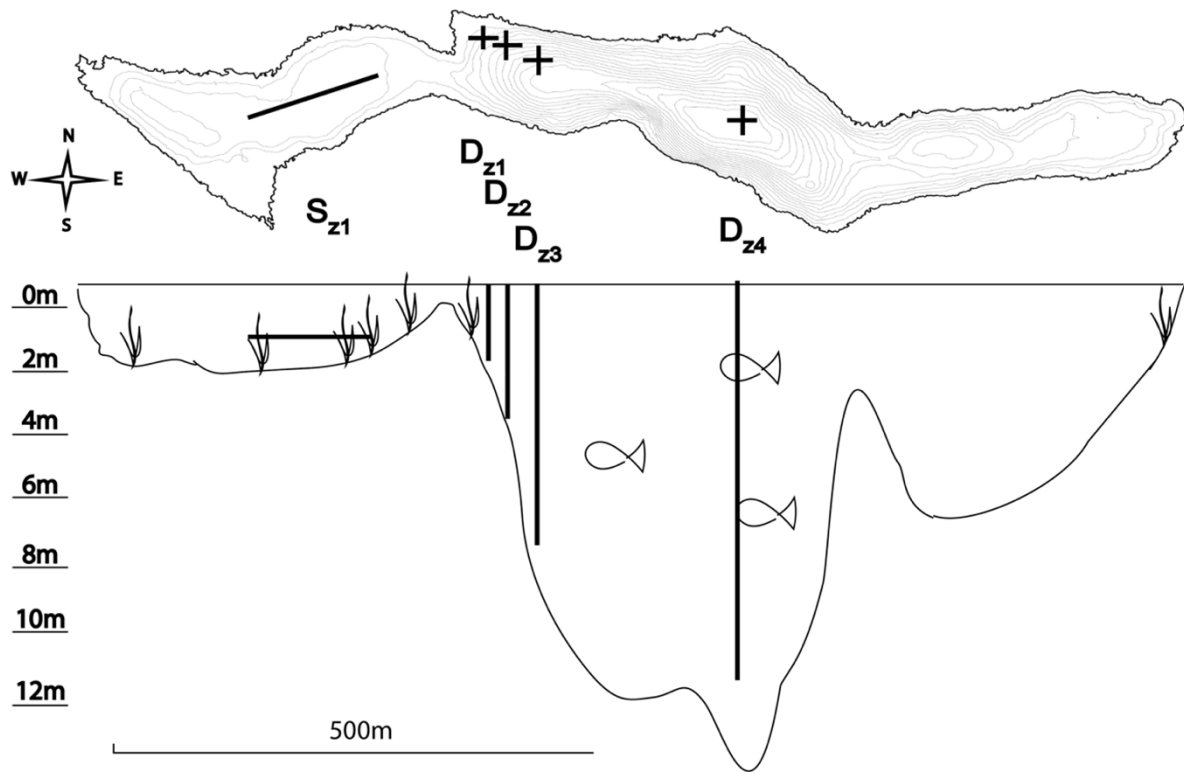


Figure 1: Schematic representation of Lake Ledoux (above: top view with 1 m isobaths; below: cross-section along its major axis). Vertical scale is exaggerated to better show depth differences. The four stations (Dz1, Dz2, Dz3, and Dz4) where vertical sampling was done are represented by crosses (above) and vertical lines (below). Horizontal sampling in the shallow basin (Sz1) is represented by lines in both views.

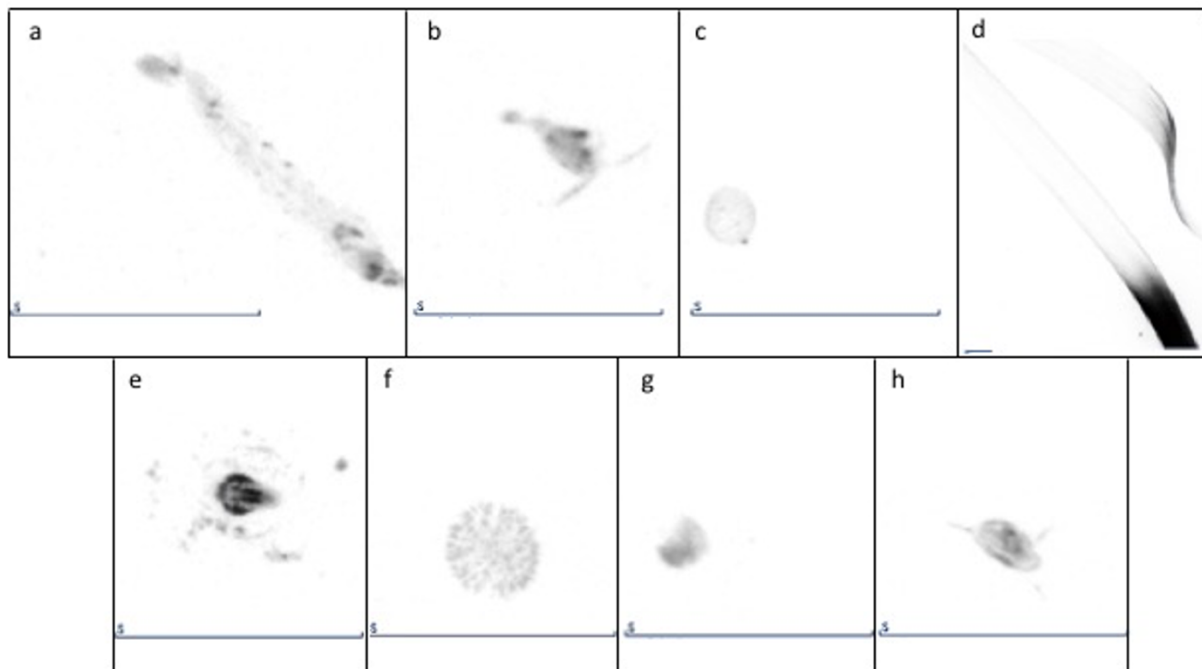


Figure 2 : Examples of underwater vision profiler (UVP) pictures (vignettes) showing the main categories found in Lake Ledoux during the sampling program. The scale bar is 5 mm. a) Chaoboridae; b) copepods; c) Volvocales; d) aquatic vegetation; e) *Holopedium glacialis* (note the gelatinous capsule partially visible in the image); f) colony of *Conochilus* sp.; g) unidentified; h) daphnids.

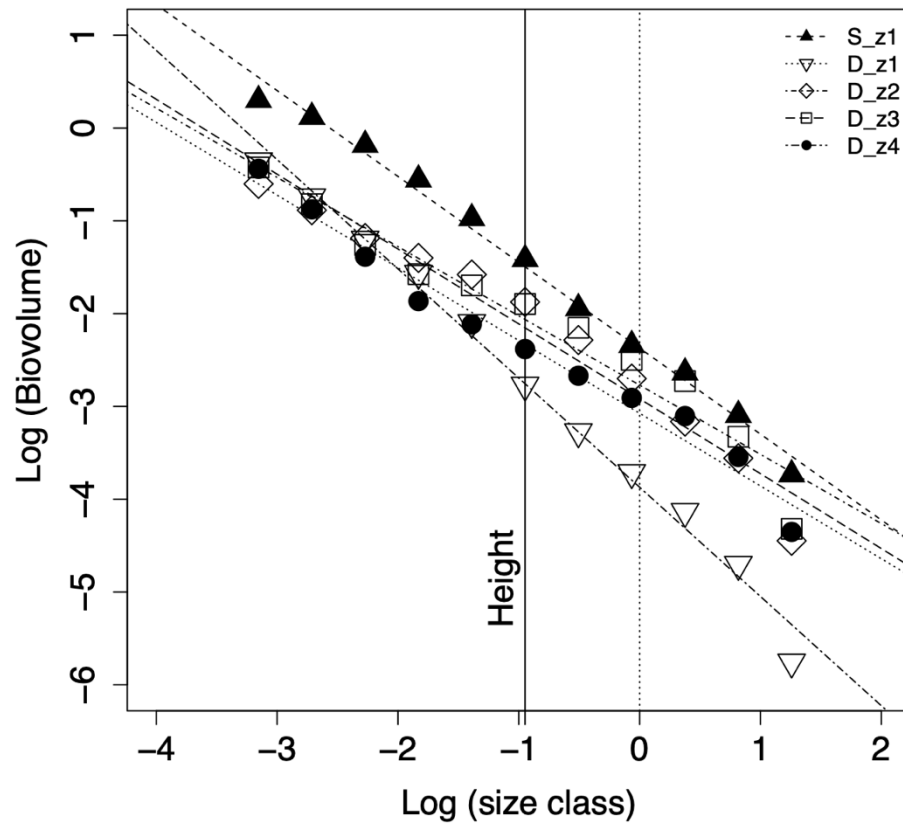


Figure 3 : Normalized biovolume size spectra (NBSS) of the five sampled stations (Sz1 in the shallow basin and Dz1 to Dz4 in the deep basin).

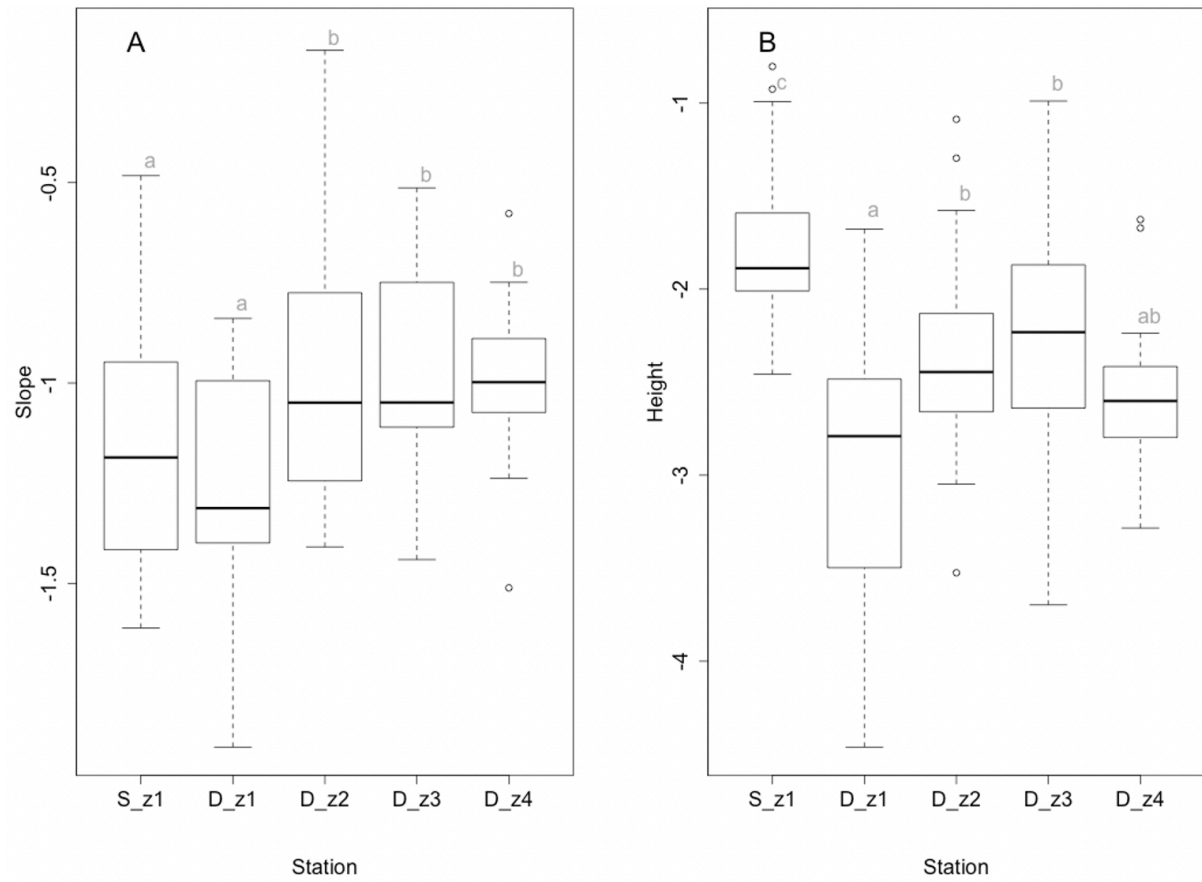


Figure 4: Boxplots showing the effect of station on the slope (A) and height (B) of the normalized biovolume size spectra (NBSS). Letters above boxplots represent groupings of the Tukey post-hoc tests.

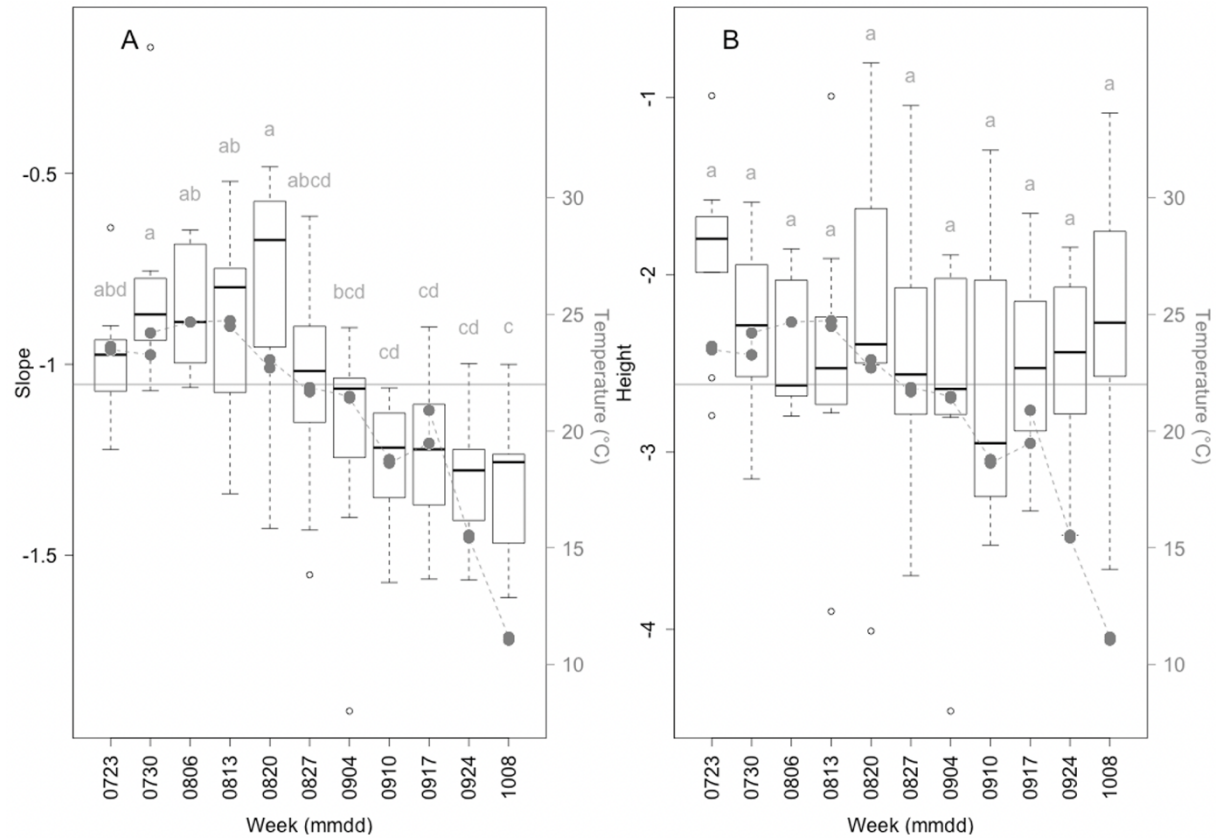


Figure 5 : Boxplots showing the effect of time (weeks) on the slope (A) and height (B) of the normalized biovolume size spectra (NBSS), all sites combined. Temperature is represented for each week, with the horizontal line representing the 22°C threshold for brook charr. Letters above the boxplots represent groupings of the Tukey post-hoc tests.

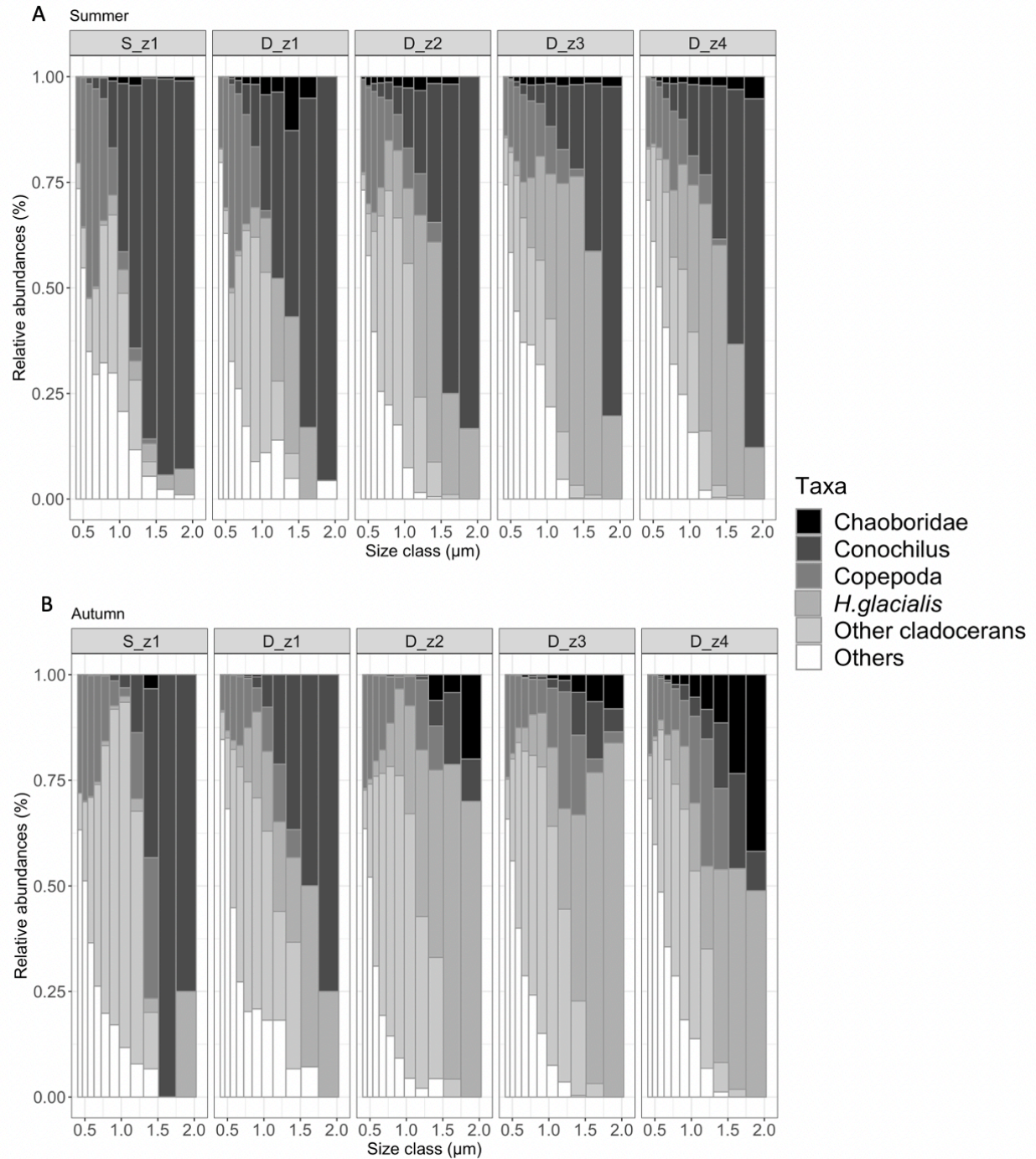


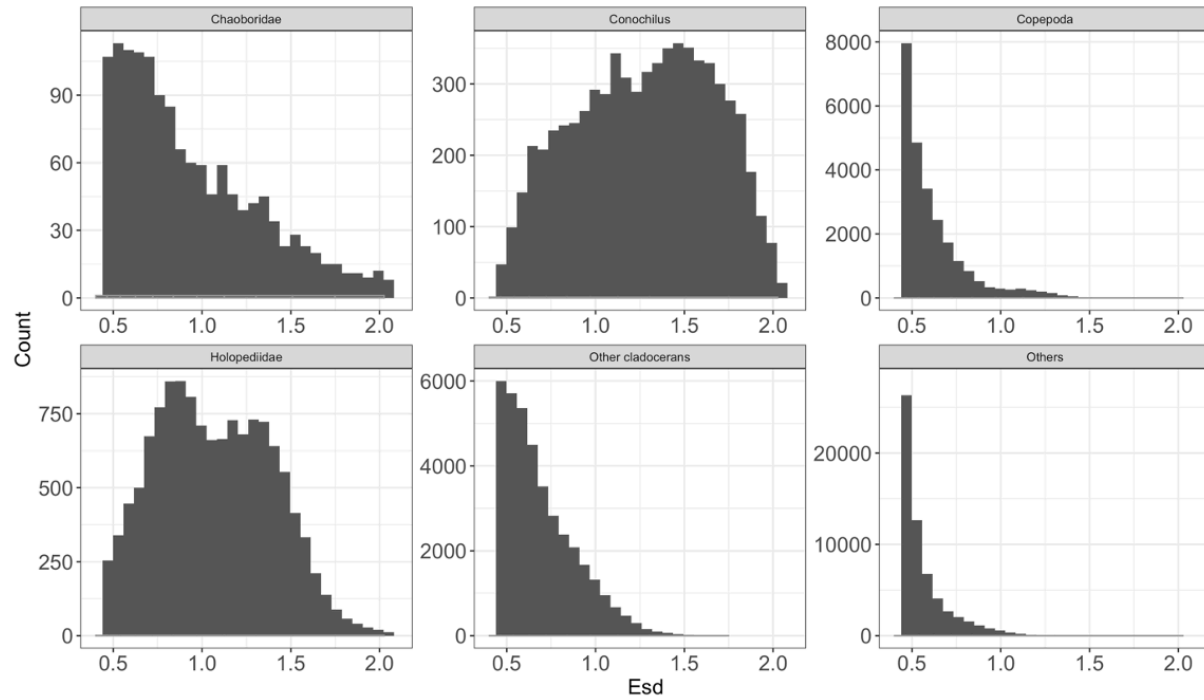
Figure 6: Histograms of relative zooplankton composition in two contrasting seasons (A: summer, B: autumn). From left to the right: shallow basin Sz1; deep basin Dz1, Dz2, Dz3, Dz4.

Supplementary

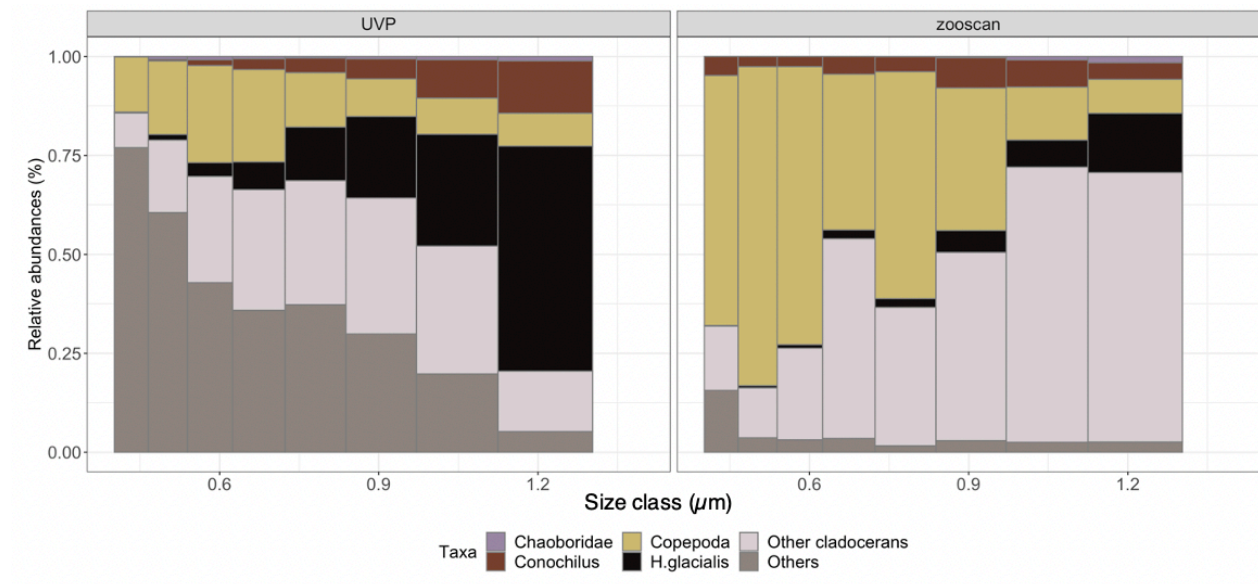
Appendix I: Underwater vision profiler (UVP) – Configuration and post-processing

Once recorded, UVP images were processed with the Zooprocess software (ImageJ adapted for UVP data) to split each image into single-particle images called “vignettes”. For each detected particle (≥ 1 pixel), area measurements were computed in pixels. For particles larger than 21 pixels (equivalent to a sphere of 310 μm diameter), vignettes were saved and transformed into gray level images. This threshold is commonly used with this UVP configuration to keep only identifiable particles, because fewer pixels is not sufficient to make a classification. Once the vignettes are processed, Zooprocess makes several biometric measurements on captured particles such as its area, perimeter, and the length of both minor and major axes of the ellipsoid fitted to the shape of the particle (see Appendices 4 and 5 from Gorsky et al. 2010). These data were imported to the Ecotaxa server (Picheral et al. 2017), where a random-forest model performed a classification of the particles to predict zooplankton taxonomy. Predictions were based on measurements of manually identified vignettes and were subsequently visually confirmed (all 358,510 predictions were verified manually).

Appendix II: Histograms of the size distribution of zooplankton individuals (Equivalent spherical diameter in mm) sampled in the study. Note that small-sized Chaoboridae were a consequence of cut images when the individual was taken in photo at the edge of the frame.



Appendix III: Histograms showing taxonomic comparisons from the Zooscan and the Underwater Vision Profiler (UVP) samples in the smaller half of the size classes, where a large amount of UVP vignettes were unidentified and classified into the “others” category.



Appendix IV: Normalized Biovolume Size Spectrum (NBSS) calculations

To calculate size spectra, areas in pixels from Zooprocess were converted into equivalent spherical diameters (ESD) by considering the particle area as a disk (Eq. 1):

$$(1) \quad ESD = 2 \times \sqrt{\frac{A \times C^2}{\pi}}$$

where A is the area of the particle and C is the conversion factor to transform pixels to millimetres (C = 0.06 for UVP acquisition and C = 0.0105833 for Zooscan acquisition). Once the ESD is calculated, it is transformed into biovolume (bv; Eq. 2) by considering the particle as a sphere:

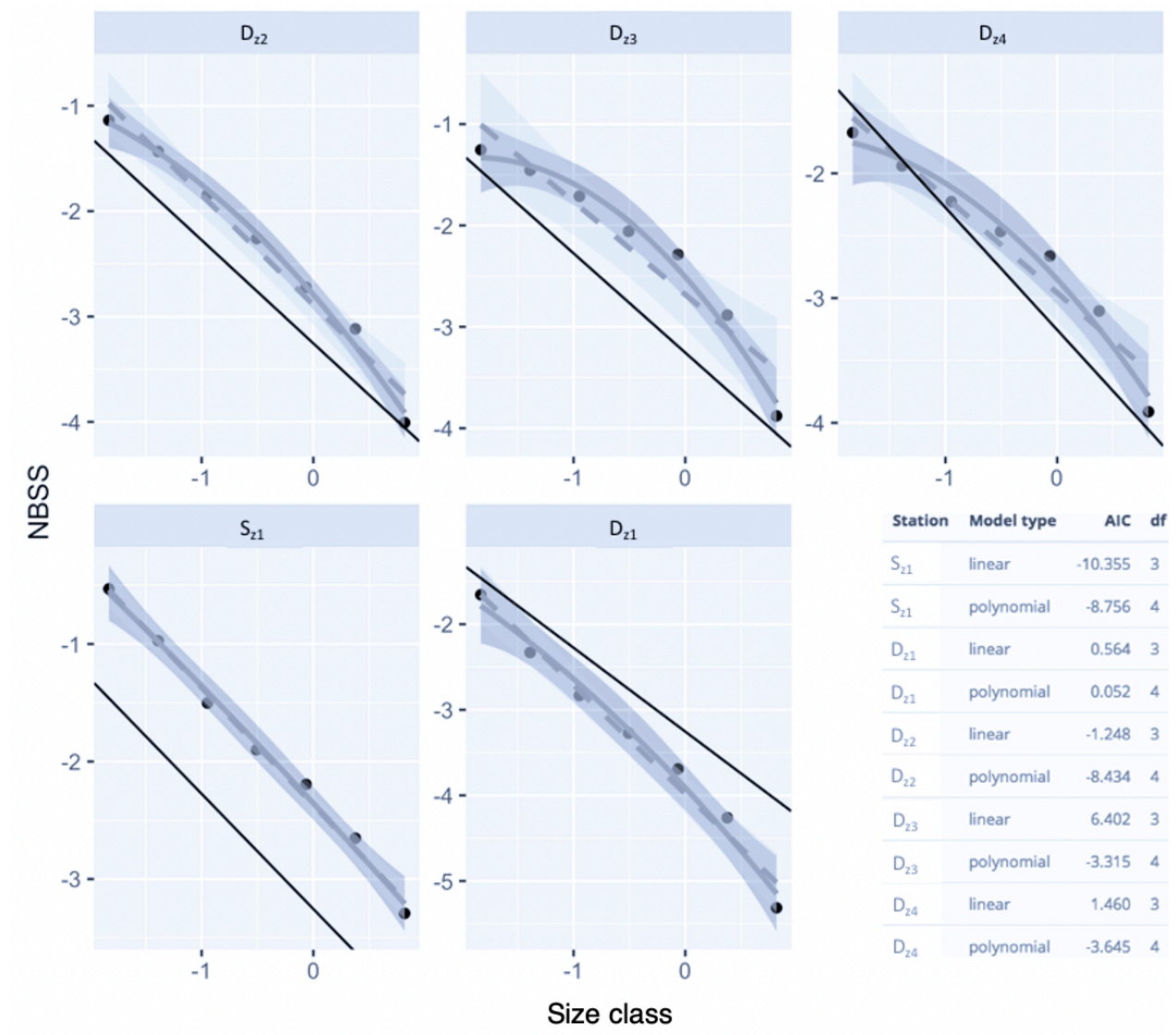
$$(2) \quad bv = \frac{4}{3} \times \pi \times \left(\frac{ESD}{2}\right)^3$$

Several mathematical methods can estimate the parameters of the power law distribution representing the NSS depending on both the units chosen and binning (Sprules & Munawar 1986, White et al. 2008, Guet et al. 2016, Barth et al. 2019). We used the biovolume to calculate NSS (hereafter NBSS) rather than biomass because biovolume is more commonly used. This choice also allowed us to simplify calculations and avoid more derived estimations. Even though continuous data were available to build the size spectrum, size classes were chosen to facilitate interpretation. Size classes were log-spaced and the total biovolume in each size class was normalized. Normalization consisted of dividing the total biovolume contained in one size class by the extent of this size class (Eq. 3). This prevents bias and allows the use of a traditional continuous model such as linear regression (Sprules & Barth 2016). The NBSS in each size class i ($NBSS_i$) was then estimated as:

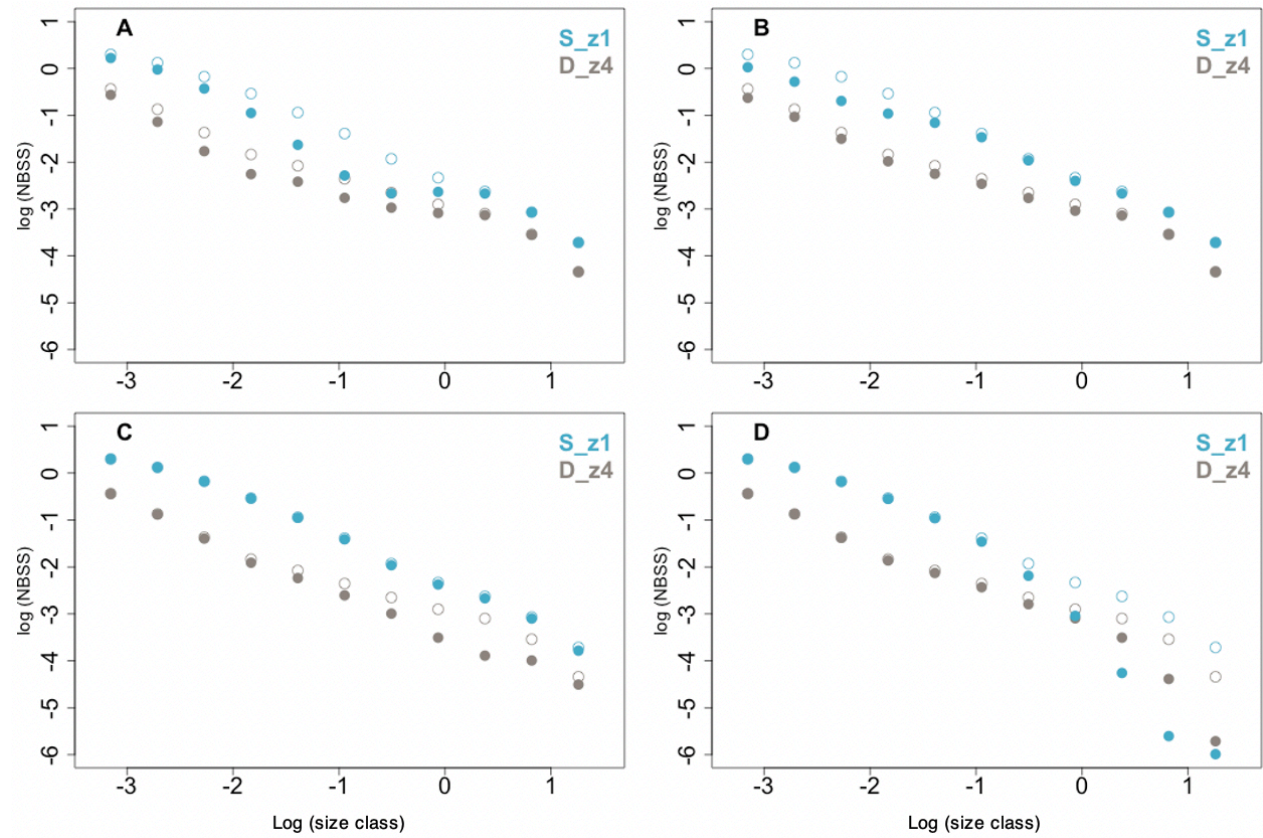
$$(3) \quad NBSS_i = \frac{\sum_i bv_i / Vol_e}{\Delta bv_i} \quad \text{with} \quad \Delta bv_i = \max(V)_i - \min(V)_i$$

where $\sum_i bv_i$ is the sum of particle biovolumes contained in size class i and Vol_e is the sample volume in litres. For the UVP data set, sample volume was the number of pictures considered multiplied by 0.18 since one UVP image samples 0.18L of water; Δbv_i is the extent of the corresponding size class, so the difference between the largest and the smallest volume of a particle within the size class.

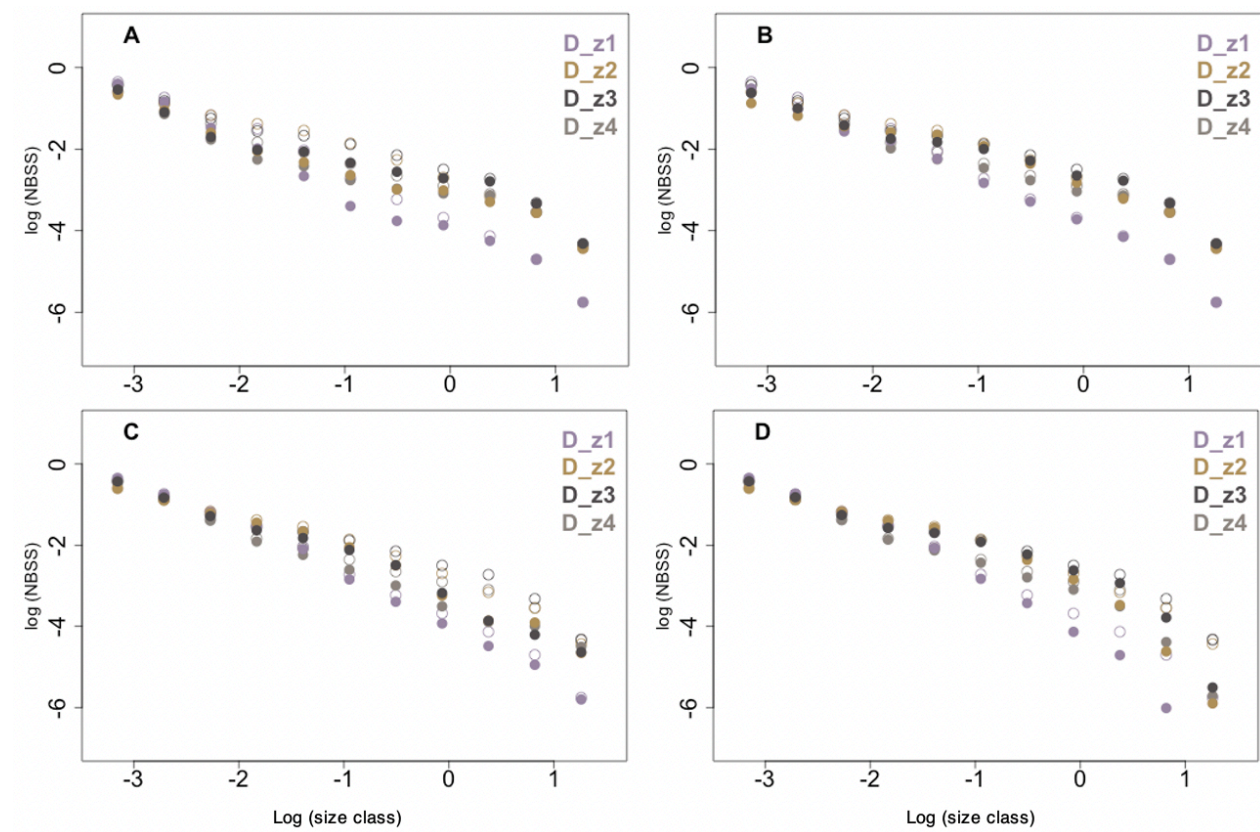
Appendix V: Comparison of polynomial (blue lines) and linear (dashed blue lines) fittings of normalized biovolume size spectra (NBSS) calculated from size classes 8 through 14 for each station. The black line represents the mean NBSS for the whole lake (all periods pooled). The table gives AICs and degrees of freedom (df) for each station and each type of fitting in order to assess whether the polynomial or linear model best fits the data from each station.



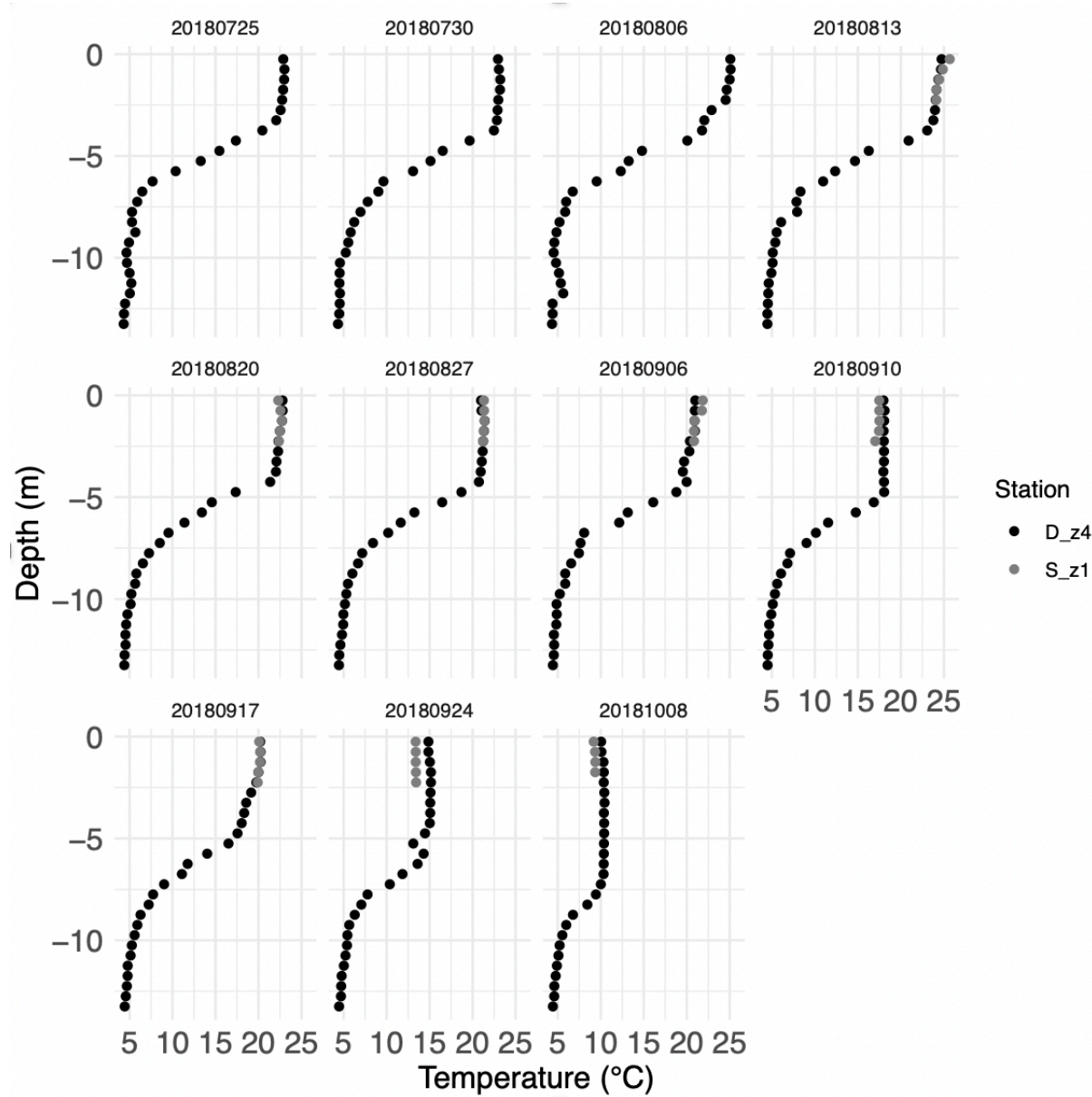
Appendix VI: Normalized biovolume size spectra (NBSS) calculated on identified zooplankton particles from all dates. To show the contribution of each taxon on the overall size spectrum (filled circles), the different taxa were removed one by one from the calculation of the NBSS (empty circles): A) Other cladocerans; B) copepods; C) *Holopedium glacialis*, and D) *Conochilus* sp. Gray symbols represent station D_{z4} and blue symbols represent the shallow basin (S_{z1} station).



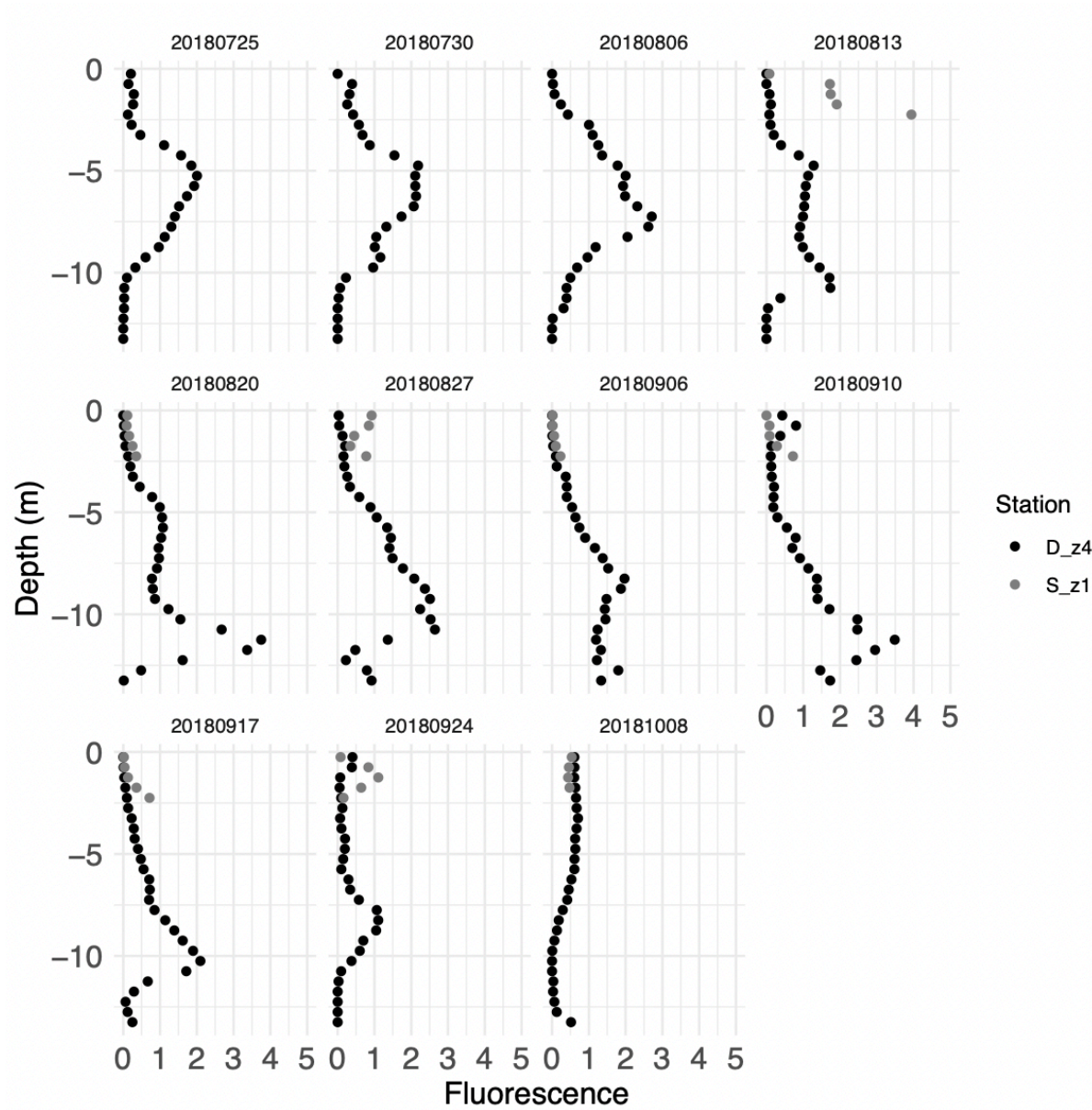
Appendix VI (cont.):



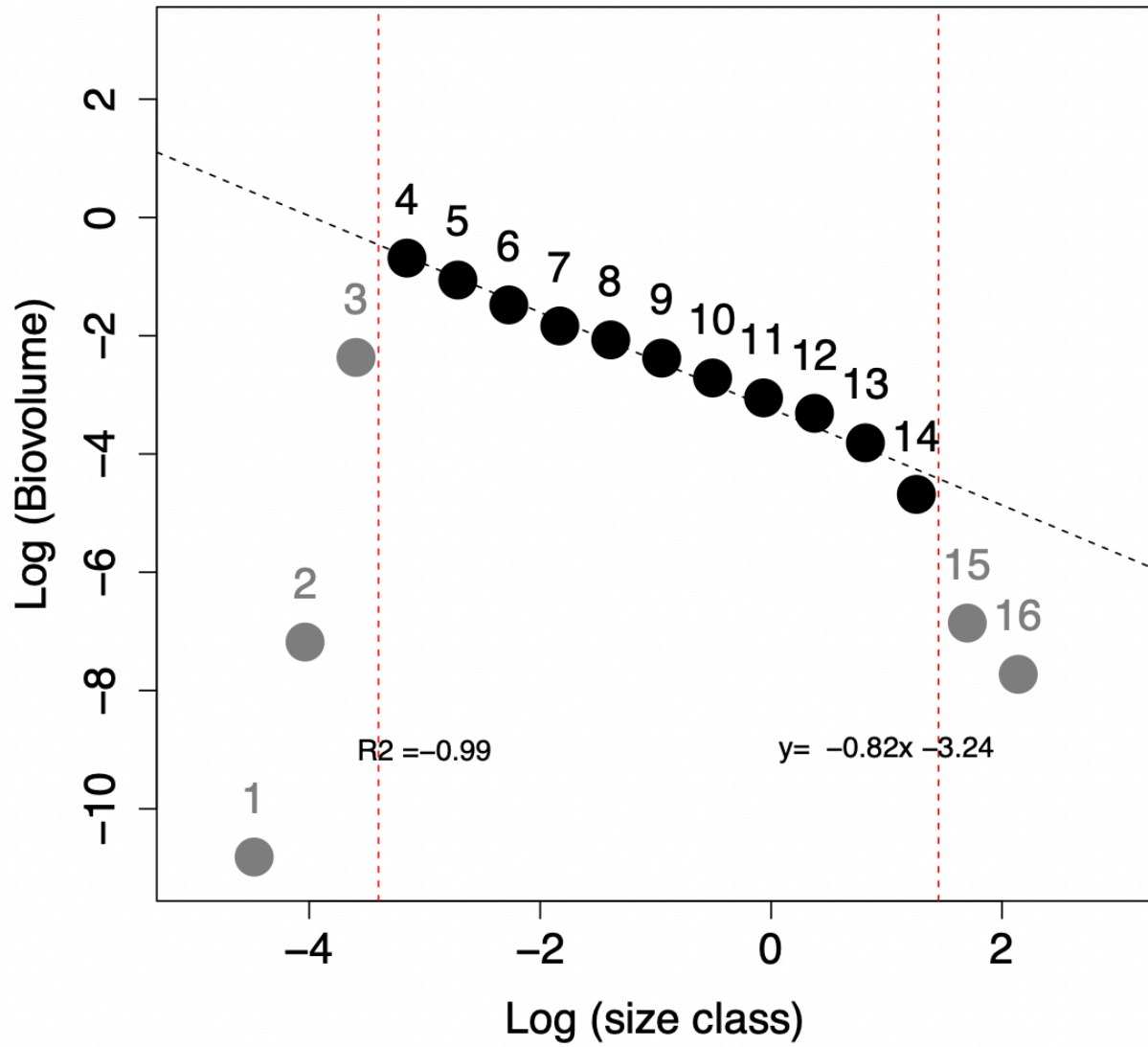
Appendix VII: Temperature profiles for each week sampled in the shallow and deep basin (respectively S_{z1} and D_{z4})



Appendix VIII: Profiles of fluorescence for each week sampled in the shallow and deep basin (respectively S_{z1} and D_{z4})



Appendix IX: Normalized biovolume size spectra (NBSS) graph of all data measured on living particles captured by the underwater vision profiler (UVP). The linear regression and Pearson coefficient of determination include only the size classes falling between the two vertical dotted lines. The size classes clearly deviating from linearity (gray circles) were removed from the analysis (see text).



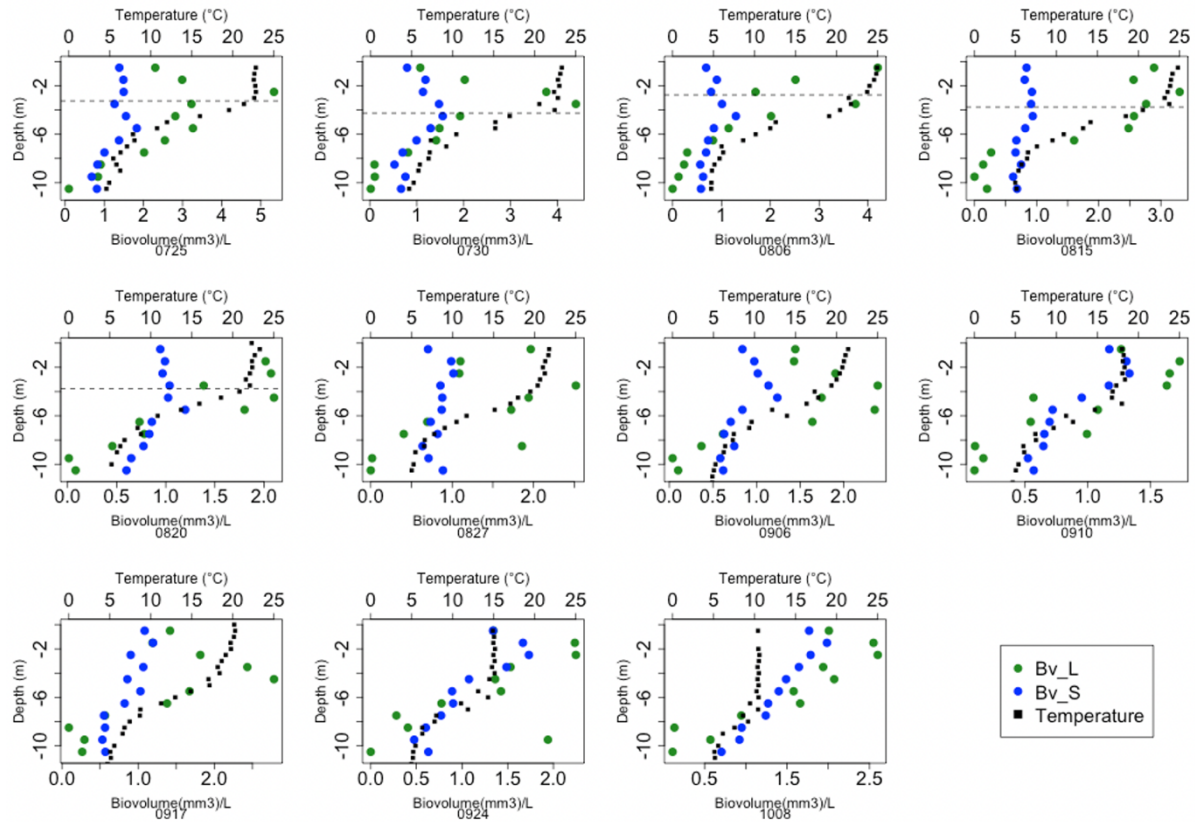
Appendix X: Summary of model (glm) AIC values used to select the best models explaining slope and height variations in the dataset. The degrees of freedom (Df) of the model gives the number of parameters in the model. Δ_{AIC} values in bold below ten indicate plausible models.

Four models could plausibly explain the NBSS slope (i.e., $\Delta_{AIC} < 10$; models M4, M7, M8, M9). Although they were all strongly supported, with Δ_{AIC} values < 4 , we retained M4 as the best since it was the most parsimonious, having the lowest AIC and fewest parameters. This model included both station and date effects, and both terms were significant ($p < 0.01$ and < 0.001 , respectively).

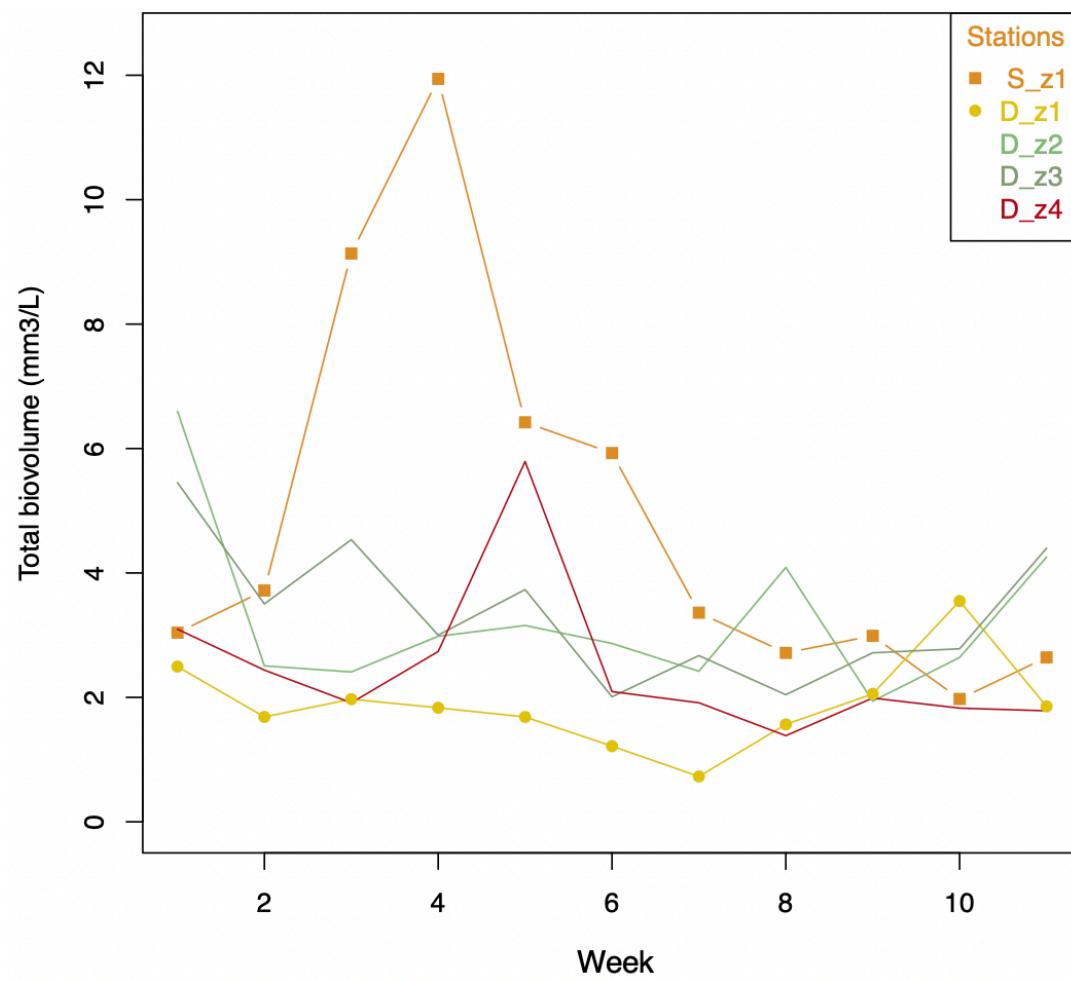
Five models could plausibly explain variations of NBSS height (models M1, M4, M6, M7, and M8). The most complex of these models, M8, included the effects of station, week, and interaction between week and station. Among these, only four models (M1, M4, M6, and M7) were strongly supported, with $\Delta_{AIC} < 4$. Model M6 was selected as the best model to explain variation in the NBSS height since it had the lowest number of parameters among models with $\Delta_{AIC} < 2$ (AIC=182.4, $\Delta_{AIC} = 1.5$, $df=7$). It included the effects of station and period of the day. Station had a significant effect ($p < 0.001$) but not the period of the day ($p = 0.060$).

Model	Df	Slope		Height	
		AIC	Δ_{AIC}	AIC	Δ_{AIC}
M0= ~1	2	42.5	53.3	219.3	40.0
M1 = ~station	6	33.9	44.6	185.6	3.3
M2 = ~period	3	44.2	55.0	218.5	36.2
M3 = ~week	3	6.5	17.2	219.3	37.0
M4 = ~station+week	7	-10.7	0	184.6	2.2
M5 = ~week+period	4	7.6	18.3	218.3	35.9
M6 = ~station+period	7	35.6	46.3	183.9	1.5
M7 = ~week+period+station	8	-9.8	1.0	182.4	0
M8 = ~week+station+station:week	11	-9.0	1.8	189.1	6.8
M9 = ~week+period+station+station:week+period:station	16	-8.4	2.4	194.0	11.7

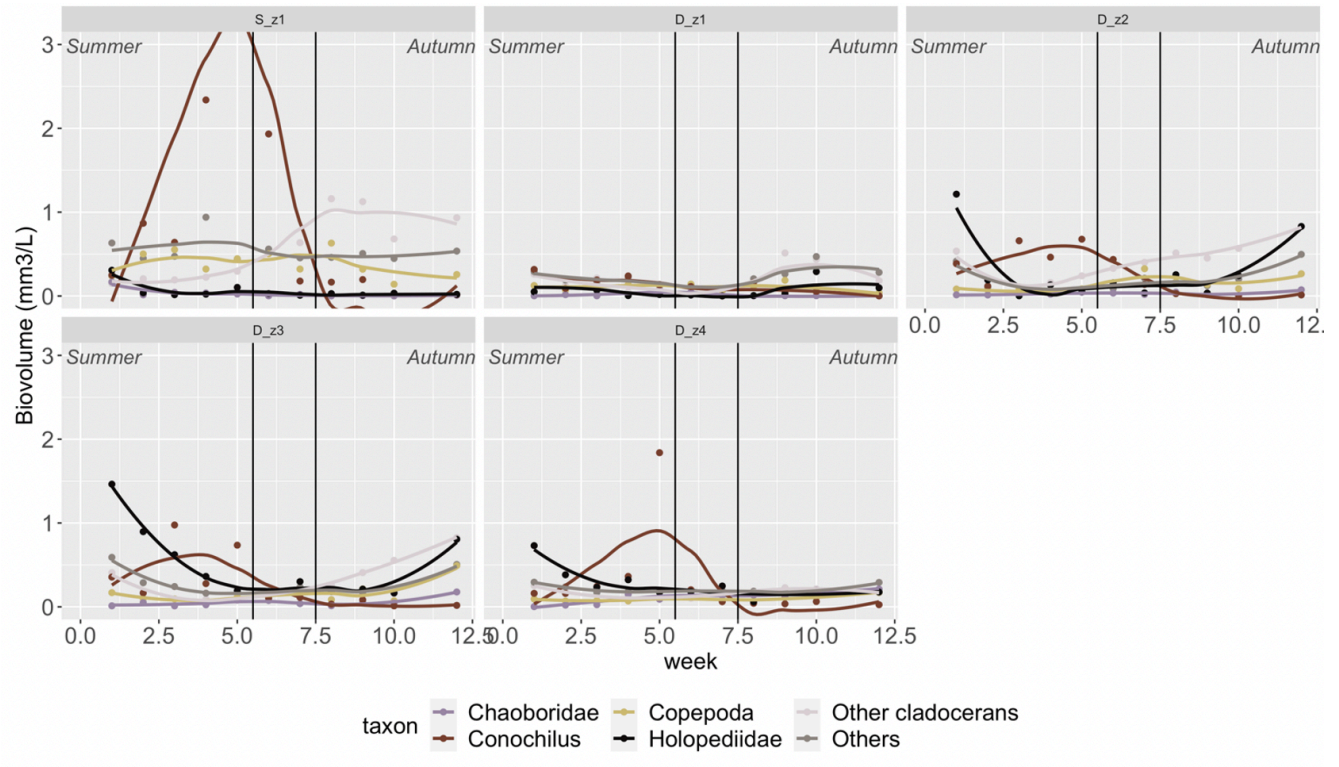
Appendix XI: Vertical distribution of the biovolumes of large (ESD > 1 mm; green circles) and small (ESD < 1 mm; blue circles) zooplankton at the deepest station of Lake Ledoux (D₂₄). Black squares represent the temperature along the vertical profile and the horizontal dashed lines represent the depth where temperature drops below 22°C. Each panel represents one week.



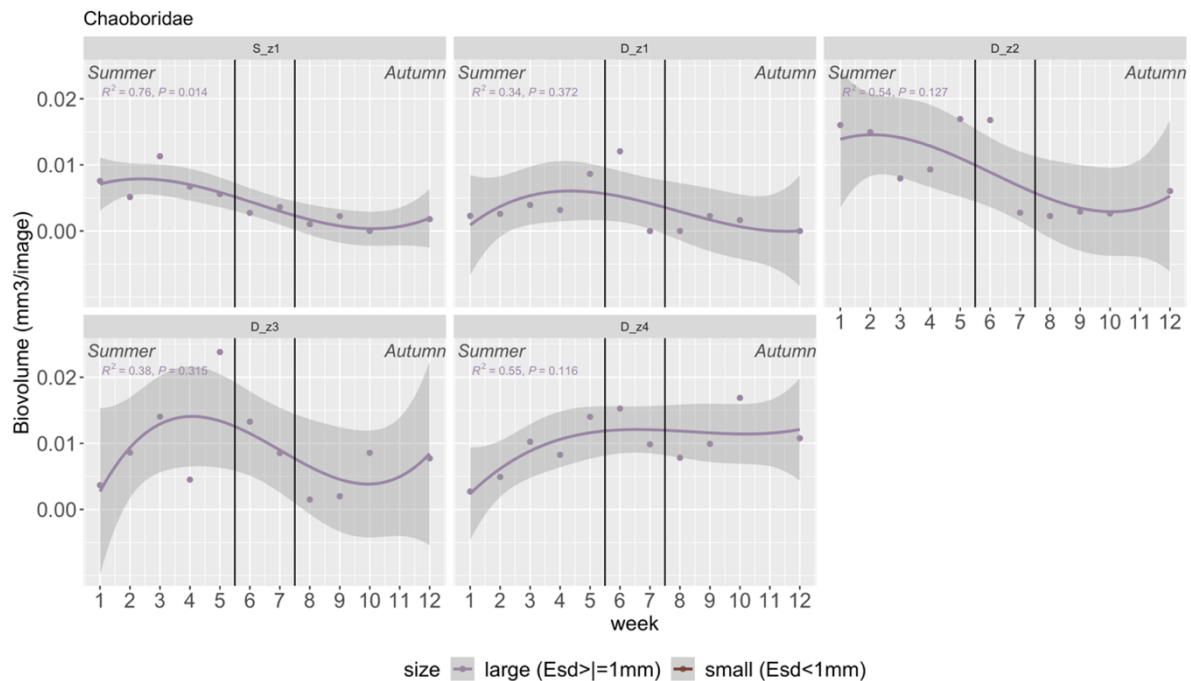
Appendix XII: Total biovolumes of all zooplankton at the stations sampled weekly in Lake Ledoux from July to October.

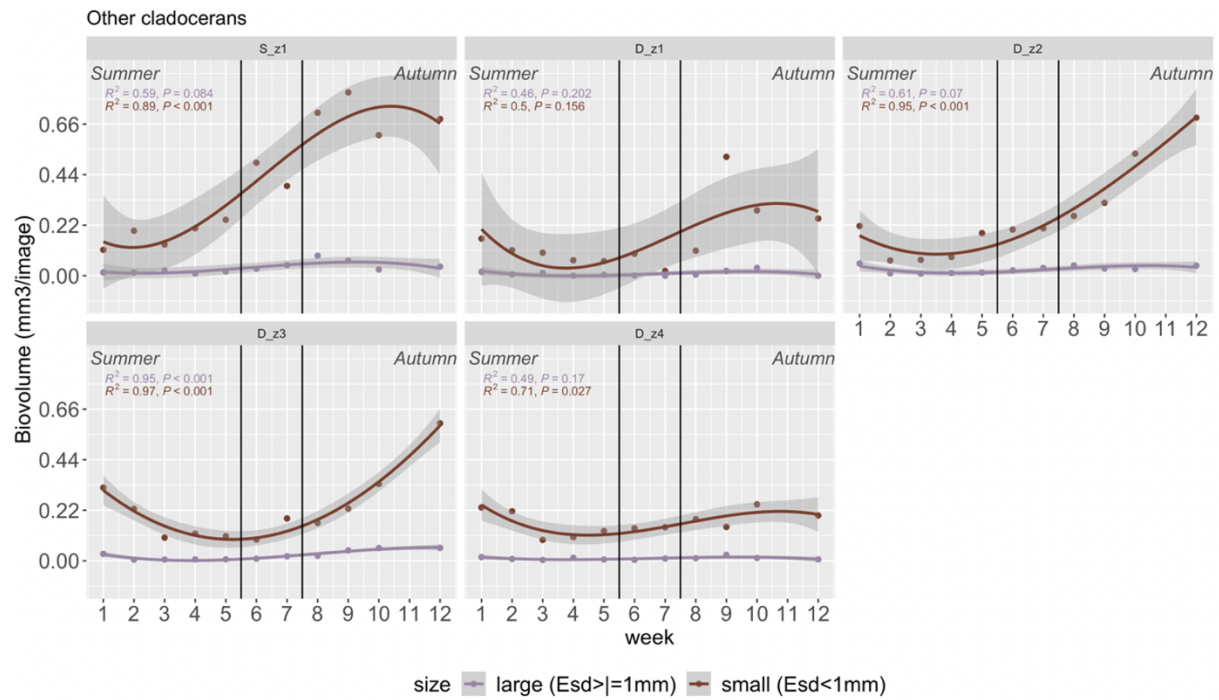
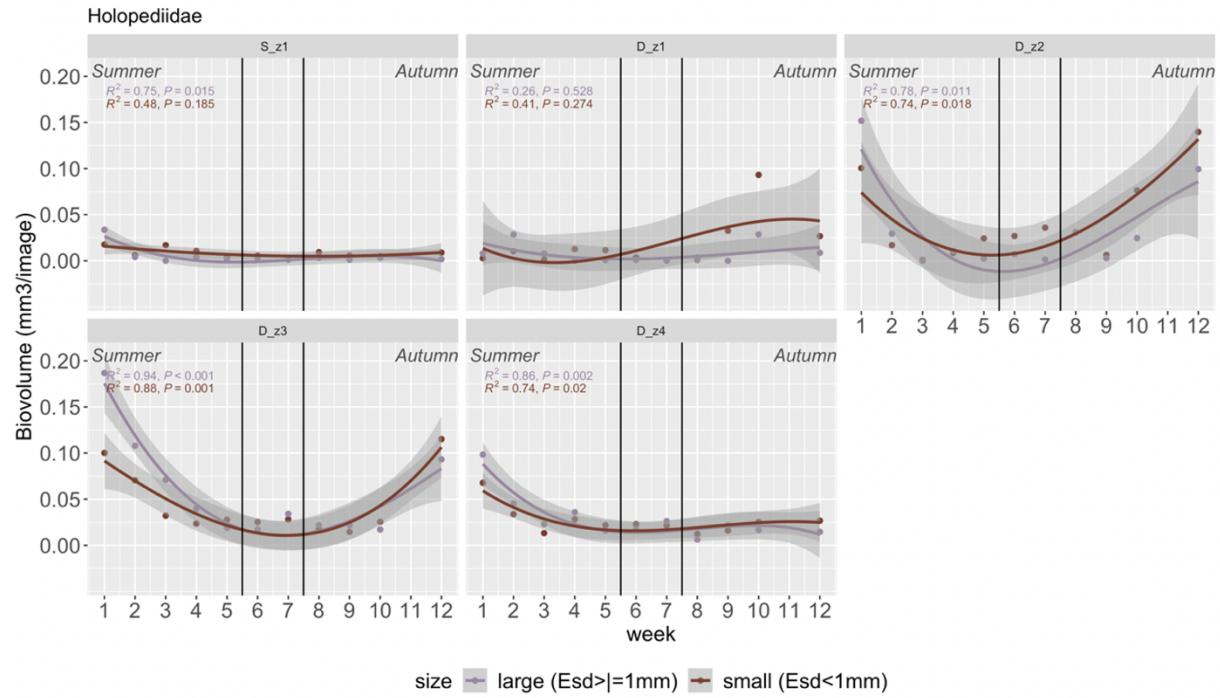


Appendix XIII: Biovolumes of each zooplankton category at each station during sampling from 23 July to 8 October for the five stations of Lake Ledoux. Loess curves were added to help visualization. The weeks between the black vertical lines (08/27 and 09/04) are the transition period, when temperatures were between 22°C and 20°C.

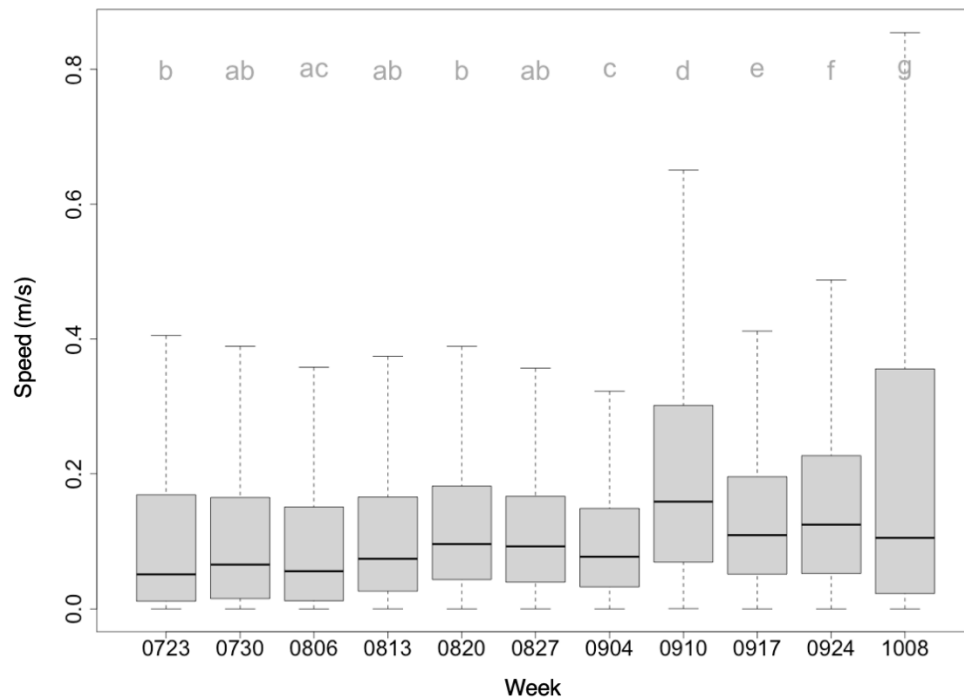


Appendix XIV: Biovolume of Chaoboridae, Holopediidae, and other cladocerans during the sampling period from 23 July to 8 October for the five stations of Lake Ledoux. Each taxon was separated into a large (> 1 mm) or small (< 1 mm) category. All Chaoboridae were considered as large individuals even if the underwater vision profiler (UVP) miscalculated their volume (the vast majority of vignettes only showed partial individuals). This calculation bias did not influence the temporal trend because similar shapes emerged from individual based plots. Data were fitted with an order three polynomial function to show if there was an abrupt shift when surface temperature dropped below 22°C, as seen with normalized biovolume size spectra (NBSS) slopes. R^2 and p values from regressions are shown at the top left of each graph. The weeks between the black vertical lines (08/27 and 09/04) are the transition period, when temperatures were between 22°C and 20°C. One image corresponds to the UVP sampling volume per image (0.18 L).





Appendix XV: Boxplot of fish swimming speed during the sampling period. Data were extracted from a fish acoustic telemetry survey that took place continuously and simultaneously with zooplankton sampling (P. Magnan, unpubl. data). Only positions recorded in the two hours prior to sunset were considered in order to simplify the analysis and because brook charr is expected to be more active at this time of the day (Bourke et al. 1996). All available positions and fish within these two hours were retained. The swimming speed calculation was the distance between each position detected by telemetry divided by the time between these two detections. If time between two detections exceeded one minute, data were discarded. Letters above boxplots are derived from a mixed model [speed ~ Weeks + (1|fish_individual)] and a subsequent group analysis (emmeans package).



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CHAPTER II: Integrating connectivity and interindividual variation in fish–zooplankton spatio-temporal coupling

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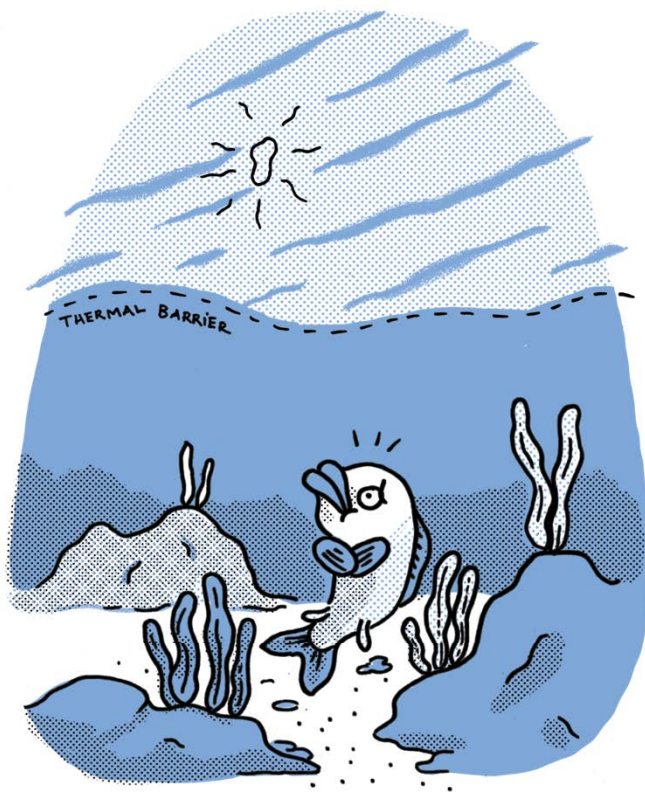
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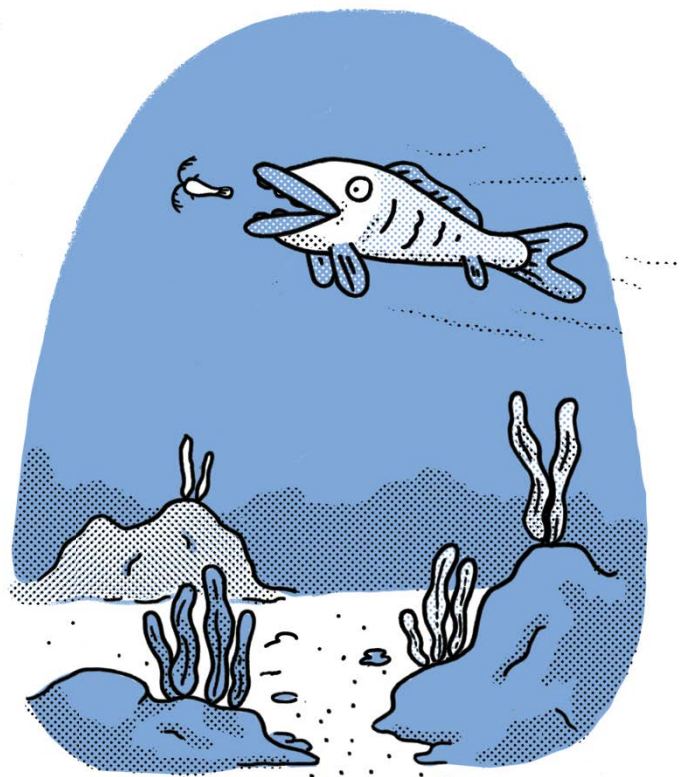
Abstract

Animal movements play a crucial role in ecosystem dynamics, regulating numerous functions from nutrient cycling to habitat coupling. Mobile predators have the potential to connect different trophic networks by moving across habitats, but it is only recently that technologies have allowed the full complexity of this phenomenon to be explored. In this study, we investigate how brook charr select habitat in a small boreal lake consisting of three basins, one of which is shallow (mean depth 1.5 m). Since brook charr do not support temperatures above 22°C, the thermal epilimnetic barrier during summer prevents them from accessing the shallow basin and littoral areas. The aim of this study was to see whether (1) fish tracked zooplankton while coping with their thermal needs; (2) fish selection depended on seasonal variations in the thermal barrier; and (3) the existing trophic polymorphism in this salmonid species allows interindividual differences in resource selection. Lake zooplankton were mapped weekly using an Underwater Vision Profiler for 2 h before sunset, corresponding to the expected crepuscular peak of fish activity. Approximately 4 km of transects collected at different depths were used to interpolate a 3D zooplankton concentration in the entire lake over 11 weeks. Acoustic tags followed the movements of 21 fish during the same period at 20 sec intervals. We found that fish mostly selected habitat close to the bottom of the water column. During summer, several fish selected the 6 m isobath in the main basin to remain close to littoral resource-rich areas while staying within a thermal refuge; fish never entered the shallow basin. When the thermal barrier disappeared at the end of the summer, they shifted from pelagic to littoral areas with more frequent selection of zooplankton patches, although some individuals never selected them. In the context of climate change, integrating behavioral responses to thermal barriers is crucial to better assess the potential impact of a longer summer in boreal areas.

Keywords: predation, habitat coupling, thermal barrier, Habitat Selection Analyses (HSA), brook charr



Summer



Fall

Introduction

Animal movement is a key component of ecosystem functioning and plays a crucial role in their dynamics (Nathan et al. 2008, Abrahms et al. 2021, Nathan et al. 2022). Individuals moving across habitats have the potential to increase fluxes and connectivity between different areas, making trophic networks more complex and enhancing nutrient cycling (Wetzel et al. 2005, Allegier et al. 2017, Atkinson et al. 2017, Williams et al. 2018). Two key drivers of habitat coupling are the movements of organisms across habitats and the spatio-temporal patterns of feeding by highly mobile organisms (Polis et al. 1997, Schindler & Sheuerell 2002, Egevang et al. 2010, Schmitz et al. 2010). Highly mobile organisms connect the trophic networks of adjacent habitats according to the predator's selection of resources (Cock et al. 1978, Schindler & Sheuerell 2002, Guzzo et al. 2017). However, understanding the mechanisms of habitat selection (including resource selection) is challenging due to the complexity of this phenomenon. Habitat selection can vary among individuals of the same species and relies on many factors, some of which are poorly understood and with some features being visible only at specific spatio-temporal scales (Nathan et al. 2008, Abrahms et al. 2021). However, once the mechanisms of habitat selection by predators are resolved, it is possible to make inferences on predator–prey relationships in space and time, and therefore on the strength of habitat coupling (Sims et al. 2008, Roberts et al. 2021, Séchaud et al. 2021).

In lakes, habitat coupling by large mobile predators such as fish is essential for connecting littoral and pelagic areas via the mutual transfer of energy, specifically by supporting production in pelagic areas (Schindler & Scheuerell 2002) and stabilizing food webs (Rooney & McCann 2012). However, in boreal lakes, the ability of several cool-water fish species to feed on littoral prey fluctuates seasonally because of water column thermal stratification. In summer, access to littoral areas is reduced, especially for cold-stenothermic fish that cannot remain too long in this habitat because of suboptimal and/or lethal temperatures (Goyer et al. 2014, Guzzo et al. 2017). During these periods, spatial heterogeneity reduces or even hinders pelagic and littoral coupling, forcing fish to make a tradeoff between a potentially resource-rich but too-warm habitat and a cooler habitat with fewer prey or harder to capture (Guzzo et al. 2017). Mechanisms behind this tradeoff remain poorly understood due to the difficulty of following fine-scale movements of individual fish both spatially and temporally. The advent of new technologies—like acoustic telemetry to monitor fish movements—offers powerful tools to investigate these decision-making processes in many aquatic species (Cooke et al. 2013, Hussey et al. 2015).

In this study, we focused on a small boreal lake where the only fish species is brook charr (*Salvelinus fontinalis*), a cold-stenothermic salmonid fish. Its main prey are zooplankton in pelagic areas and zoobenthos in littoral areas (Magnan 1988, Bourke et al. 1999). Previous studies suggested that brook charr cannot access the epilimnion when it exceeds a threshold temperature of 22°C, thus almost entirely preventing access to the large, warm, shallow area of the lake during summer (Bourke et al. 1996, Bertolo et al. 2011, Goyer et al. 2014). To avoid thermal stress, fish exhibit behavioral thermoregulation, which varies according to individuals (Goyer et al. 2014). These individual variations could have cascading effects on brook charr feeding strategy at the within-population level.

The main objective of this study is to understand how fish adapt their food acquisition tactics to cope with their thermoregulatory needs. To investigate this process, we used Habitat Selection Analysis (HSA; Boyce & MacDonald 1999, Fieberg et al. 2021) and tested the three following hypotheses.

First, we tested the hypothesis that fish select areas with more food resources (e.g., zooplankton abundance) as long as the food resources are in a suitable physical habitat (e.g., depth, temperature). It is expected that fish will be non-randomly distributed in the pelagic area, being associated with zooplankton hotspots, especially those composed of large (i.e., >1 mm) and more conspicuous (e.g., *Daphnia* spp.) zooplankton taxa (Confer et al. 1978, Magnan 1988). It is also expected that fish will often be found close to littoral areas, where we assumed a greater abundance of benthic invertebrates are found, in order to access this highly profitable resource via short forays into warm waters (Bertolo et al. 2011, Goyer et al. 2014, P  pino et al. 2015).

Second, we tested the hypothesis that fish selection depends on seasonal variations in the “permeability” or strength of the thermal barriers between the littoral and pelagic zones and between the epilimnion and metalimnion, with the latter being more permeable for fish (P  pino et al. 2015). We expect that the selection pattern by fish on zooplankton hotspots will be weaker when littoral areas become available to fish. When the thermal barrier is not present (i.e., starting in September in our study system), benthic invertebrate become available and should be preferred by fish (Magnan 1988, Bourke et al. 1999).

Third, we tested the hypothesis that habitat and resource selection will vary among individuals because of trophic polymorphism in brook charr populations of this system (Bourke et al. 1999, Dynes et al. 1999, Rainville et al. 2021). We expect that some individuals will preferentially select pelagic zooplankton patches (Bourke et al. 1997, 1999, Dynes et al. 1999, Rainville et al. 2021).

Sorting out the relative roles of habitat features and resources in driving individual fish behavior should improve our understanding of the contribution of animal movements to habitat coupling. Global warming has an increasingly strong impact on lake temperature, especially for boreal lakes (O'Reilly et al. 2015, Dokulil et al. 2021), and should increase future spatial and seasonal differences of lake thermal regimes, with potential consequences on the trophic coupling between the littoral and pelagic habitats. In this context, integrating behavioral responses and thermal barriers is crucial to better assess the potential impact of warmer and longer summers in boreal regions.

Methods

Study site

The study was done in Lake Ledoux, Mastigouche Wildlife Reserve, Quebec, Canada (46.802381°N, 73.277136°W), a small oligotrophic boreal lake for which a large amount of data on brook charr habitat use is available (e.g., Bourke et al. 1996, Dynes et al. 1999, Bertolo et al. 2011, Goyer et al. 2014). Lake Ledoux has a main deep basin (max. depth 18 m) and two shallow basins with mean depths of about 1.5 m and 5 m at the west and east sides, respectively (Figure 1). The lake is typical of Canadian Shield lakes and is located in a relatively protected area, where logging and fishing are strictly controlled by the Quebec Government. Sampling occurred from July to October 2018, and the lake was closed to fishing during the study. Submerged aquatic vegetation was relatively abundant in the shallow western basin (hereafter “shallow basin”) and only patchily distributed in the littoral areas of the deep basin.

Brook charr is the only fish species in the lake and has two main prey sources, zoobenthos and zooplankton (Magnan 1988, Lacasse & Magnan 1992, Rodríguez et al. 1993, Bourke et al. 1999). Given that brook charr avoid temperatures above 22°C (Goyer et al. 2014), it encounters strong thermal constraints to feeding in the shallow western basin and in littoral areas during the stratification period (based on recent high-frequency hydroacoustic telemetry surveys; P. Magnan, unpub. data). During summer, individuals in the main basin make forays into the epilimnion but often stay at depths of 5–6 m (Bertolo et al. 2011, Goyer et al. 2014), which corresponds to their preferential temperature in the metalimnion (Smith & Ridgway 2019). During this period, zoobenthic prey were largely inaccessible to fish, and they had to rely on zooplanktonic resources. The deep basin offers a thermal refuge (i.e., cold waters of the metalimnion), allowing fish to thermoregulate (Bertolo et al. 2011, Goyer et al. 2014). Therefore, while the thermal barrier is not impassable within the deep basin—fish are able to make short excursions to the

epilimnion or littoral area—it is impassable between the two basins due to the distance to the thermal refuge.

Zooplankton sampling

To match the spatio-temporal resolution of fish data (see *Fish tracking* section), zooplankton was sampled weekly with an Underwater Vision Profiler (UVP; Hydroptic, L'Isle-Jourdain, France), which take up to 11 pictures per second. The UVP was modified to better detect small freshwater zooplankton by reducing the focal length to improve the resolution while reducing the sampling volume (M. Picheral, LOV Oceanographic Observatory, Villefranche sur Mer, France, pers. comm.). With this modification, the UVP was able to take pictures of zooplankton individuals larger than 262 μm , which is roughly the particle size range visible to brook charr. Sampling consisted of shore-to-shore zigzag transects to optimize sampling time and cover the maximum lake area (Figure 1). Six depths were selected to cover the lake's water column: two in the epilimnion (0.5 and 1.5 m), two in the metalimnion (3 and 5 m), and two in the hypolimnion (8 and 12 m). The three deepest transects were straight east–west lines in to avoid contact between the UVP and rocks on the bottom next to the shores (Figure 1). Weekly sampling occurred on 11 occasions between 23 July and 8 October and lasted 2h each. Transects started 2 h before sunset, to cover the time of day when fish are presumably the most active (Bourke et al. 1996). This protocol maximized the probability that brook charr were actively feeding when zooplankton was mapped. We used a dGPS (GENEQ Inc., model Sx-Blue) to be sure that we followed the same transect path each week. The beginning, the end, and each turning point of the transect (hereafter turning points) were time-stamped to match the UVP time recording to GPS positions. Boat speed was maintained at 1 m/s (i.e., 3.6 km/h) to interpolate the positions between two GPS points.

Details of the image processing and semi-automatic classification protocols are provided in Leroux et al. (2022). The 651,255 images (hereafter referred to as vignettes) were classified with the Ecotaxa random forest model (Picheral et al. 2017), which is based on a training using 358,510 manually identified vignettes. The equivalent spherical diameter (ESD) was calculated for each zooplankton picture and transformed into biovolume according to the equations described in Leroux et al. (2022). To minimize the incertitude associated with image resolution, vignettes were not identified to the lowest possible taxonomic level but rather pooled into the following groups: copepods, Holopediidae (*Holopedium glacialis*), other cladocerans, Chaoboridae, colonial rotifers (*Conochilus* sp.), and non-zooplanktonic

categories (e.g., Volvocales, aquatic plant, Arachnidae, and detritus). “Other cladocerans” included mainly Bosminidae and Daphniidae, the latter being largely dominant in large size classes.

To explore different hypotheses about fish preferences, we examined our zooplankton data in different ways. Three zooplankton categories were created using three different criteria; each category was separated into two subcategories that did not overlap to avoid biased covariations in the Habitat Selection Analysis (HSA) described below. The first zooplankton category was based on size and included the whole zooplankton community. Individuals ≥ 1 mm were grouped into a “large” group without taxonomic discrimination while the remaining individuals were the “small” group (< 1 mm). The size threshold of 1 mm was chosen according to predation performance of brook charr on zooplankton (Confer et al. 1978, Magnan 1988). It is important to note that even the small group contains potential zooplankton prey because the minimum particle diameter sampled by the UVP is 262 μm , and 99% of particles had an equivalent spherical diameter (ESD) greater than 389 μm . Therefore, we expect that brook charr have the potential to select both small and large zooplankton categories, but with a stronger preference for the latter. Because the jelly capsule of *Holopedium* spp. is transparent, UVP size measurements represented carapace size rather than the larger capsule.

The second zooplankton category was based on a rough taxonomic selection criterion and was meant to help reveal the taxon preference of brook charr. It was composed of *Holopedium glacialis* (a taxon that is particularly abundant in Lake Ledoux compared to other lakes of the region; Gignac-Brassard et al. 2022) and “other cladocerans” (OC), with no distinction based on size.

The third zooplankton category was a mixture of taxonomic and size criteria, with “large other cladocerans” (hereafter “LOC”; composed of “other cladocerans”, i.e., mostly *Daphnia* spp., with an ESD > 1 mm) and “other large zooplankters” (hereafter “OLZ”; all zooplankton taxa > 1 mm excluding cladocerans). *H. glacialis* was included in the OLZ category.

Zooplankton mapping

On a few occasions, our dGPS recording system failed. However, since we followed the same path every week for each transect by using easily identifiable landmarks on the shore, we retrieved the missing sampling positions by matching the time-stamped turning points to their previously recorded locations. Available GPS coordinates of turning points were averaged to assign a coordinate to the time-stamped key

points for all weeks. This approach is supported by a graph of available turning point coordinates, which showed that sampling trajectories were consistent across weeks and transects (Figure S1). The position of each UVP image between turning points was estimated by interpolating coordinates between the two neighboring turning points with the time recorded for each UVP image. This allowed us to retrieve data collected every few centimeters along the transects. However, the resulting zooplankton biovolumes obtained from these raw data were averaged every 5 m to have a relevant spatial resolution, thus reducing biases linked to the uncertainties related to dGPS positioning and the approach of turning points. For each 5 m segment, zooplankton biovolume measurements were available for each of the categories defined above. Biovolume was calculated by summing all zooplankton biovolumes sampled in the 5 m segment divided by the sampled volume (i.e., the number of UVP images). On average, one complete UVP weekly sampling resulted in 722 ± 119 5 m segments, meaning that an average of 3.6 km was sampled each week. Some weeks had fewer data due to UVP shutdown during cleaning operations when surface transects passed through vegetation beds.

For each weekly sampling, all available 5 m segments were used to build an Empirical Bayesian Kriging 3D model on ArcGIS Pro (EBK3D; Krivoruchko 2012, Krivoruchko & Gribov 2019). The horizontal spatial resolution of the EBK3D corresponded to the resolution of the segments with pixels of 5 m side. The vertical spatial resolution was set to 1 m to be sure to include transitions in stratification. The EBK3D provided an estimation of zooplankton biovolume over the entire lake in $5 \times 5 \times 1$ m parcel. Detailed parameters are presented in supplement S2. A main advantage of the EBK3D is that most parameters do not require prior assumptions. EBK3D error was estimated for each kriging to determine the model's strength. Kriging was performed weekly for each category of zooplankton and was then imported to R (R Core Team 2021) and converted to a raster layer for each week and depth to associate a value for each fish position using the *extract()* function of the raster package.

Fish tracking

Fish were tracked by 23 acoustic receivers (HR2-180k-100; VEMCO Inc., Halifax) installed around the lake (supplement S3). A total of 21 fish equipped with acoustic transmitters (V9TP-2x-180k-xxxm; VEMCO Inc., Halifax) were tracked during this study. The minimum time between two recordings of a single fish was 10–14 s (i.e., delay between two consecutive tag signal transmissions), but gaps could occasionally appear in the data if a fish was not recorded by any receiver. More details on the methods and tagging protocol are presented in supplement S3. Only fish positions from 1 h before and 1 h after zooplankton

sampling were analyzed to ensure the temporal match between zooplankton and fish sampling; as such, each fish was monitored for 4 h per week. We will refer to this 4 h position dataset from one individual during a given week as a “sequence” hereafter. A total of 194 sequences were available for this study during the study period. All position measurements were converted to the Coordinate Reference System used by the receivers. Fish depth was also estimated by the receivers from the signal sent by the transmitters.

Habitat selection analysis

Selection analysis frameworks are suitable for managing the huge amount of high-frequency data generated by acoustic telemetry (e.g., Resource Selection Function [RSF], Step Selection Function [SSF]; Thurfjell et al. 2014, Fieberg et al. 2021, Munden et al. 2021). This analytical framework relies on the comparison between an animal’s actual habitat use or real movements and the movements of a virtual animal that would randomly move in an environment (Fieberg et al. 2021), so the virtual animal will encounter ecological features that could be similar to or different from what a real animal would encounter (e.g., temperature, biotope, resource density). It is then possible to determine whether the animal in question actually selects particular features. All fish, zooplankton (hereafter referred as “resources”), and habitat (i.e., lake morphometry, hereafter referred as “physical variables”) data were integrated into the framework of the Habitat or Resource Selection Analysis (hereafter referred to jointly as HSA; Boyce & MacDonald 1999; Fieberg et al. 2021). This approach is designed to handle two-dimensional data only, but we circumvented this limitation by implicitly considering a third dimension (i.e., depth) to fit fish movements (see *Assigning depth to simulated trajectories* section). The most common statistical method to make this comparison is the conditional logistic regression (Thurfjell et al. 2014, Avgar et al. 2016, Fieberg et al. 2021). We chose HSA rather than SSF because of the high-frequency acquisition of fish positions. The Step Selection Function, which integrates movement parameters such as step length and turning angles, compares each observed step from a trajectory with simulated steps. Thus, measurements of habitat features must have a finer resolution than the average step length to make SSF relevant. In our study, this would have meant a significant decrease in the temporal resolution of fish locations for us to find environmental differences when comparing an observed step to simulated ones. In the literature, SSF have been used in the long-term monitoring of terrestrial mammals, with position recordings ranging from 15 min to every few hours; this results in average step lengths of hundreds of meters (Sims et al. 2008, Thurfjell et al. 2014, Avgar et al. 2016).

Generating simulated positions

HSA compares habitat selected by a tracked animal within a given area based on habitat availability. The vast majority of the HSA literature uses the minimum convex hull method to define available habitat (Fieberg et al. 2021). In a small lake, we found it more appropriate to consider the whole lake as the available habitat, but we bounded the movement of tracked individual fish according to their thermal preferences. Therefore, we adapted HSA by generating simulated trajectories instead of picking random points within a polygon. These simulated trajectories were used to build the dataset of “random points”. To do this, we used the *adehabitatLT* package in R (Calenge 2015) to first transform fish positions (“observed points”) into observed trajectories. Each trajectory was then standardized to have a regular time lag between successive 1 min locations (i.e., 240 locations per sequence) and thus avoid biases in further analyses (Avgar et al. 2016, Fieberg et al. 2021). Standardization was obtained by linear interpolation of x, y, and z values between two locations neighboring the selected time. Step length and turning angle distributions were subsequently extracted for each observed trajectory and sampled randomly to build simulated trajectories with the same number of locations using the function *sample()*. Available habitat was constrained by the contour of the lake at the shallowest (i.e., warmer) isobath of the observed fish trajectory to account for their thermal tolerance. This constraint allowed us to match the vertical distribution of observed and simulated trajectories and thus implicitly account for a third dimension in the HSA (see *Assigning depth to simulated trajectories* section). Furthermore, all simulated trajectories started from the same initial location for a given individual. This procedure was repeated 5000 times for each of the 194 sequences to ensure a sufficient number of simulated trajectories for statistical testing (see *Conditional logistic regressions* section, supplements S4 and S5). Since the computation time is too cumbersome to be handled by a personal computer (several months), all these first steps were done with parallel operations on a multicore computer (Titan S599 with 20 cores (+40 Threads) and 96 GB of RAM; Titan Computers).

Assigning depth to simulated trajectories

To the best of our knowledge, there is no existing computation method that can explicitly integrate a third dimension of space to HSA or a Step Selection Function (Johannes Signer, Georg-August-Universität Göttingen, pers. comm.). However, to simulate random 3D trajectories, assigning a random depth could be a straightforward way of considering the third dimension in a system like ours. Due to the relatively small maximum lake depth (16 m), an adult fish should be physically able to travel the water column from surface to bottom in less than a minute (i.e., between each location; Tudorache et al. 2011, Figure S6).

However, the thermal constraints of brook charr reduce the probability of encountering the fish in the relatively warm upper layers of the water column. Similarly, the probability of encountering the fish in the cold deeper layers should also be reduced due to both the fish's thermal preference and the absence of light in hypolimnion that prevents the fish from seeing its prey. This study thus seeks to determine if fish select habitat features within the bounds of this temperature constraint. Therefore, for each location within a given simulated trajectory, a depth value was randomly sampled from the depth distribution of the corresponding observed trajectory (constrained by the maximum depth of the water column at each location). This procedure allowed us to mimic a realistic depth distribution of simulated fish by accounting for both the thermal tolerance of the observed fish and lake morphometry. Therefore, if a given fish displayed a behavior characterized by forays into the epilimnion, simulated trajectories would also show this same behavior.

Assigning physical variables to trajectories

Once we reached 5000 3D simulated trajectories for each sequence, observed and simulated locations needed to be coupled with physical variables to assess fish selectivity of the variable. Three physical variables were calculated: (i) the distance of the location from the shore on the horizontal plane (D_{sh}), as a rough measure of association with the littoral or the pelagic area, (ii) the vertical distance of the location from the bottom of the lake (D_{bo}), as a measure of access to benthic resources (i.e., bottom of the lake rather than the water column), and (iii) the deviation in the horizontal plane of the location from the 6 m isobath (D_{6m}), as a measure of the access to a thermal refuge while remaining close to littoral resources. This latter variable is the horizontal distance to the 6 m isobath, which was chosen based on summertime Lake Ledoux temperature profiles and the optimal preferred temperature of brook charr (supplement S7; Smith & Ridgway 2019). This contour represents the optimal position for fish to make forays in littoral areas while spending most of their time in cool temperature layers (Bertolo et al. 2011; Goyer et al. 2014). We expect a shift of D_{sh} selection when the thermal barrier weakens, with a preference for littoral areas (i.e., more negative selectivity) as well as a simultaneous reverse shift for the selection of D_{6m} (i.e., stronger positive selectivity), suggesting a preference for the shallow basin. We expect that the brook charr population will select areas close to the bottom within the metalimnion, especially during summer, since this would combine thermoregulation in cool areas with access to littoral resources. At the individual level, there could be less selection for D_{6m} by some fish if zooplankton aggregate in the center of the lake. Depth or temperature were not included here since the generation of simulated trajectories was already constrained to mimic the observed depth distribution, and, in turn, the thermal tolerance of brook charr.

The six zooplankton groups defined in the *Zooplankton mapping* section were also included in the analyses to express resource selectivity. For each location, we extracted the values from the relevant raster calculated for each zooplankton class (see above).

Conditional logistic regressions

For each sequence, a conditional logistic regression was computed to test whether a fish selected some of the variables (i.e., if regression estimates were different from 0; Fieberg et al. 2021). However, several pitfalls had to be avoided before extracting regression estimates. First, it is necessary to choose the right number of simulated points (Fieberg et al. 2021), which comes down to choosing the right number of simulated trajectories. Therefore, we determined the threshold of simulated locations needed to stabilize estimates following Fieberg et al. 2021. Our preliminary analysis showed that 150 trajectories were sufficient to obtain a robust regression estimate (see supplement S4 for details). It is then important to weight the simulated points relative to the observed ones (5000 to 1, respectively) to ensure that the regression will converge (Fithian & Hastie 2013, Fieberg et al. 2021). Secondly, it is important to correct for the overly large number of samples to avoid meaningless p-values in the regression analysis (i.e., always significant due to extremely large sample size). Therefore, we compared the regression estimates for each sequence and variable to a distribution of regression estimates (hereafter H0 distribution) calculated using a reference simulated trajectory versus other simulated trajectories (hereafter “null estimates”). This mimics the theoretical distribution of regression estimates when no selection has occurred (see example Figure 2).

We built the H0 distribution as follows. For each sequence and each variable, null estimates were calculated by repeating a regression with one randomly picked simulated trajectory among the 5000 as the reference and another 150 simulated trajectories among the 4999 left (see example Figure 2). We determined that 500 null estimates were enough to build an H0 distribution of null estimates from a conditional logistic regression without any further variations in the 2.5/97.5% quantile range (see supplement S5). Once the distribution of null estimates was built, the 2.5% and 97.5% quantiles were extracted. If the estimate from the regression comparing the observed trajectory with simulated ones fell outside these boundaries, it was considered to be different from zero, meaning there was active selection (positive or negative) of the corresponding variable by the fish. Several weeks of computing time were necessary to handle all the sequences.

Analysis of HSA results

Once all HSA estimates were available for each sequence, we looked at positive and negative selectivity occurrences (regression estimates higher and lower than 0, respectively) among fish and throughout the sampling period. For the interpretation of these results, we should keep in mind that a positive estimate for distance variables (D_{bo} , D_{sh} , and D_{6m}) means that fish selected areas farther from the reference (i.e., bottom, shore, and 6 m isobath, respectively). In contrast, a negative estimate means that fish selected areas close to the reference. A PCA was built to determine which selectivity estimates covaried among the variables tested (package FactoMineR); this helped us to understand the possible tradeoffs made by brook charr during the season. For resource variables, a positive selection is interpreted as the selection of a food patch, whereas the negative selection of a zooplankton category is probably due to a spurious correlation because of the selection of another variable by fish that is negatively correlated with a given zooplankton category (i.e., avoidance of a food patch that seems to have no sense ecologically). The selection of zooplankton-poor areas is possibly an index of selection for potentially unmeasured physical variables or for other zooplankton categories. All data on the density of the different zooplankton categories from the mapping were used to build a PCA to assess whether zooplankton categories were distributed randomly or homogeneously in the lake. This helped us to better grasp the meaning of negative selection estimates for resource variables.

Results

Thermal constraint in littoral–pelagic habitat use

During the sampling period, Lake Ledoux exhibited contrasting thermal conditions relative to fish thermal tolerance. From the beginning of sampling on 23 July to 4 September, epilimnion temperatures were above the 22°C upper optimal threshold for the species, thus preventing brook charr from spending a long time foraging in the surface layer or the shallow basin (Figure 3 and supplement S8). The mean depth of fish locations recorded was 5–6 m until 10 September (i.e., when fish began to enter shallower waters following the weakening of the thermal barrier), and up to 1–2 m in October. Furthermore, from 6 August to 4 September, no fish were recorded in the shallow basin or in littoral areas (depths < 3 m) whereas more than 30% of their locations occurred in the shallow basin in October; this value rose to 60% when all shallow areas were considered (Figure 3).

Zooplankton spatial distribution and associations between resource and physical variables

The PCA based on zooplankton categories showed that zooplankton variations in the lake were heterogeneous (Figure 4A). This analysis revealed the covariation of two zooplankton groups, OC and LOC. The first two axes together explained more than 80% of the variation in the data, with all zooplankton categories correlating with the positive part of the first axis (61%), suggesting that, overall, all zooplankton groups followed the same pattern of abundance distribution at the lake level. Furthermore, the large (together with *Holopedium* and OLZ) and small (together with OC and LOC) zooplankton groups were correlated with the negative and the positive part of the second axis, respectively (21%). This result suggests that the fish–zooplankton coupling patterns can be elucidated by considering only one of the zooplankton categories we determined. As such, we will hereafter mainly present results from the large and small category, since this is the only pair that includes the whole community. Fish coupling with the *Holopedium*/OC and OLZ/LOC pairs will be discussed when appropriate.

HSA results showed that the coefficients for variables associated with fish trajectories showed some degree of covariation among them. As expected considering the PCA on zooplankton concentrations (Figure 4A), resource variable coefficients showed a clear pattern of correlation, forming two opposed groups: large zooplankton, *Holopedium*, and OLZ were on one side, and small zooplankton, OC, and LOC on the other side (Figure 4B). On the first two axes of the PCA, which together explained more than 50% of the variation in the data, zooplankton variables were mostly correlated with the first axis (and partly with the positive part of the second), whereas the physical variables correlated mostly with the second axis. More specifically, the selection of large zooplankton correlated with the positive part of both the first and second PCA axis, whereas selection of small zooplankton correlated mostly with the negative part of the first axis (Figure 4B).

Habitat selectivity

HSA showed nonrandom associations with different habitat and resource variables for several individuals during the sampling period (Table 1; supplement S9). The stronger selection pattern involved habitat variables whose variances were more explained by the second PCA axis (Figure 4B). Among all the fish trajectories examined, 72% exhibited nonrandom selection for at least one habitat variable, especially D_{bo} . This variable was selected by fish 51% of the time (positive or negative selection), There was a prevalence for negative selection (87%), suggesting a preference for the bottom of the water column. A

similar pattern for D_{6m} showed that almost a third of fish trajectories were bound to this isobath. No clear pattern emerged from HSA for D_{sh} , with no selection in 75% of cases and equal numbers of positive and negative selection.

Across the season, individual fish behaviors were rather irregular, with selected variables varying in both nature and direction (Figure 5). First, among the individuals showing a non-random association with some variables, most preferred to be near the 6 m isobath during summer (i.e., from the start of sampling on 23 July through 10 September). When the thermal barrier disappeared in the fall, this preference was inverted, with an increased proportion of individuals selecting areas far from the 6 m isobath (Figure 5A). Negative selection was found more frequently for the distance to the bottom of the water column, with relatively more fish being closer to the bottom in autumn compared to summer (55 and 40% of individuals selected the D_{bo} , respectively; Figure 5C). Finally, no clear temporal pattern emerged when comparing the selectivity of fish movements according to the distance to the shore (Figure 5B).

Fish–zooplankton coupling

The selection pattern was less clear-cut regarding the coupling between brook charr and potential zooplankton prey, with 11 to 19% of fish trajectories exhibiting a selection, depending on the zooplankton category. However, when we consider the coupling with the overall zooplankton (i.e., large and small zooplankton combined), 35% of fish trajectories showed active selection of zooplankton patches (small and/or large individuals; Table 1). Unexpectedly, there was almost no difference in the overall coupling between fish and small or large zooplankton (Table 1), but brook charr showed interindividual variability in zooplankton selection behavior (Figure S10). For the time period analyzed, there was a clear gradient ranging from individual fish that never selected zooplankton areas to fish that selected patches with large and/or small zooplankton 80% of the time. Most individuals fed indiscriminately on zooplankton prey during the study period, with no individual being specialized in only one zooplankton category (Figure S11). However, some individuals seemed to show a preference for one category (e.g., fish 56 followed at least one zooplankton category for eight weeks out of 11, especially LOC for five weeks; Figure S11).

In addition to the interindividual variability of zooplankton preference, there were also temporal differences (Figure 5D-E) that ranged from 12% of fish selecting large and/or small zooplankton on 27 August to 56% on 8 October (Figure S12). The proportion of fish selecting zooplankton increased on 4

September, just before the thermal barrier disappeared. The proportion of fish selecting zooplankton-rich areas before and after this week differed significantly (Wilcoxon test, $p = 0.02$). No clear pattern emerged between fish and each zooplankton category when data were visualized graphically (Figure S13). However, some weeks were characterized by a stronger coupling between individual fish and a specific zooplankton category (e.g., on 20 August, almost half of the individuals selected *Holopedium*-rich areas).

Considered individually, none of the resource or physical variables seemed to be associated with a large number of individual fish during the study period. However, when focusing on the overall selectivity of each fish trajectory, it appears that most fish selected at least one variable and only 14% of the trajectories were not associated with any of the variables considered in the HSA (Figure S14).

Discussion

This study shows how temperature and interindividual variation impacted littoral–pelagic habitat coupling in a stenothermic fish species from summer to fall. By matching the resolution of zooplankton sampling with fish monitoring, we were able to show a temporal variation in habitat or resource selection in the brook charr population of a boreal lake. On one hand, brook charr often selected physical variables that suggested a preference for the bottom of the water column, near the 6 m isobath, especially during summer. Brook charr behavior changed when the thermal barrier disappeared in September, with a shift toward shallow waters. On the other hand, we found a large variability in the selectivity of individual fish to the zooplankton categories, from no selection to a quasi-constant selection. Moreover, fish–zooplankton coupling increased with the disappearance of the thermal barrier.

Selection of habitat variables

Since we accounted for brook charr thermal tolerance when building the HSA, we did not explicitly verify the selectivity of fish for depth (which is related to temperature in a nonlinear way). However, our telemetry data clearly showed that fish preferred the metalimnion when water temperatures of the epilimnion and littoral areas exceeded 20°C, confirming results from previous studies (Figure 3; Bourke et al. 1996, Bertolo et al. 2011, Goyer et al. 2014).

Among the three variables chosen to describe habitat selection by brook charr, distance to the shore was rarely selected by the individuals. We could have expected a positive selection of this variable

during summer because fish avoided the too-warm littoral areas (Bourke et al. 1996; Bertolo et al. 2011; Goyer et al. 2014). However, since we controlled the HSA with the thermal tolerance of brook charr (by bounding the depth distribution of simulated trajectories), it was expected that the analysis on selection of the distance to the shore would not reveal clear fish avoidance of littoral areas when temperatures were too high. Nevertheless, some individuals still exhibited positive or negative selection for the distance to the shore. This result can be interpreted as an aggregation of fish at the lake's center (positive selection) or close to a thermal refuge near the littoral area (negative selection), as suggested by the covariation of D_{sh} and D_{6m} . The results were particularly clear for the latter variable, showing more negative than positive selection during summer, thus depicting a preference of individuals for areas close to the 6 m isobath. These individuals were likely selecting areas close to littoral areas rich in benthic invertebrates while remaining in relatively cool waters (Bourke et al. 1997, Bertolo et al. 2011, Goyer et al. 2014). This allowed individuals to make quick excursions into littoral areas (Figures 3, supplement S8), probably to feed on benthic invertebrates. Fish that did not actively select distance to the 6 m isobath (D_{6m}) might have preferred more pelagic habitats and focused on zooplankton prey. However, the HSA results did not show a covariation between D_{6m} and resource selection. This could suggest more complex prey–predator interactions between fish and zooplankton in boreal lakes, where pelagic zooplankton could remain relatively inaccessible to cold-stenothermic predators during the summer by staying in the epilimnion (see Figure S11 in Leroux et al. 2022). This contradicts the current zooplankton migration paradigm (Hays 2003), with a different migration strategy in boreal lakes, where zooplankton would be in the epilimnion during the day, relatively free from visual predators, even if there are better light conditions for predation.

When the thermal barrier weakened and disappeared, active selection for distance to the 6 m isobath (D_{6m}) switched from negative to positive. This suggests a greater tendency for individuals to enter previously inaccessible littoral areas and the shallow basin when surface temperature cooled, rather than a simple preference for locations far from the 6 m isobath. This preference for littoral habitats is coupled with an increased preference for the bottom of the water column (D_{bo}), presumably to feed on benthic invertebrates (Magnan 1988, Bourke et al. 1999).

Selection of resource variables

A third of the fish trajectories showed a coupling with zooplankton (large or small) during the study period, meaning that most individuals were not coupled with zooplanktonic prey in Lake Ledoux. Considering the relative profitability of resources, we would expect that brook charr would select

zooplankton patches: these are the main prey available during summer because littoral invertebrates are relatively inaccessible due to the thermal barrier. Nevertheless, this result agrees with the observation that brook charr exhibit a subtle resource polymorphism in different systems (including Lake Ledoux), where a littoral ecotype feeds mainly on zoobenthos and a pelagic ecotype feeds mostly on zooplankton (Bourke et al. 1997, 1999, Dynes et al. 1999, Rainville et al. 2021). While these previous studies were based on stomach content analyses, our results confirmed that some individuals were coupled with zooplankton patches on a long-term basis compared to others that were never coupled with zooplankton patches. However, it was unexpected that no fish selected zooplankton on every sample dates. The zooplankton categories defined in this study help to explain this result. Almost two-thirds of fish trajectories were related to at least one zooplankton category. In future studies, increasing the taxonomic resolution of zooplankton categories could allow an improved assessment of fish selectivity on specific zooplankton groups. This is particularly relevant considering how planktivorous fish can rapidly shift targets depending on prey densities and conspicuousness (Gliwicz 2002). Furthermore, regardless of the brook charr resource polymorphism, it has been shown that most individuals used both resources at some period of the year (based on stable isotopes; Rainville et al. 2021). This could explain why individuals selecting zooplankton patches most of the time were not selective during some periods. Finally, our study only considered the 4h crepuscular period. While brook charr are expected to feed more actively during this period (Bourke et al. 1996), it is possible that some individuals were feeding at different times and using different thermal strategies, which would explain the apparent lack of zooplankton selection by most individuals (Goyer et al. 2014).

Our results clearly show an increase in fish–zooplankton coupling as soon as the thermal constraint weakened, with a five-fold increase between 27 August and 04 September. This result goes against our hypothesis that brook charr preferred zooplankton in summer and zoobenthos in autumn. It rather suggests that individuals increased their search for zooplanktonic food that had found refuge in the epilimnion, which is not inaccessible to fish during summer, constituting what could be called “*the boreal anomaly*”. However, the following decrease in fish–zooplankton coupling in September is confusing but could be explained by the trophic polymorphism of brook charr and the propensity of littoral fish to focus on benthic invertebrates rather than zooplankton when they can access the shallow basin. Movement analyses using state–space models could help validate this interpretation by linking habitat used with changes in fish behavior, from resting to active searching (e.g., Jonsen et al. 2006, Gurarie et al. 2016).

Finally, our results failed to demonstrate a stronger selection by brook charr for more conspicuous prey such as large profitable zooplankters (e.g., *Daphnia* sp.). Despite the gelatinous capsule, which make *H. glacialis* less beneficial for fish because their stomach would be filled with relatively diluted nutritive matter (Detmer et al. 2017), some individuals selected *H. glacialis* in proportions similar to or greater than *Daphnia* sp. (i.e., OC). Furthermore, small zooplankton were more abundant than large ones, suggesting that both categories might be perceived as equally abundant by this visual predator, as proposed in previous work on reaction distance (Gliwicz 2002). It appears that brook charr prey selection relies not only on conspicuousness but also on prey density, with a selection of less valuable prey when they are sufficiently numerous (Gliwicz 2002).

Conclusion

Habitat Selection Analysis and Step Selection Function are gaining interest in animal ecology since the development of high frequency tracking devices (Thurfjell et al. 2014, Fieberg et al. 2021). However, it is still difficult to couple telemetry data with spatio-temporal variations in potential prey like mobile microorganisms. To the best of our knowledge, this study is the first to have such a fine spatio-temporal scale for two different trophic levels—the food resources of the tracked fish species. In addition to revealing some specificities of boreal lakes (e.g., the importance of thermal barriers in fish–zooplankton coupling), we showed the complex interplay between habitat availability, fish–zooplankton coupling, and the expected resulting interindividual preferences in a fish population. This fine resolution, which was not possible even a few years ago, enables a better understanding of ecosystem functioning and is required for studies considering the changes occurring in the biosphere. The brook charr population is almost entirely cut off from the littoral area during summer, so coupling between pelagic and littoral ecosystems via mobile predators ceases until the thermal barrier disappears (e.g., mid-September in Lake Ledoux); this same phenomenon was also observed in lake trout (*Salvelinus namaycush*) (Guzzo et al. 2017). In the context of global change, summers could last longer, depriving cold-stenothermic fish species of benthic resources and preventing coupling between the littoral and pelagic habitats for a longer period of time, with unknown consequences for system functioning.

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Data availability statement

The datasets generated during the current study are available from the corresponding author on reasonable request.

Conflict of interest

The authors declare that they have no conflicts of interest.

Ethical approval

All applicable institutional or national guidelines for the care and use of animals were followed. This protocol was approved by the Animal Care Committee at the Université du Québec à Trois-Rivières.

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Tables

Table 1: Number of trajectories where Resource Selection Function showed a positive and significant, negative and significant, or non-significant estimate for each tested variable. D_{bo} : Distance to the bottom, D_{sh} : Distance to the shore, D_{6m} : Distance to the 6 m isobath, Large: zooplankton ≥ 1 mm, Small: zooplankton < 1 mm.

Parameter	Selectivity		
	Positive	Negative	Neutral
Habitat			
D_{bo}	13	86	95
D_{sh}	25	23	146
D_{6m}	19	39	136
Resource			
Large	36	30	128
Small	31	33	130

Figures

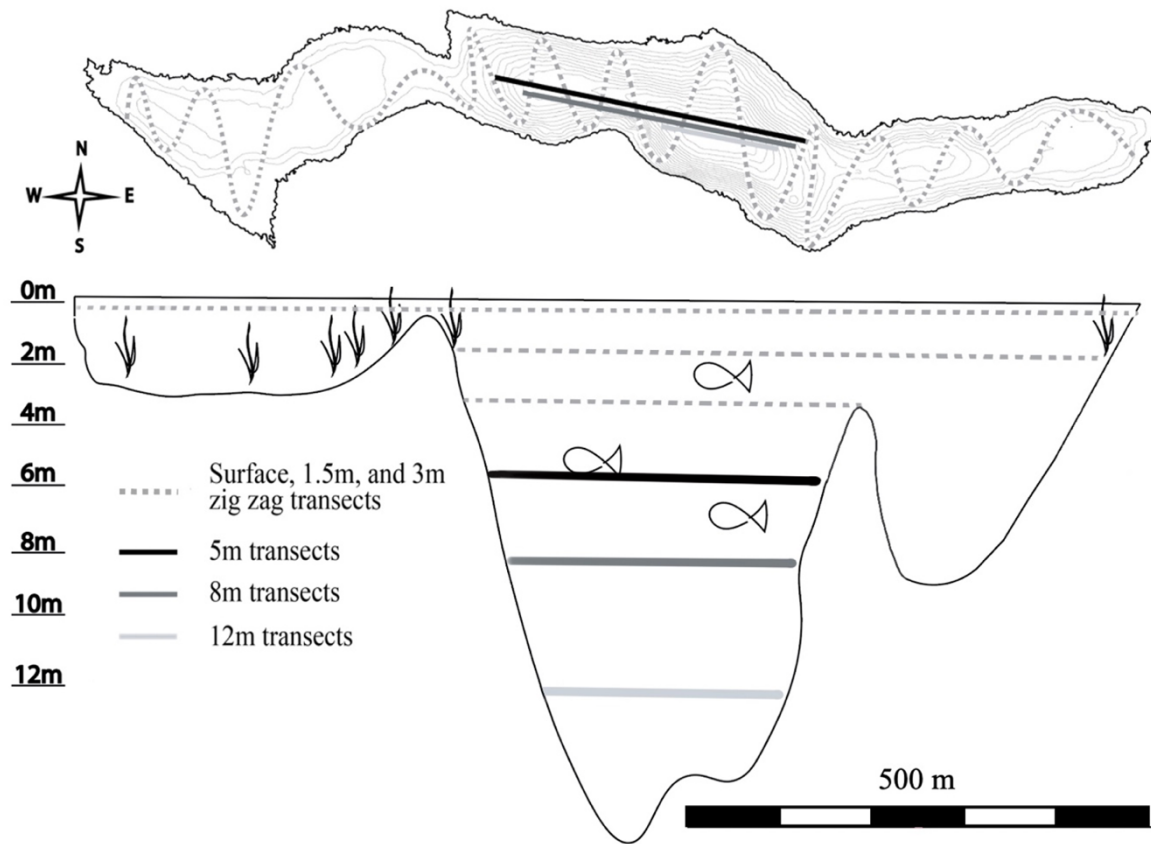
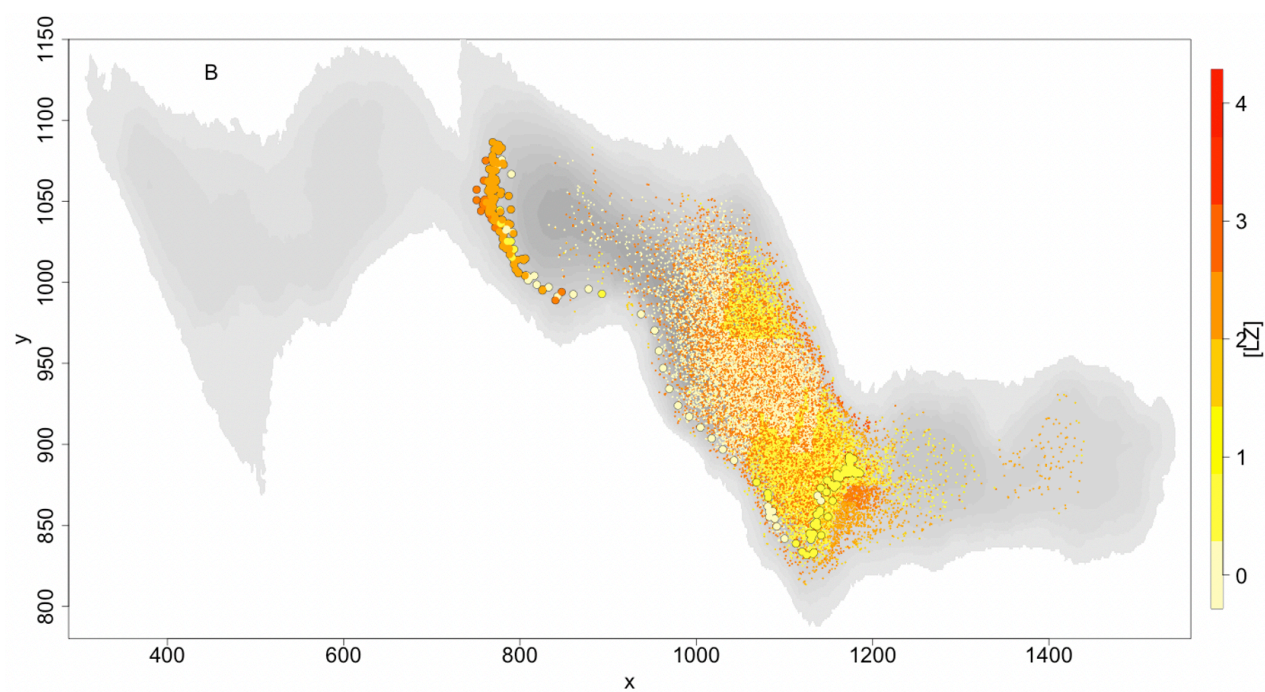
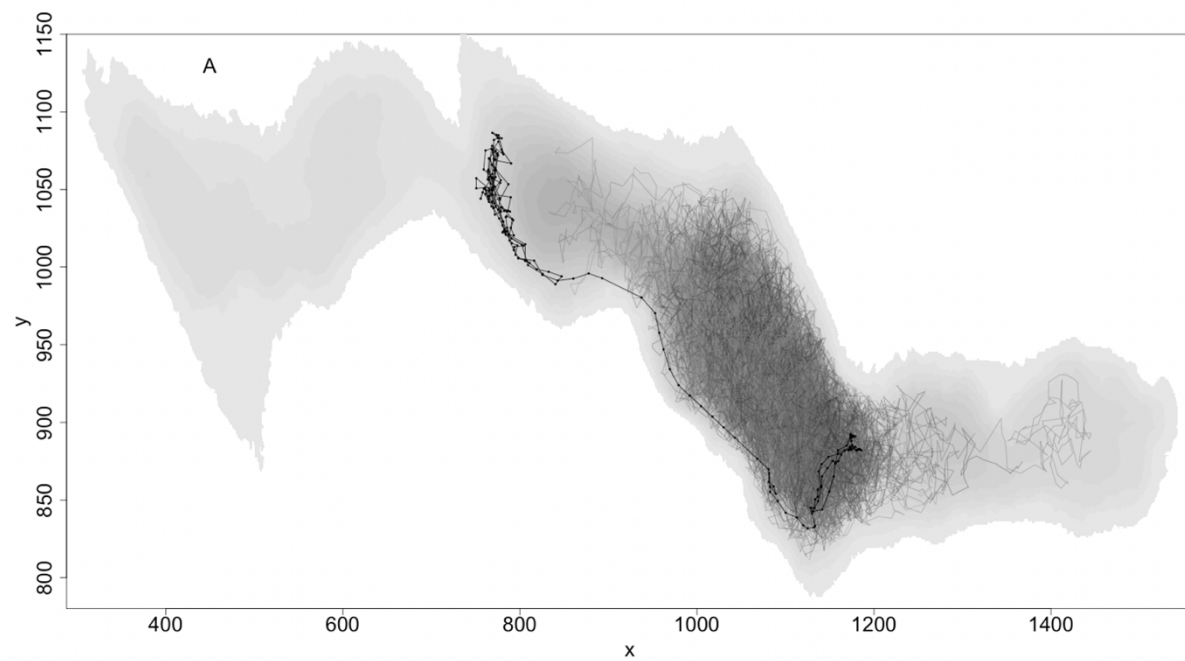


Figure 1: Top (upper) and transversal (lower) views of Lake Ledoux showing the zooplankton sampling track with the Underwater Vision Profiler. Isobaths (upper) denote 1 m intervals.



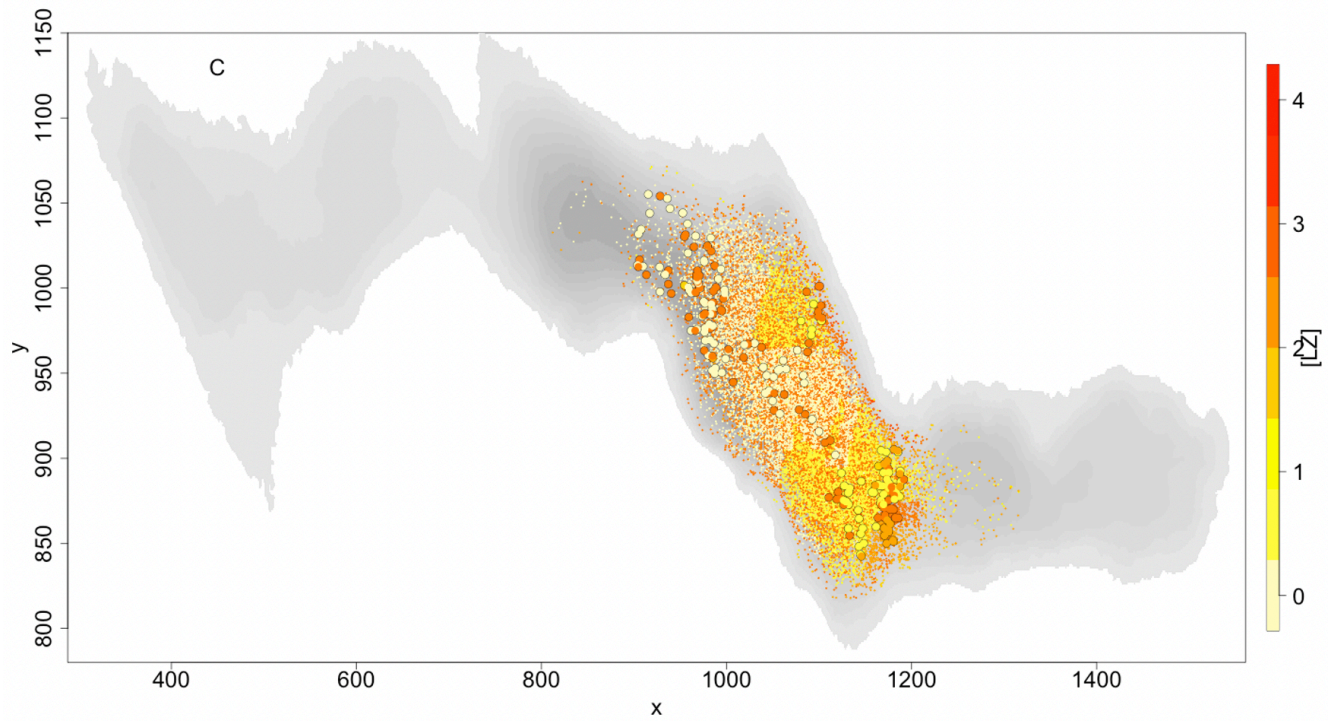


Figure 2: A: The observed trajectory of fish n°72 on 6 August 2018 (black) used to simulate the 5000 trajectories for the Habitat Selection Analysis (HSA) analysis (grey lines). B: The 150 simulated trajectories (small points) used to get the HSA estimates for the observed trajectory (large points). C: A hypothetical observed trajectory (large points) was selected among the 5000 simulated ones. This trajectory was compared to a random selection of 150 simulated trajectories (small points) among the 4999 remaining. This comparison was repeated 500 times to build the H0 hypothesis. Colors in B and C represent the corresponding concentration of the large zooplankton category for each location ($\text{mm}^3/\text{unit volume}$). The x and y axes represent the reference coordinates (m).

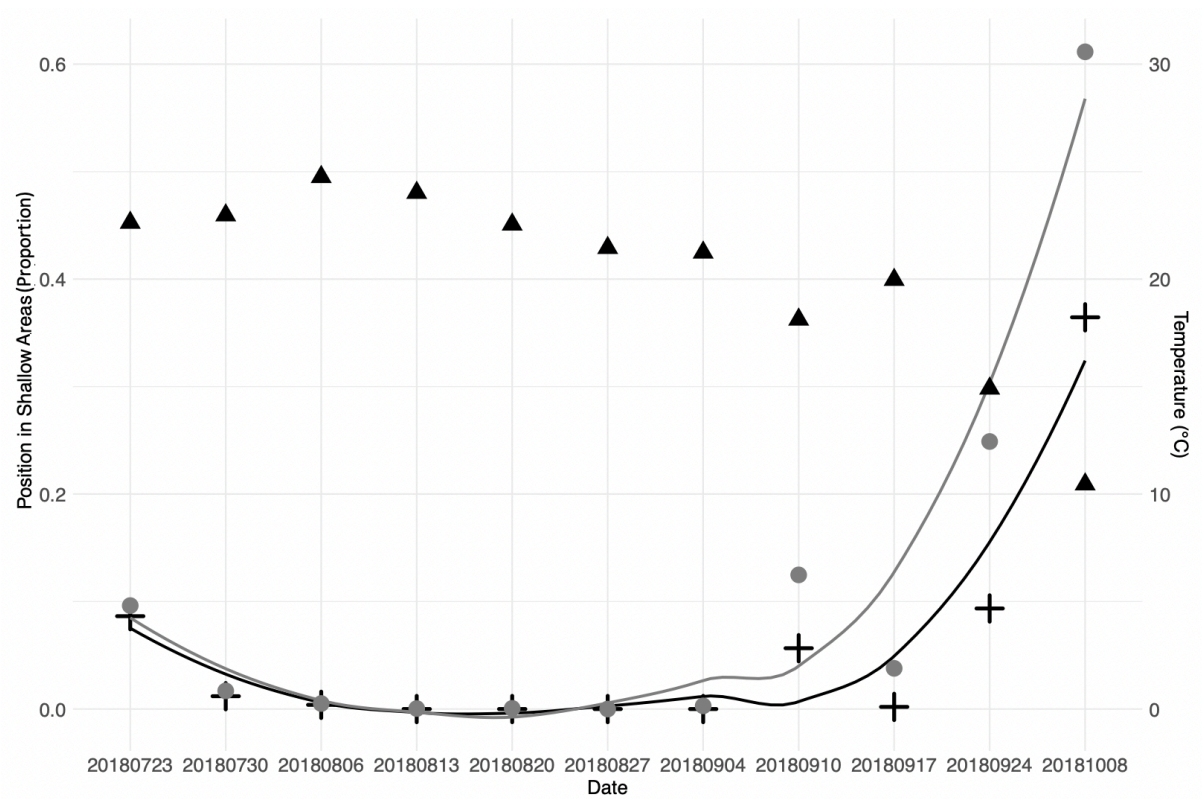


Figure 3: Proportion of all positions recorded in the shallow western basin (black crosses) or in shallow areas (i.e., water column depth < 3 m; gray dots). The surface temperature for each date is represented by triangles.

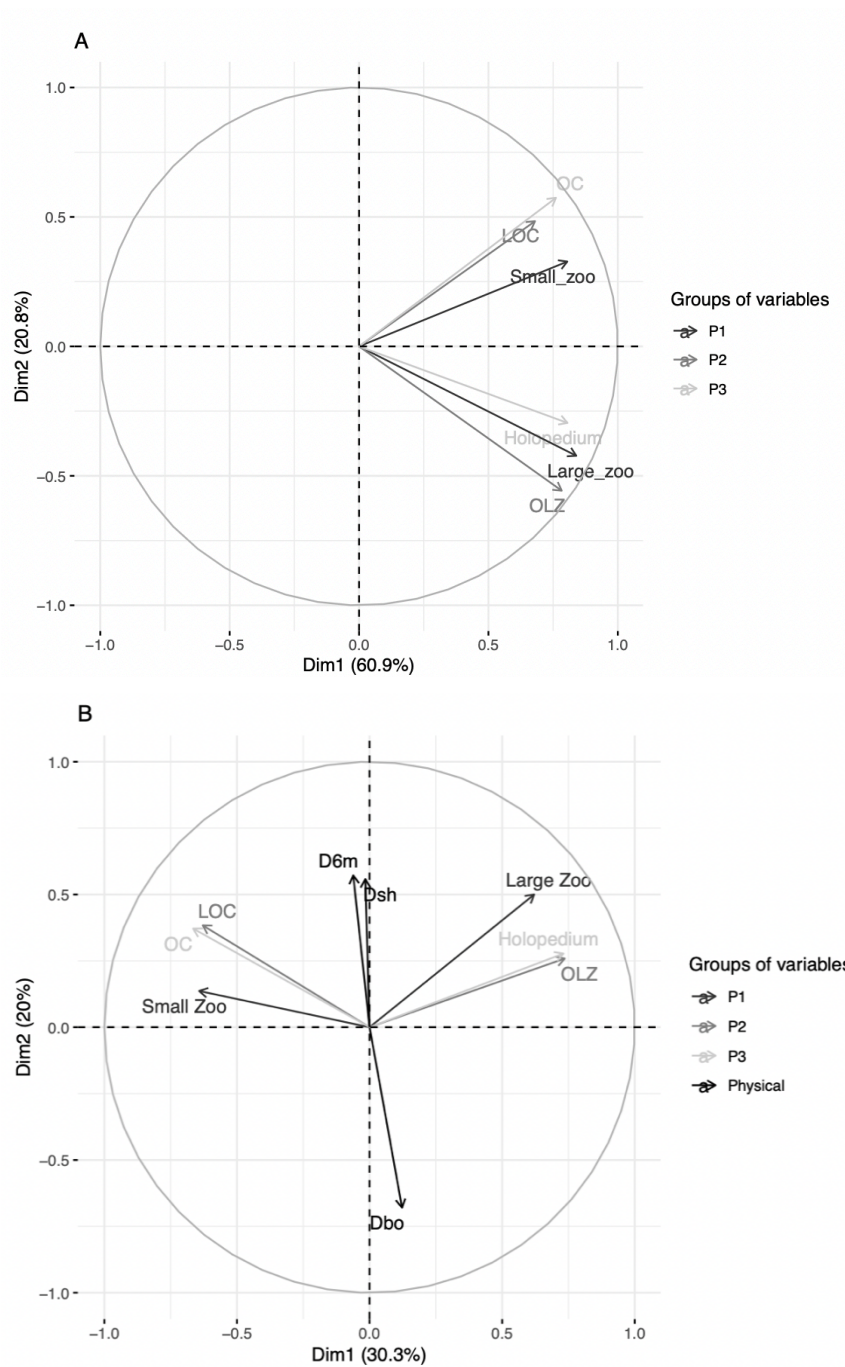


Figure 4: A: PCA based on the zooplankton concentrations in Lake Ledoux, showing covariations of the different zooplankton categories. B: PCA based on Habitat Selection Analysis estimates for habitat and resource variables, showing covariations of selection estimates for these variables. P1, P2 and P3 represent the three categories defined in the text. P1: small and large zooplankton (Small_zoo and Large_zoo resp.); P2: large other cladocerans and other large zooplankters (LOC and OLZ resp.); P3: other cladocerans (OC) and Holopedium.

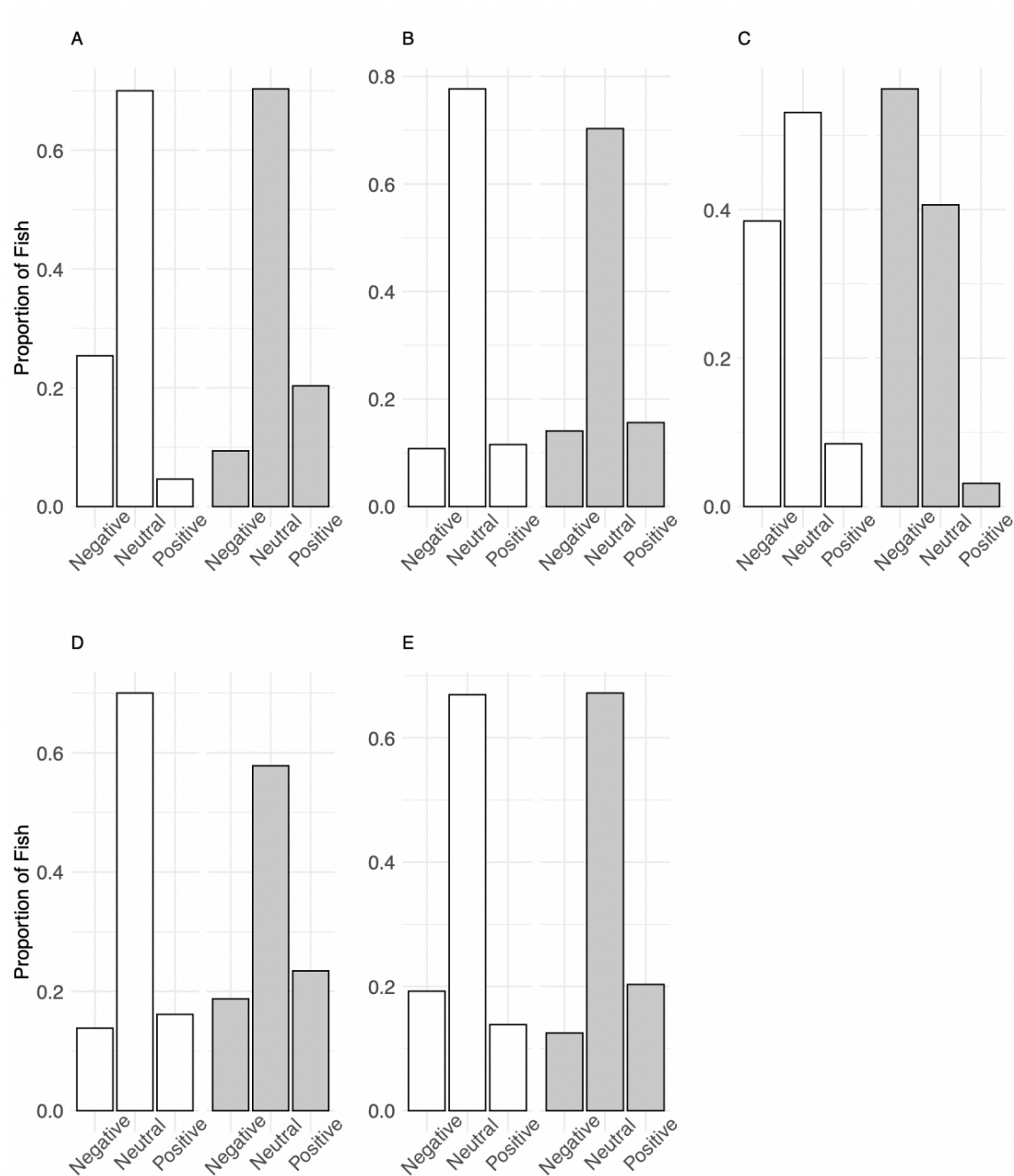
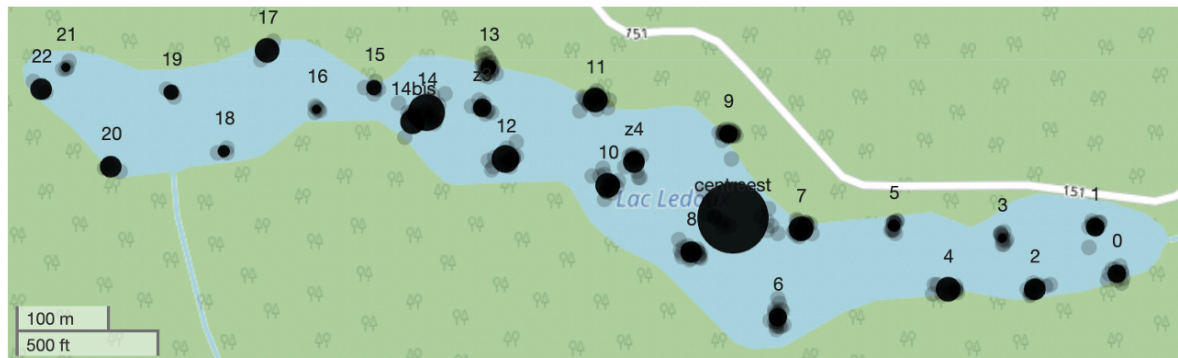


Figure 5: Distribution of the number of non-random negative, non-significant, and non-random positive coefficients calculated among the 194 trajectories according to the period of the season (white: before 10 September; grey: 10 September and later). Distribution coefficients of the selection for (A) distance to the 6 m isobath, (B) distance to the shore, (C) distance to the bottom of the water column, (D) large zooplankton, and (E) small zooplankton.

Supplementary

S1: Key-point positions for each transect (from 0 to 22; pale grey circles) and their respective average positions (black circles). The size of the black circles represents the standard deviation of key-point positions for a given number.



S2: Parameters chosen for the 3D Empirical Bayesian kriging (EBK3D).

Layer Properties: EBK_Small

General
Metadata
Source
Elevation
Cache
Summary

Input datasets

Dataset D:\Recherche\Riwan\Ledoux_Oct2021
\\Ledoux_Oct2021.gdb\DataRiwan_UTM

Type Feature Class
Elevation field LedouxDepth
Data field 1 small
Coincident Point Method Use Mean
Records 7844

Method EmpiricalBayesianKriging3D

Output type Prediction
Transformation Type None
Semivariogram Model Type Power
Subset Size 100
Overlap Factor 1
Number of Simulations 100
Elevation Inflation Factor 1,000

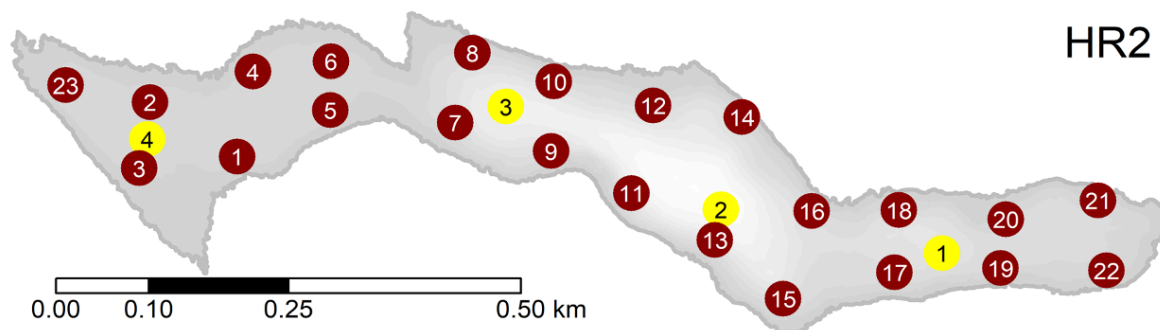
Searching neighborhood Standard 3D

Neighbors to include 2
Include at least 1
Sector type 12 Sectors (Dodecahedron)
Major semiaxis 163.567412672843

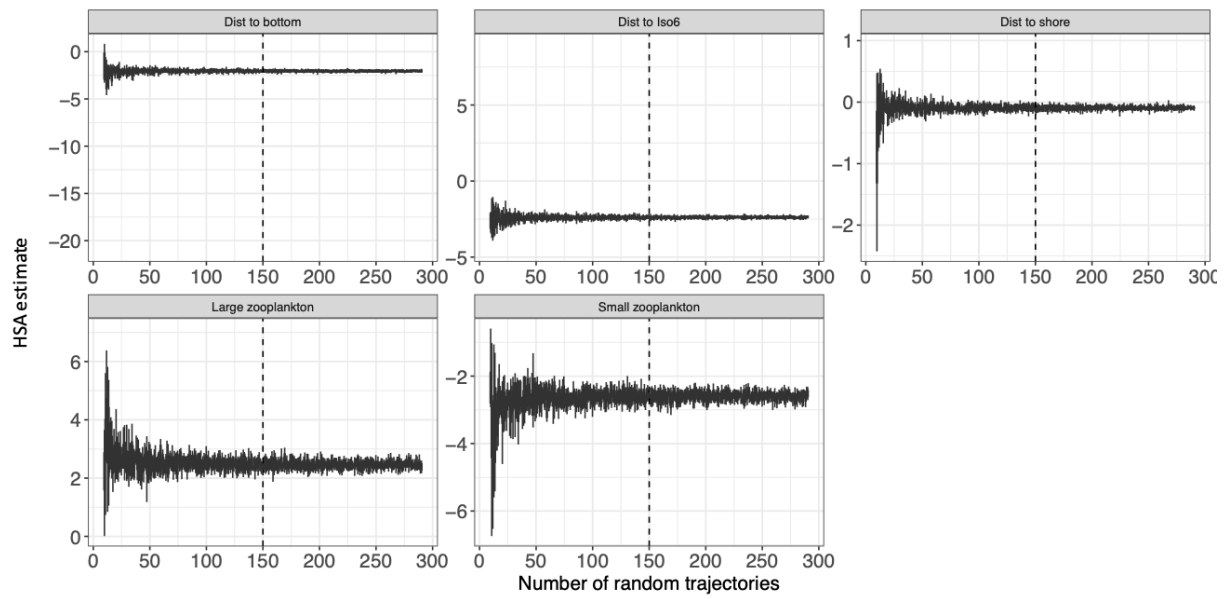
OK Cancel

S3: Brook charr hydroacoustic survey protocol and hydrophone positions on the map.

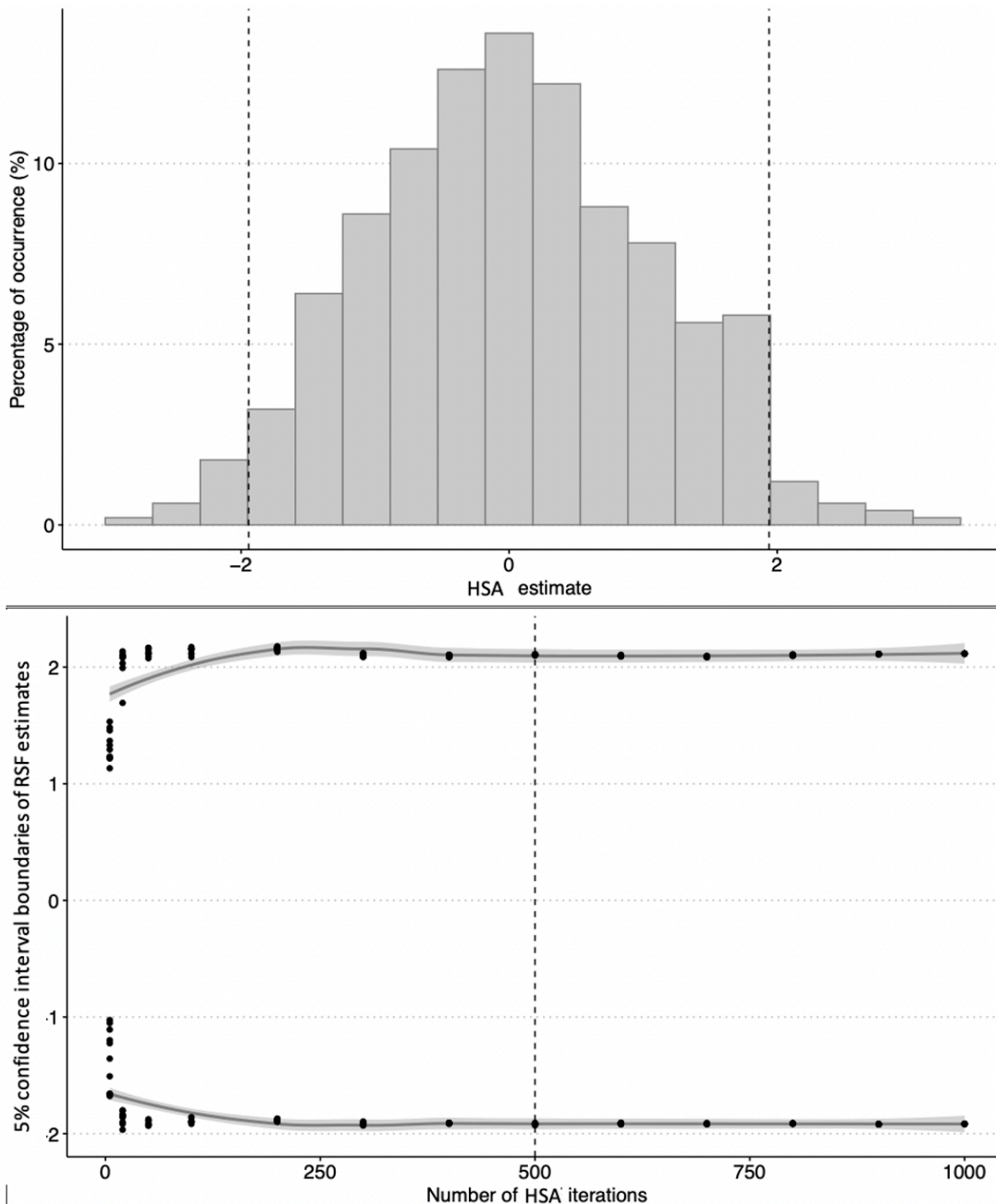
From 07 to 08 June 2017, we deployed 23 receivers (HR2-180k-100; VEMCO inc., Halifax, see figure below). The receivers were attached to a floating line (3/8" multifilament polypropylene rope) with four to five Tie-Wrap cables (14.6" x 0.30" Type 21S) and anchored to a 50 lbs patio deck bloc equipped with a 5/16 galvanised swivel to ensure a free rotation of the floating line. The floating line were attached to a buoy (7 lbs weight, 3 200 grs buoyancy; PESCA N-90/8A) with a stainless locking safety snap SS. Two welded stainless-steel ring were used, one to attach the floating line to the buoy with the carabiner and one stared at the swivel of the anchor to allow the rope to slide. The setup allowed us to retrieve the receiver without moving the anchor that stayed in place during the whole study period. The buoy was installed below the water surface to avoid boat collision and best maintain the receiver in vertical position. The geographic coordinates of the receivers and reference tags were given by the company to obtain a full coverage of the lake. The water depth of the receivers was chosen to represent the water depth mainly used by fish. The built-in-transmitters of the receivers were activated in Sync Tag mode with very high-power level following the recommendations of the company to ensure high detection and synchronisation among the receivers. On 21 June 2017, we installed four reference tags (V9TP-2x-180k-xxxm; VEMCO inc., Halifax) to complete the VPS. This full-scale research study was the first to test this tag in a natural system. In both setups, power level was high.



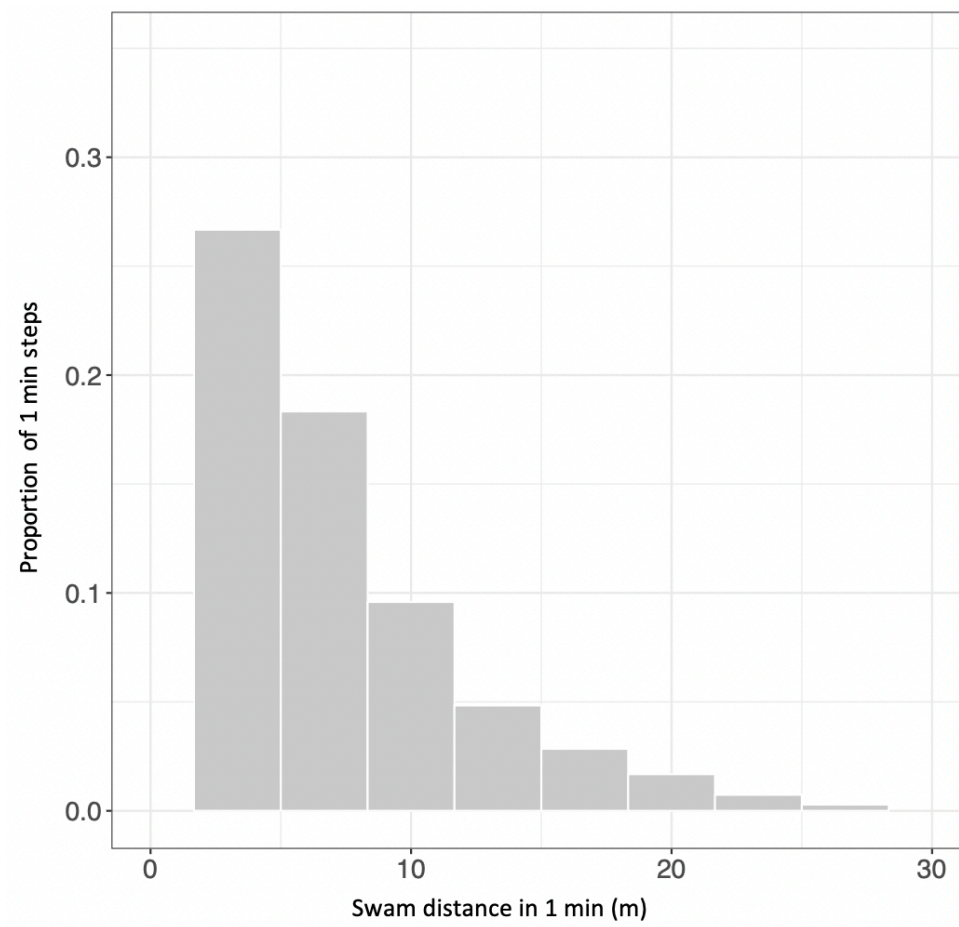
S4: Example of Habitat Selection Analysis (HSA) estimates for one trajectory according to the number of simulated trajectories used in the calculations. The dashed line represents 150 simulated trajectories compared with the observed trajectory.



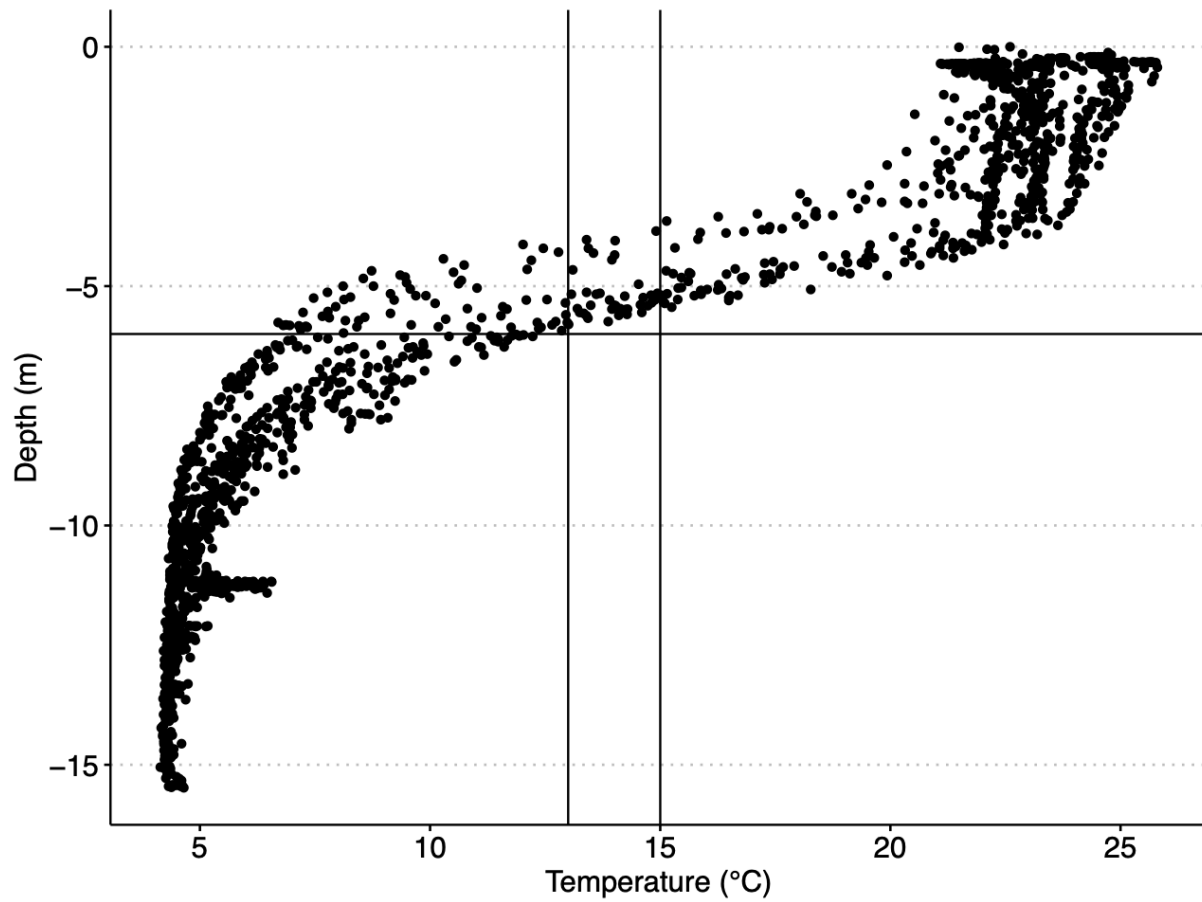
S5: Example of Habitat Selection Analysis (HSA) estimates for one burst calculated by comparing simulated trajectories with one reference simulated trajectory (i.e., H0 estimate). Top: distribution of HSA estimates for one variable when it is compared to 500 chosen reference simulated trajectories with 150 other simulated trajectories. The dashed vertical lines represent the 2.5–97.5 quantiles of the H0 estimates distribution. Bottom: evolution of the 2.5–97.5 quantiles of H0 estimates distribution according to the number of iterations.



S6: Histogram of step lengths for the observed trajectories. The X axis was reduced to [0;30], but some steps had greater lengths (50 m or more).



S7: Temperatures profiles of Lake Ledoux from 23 July to early September 2018. The horizontal black line represents the 6 m depth and vertical black lines represent the 13–15°C interval of optimal temperature for brook charr determined by Smith & Ridgway (2019).



S8: Boxplot of (A) the depth of the water column and (B) depth recorded for the all the population locations according to the date of sampling. Colors represents different individuals.

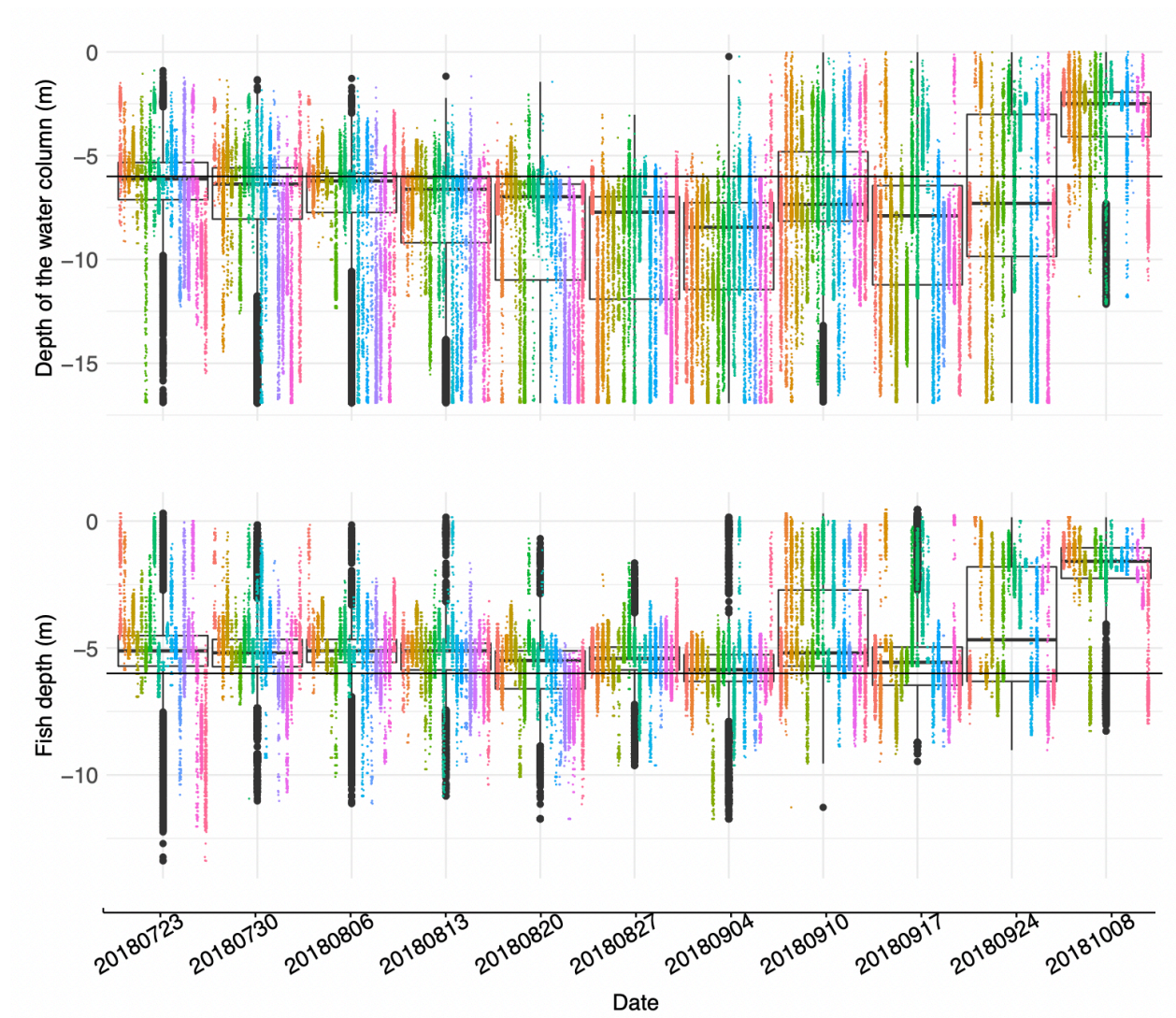
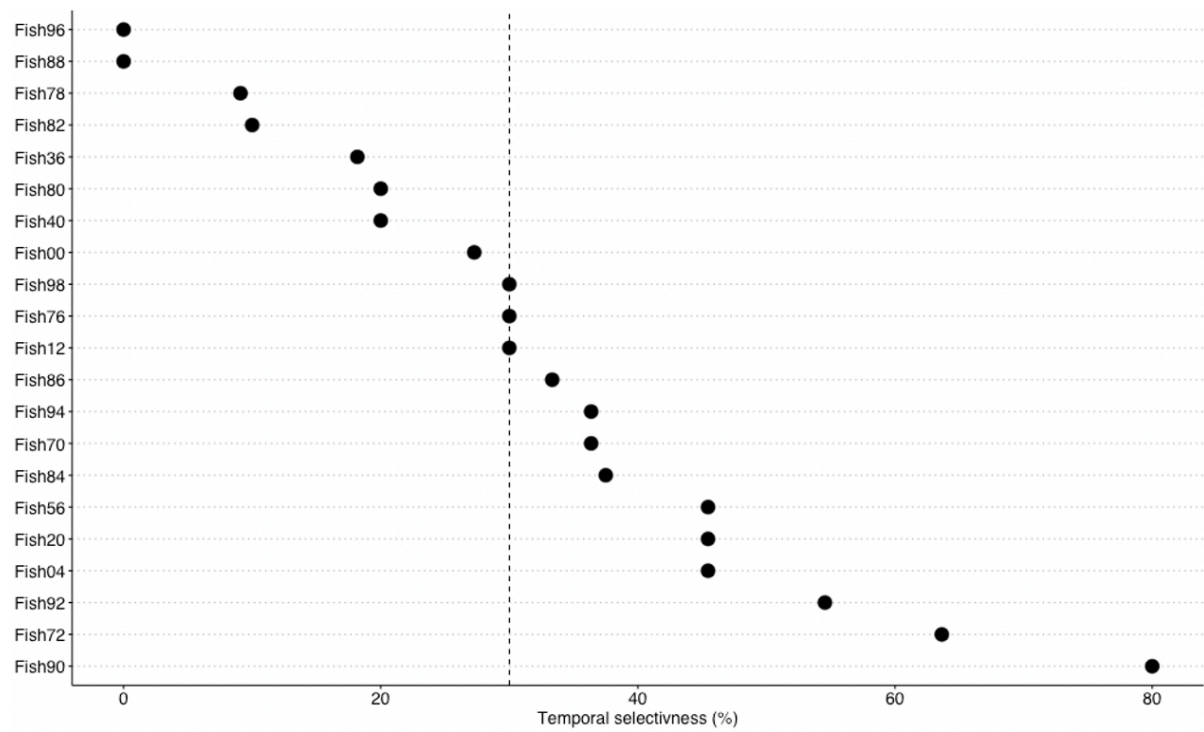


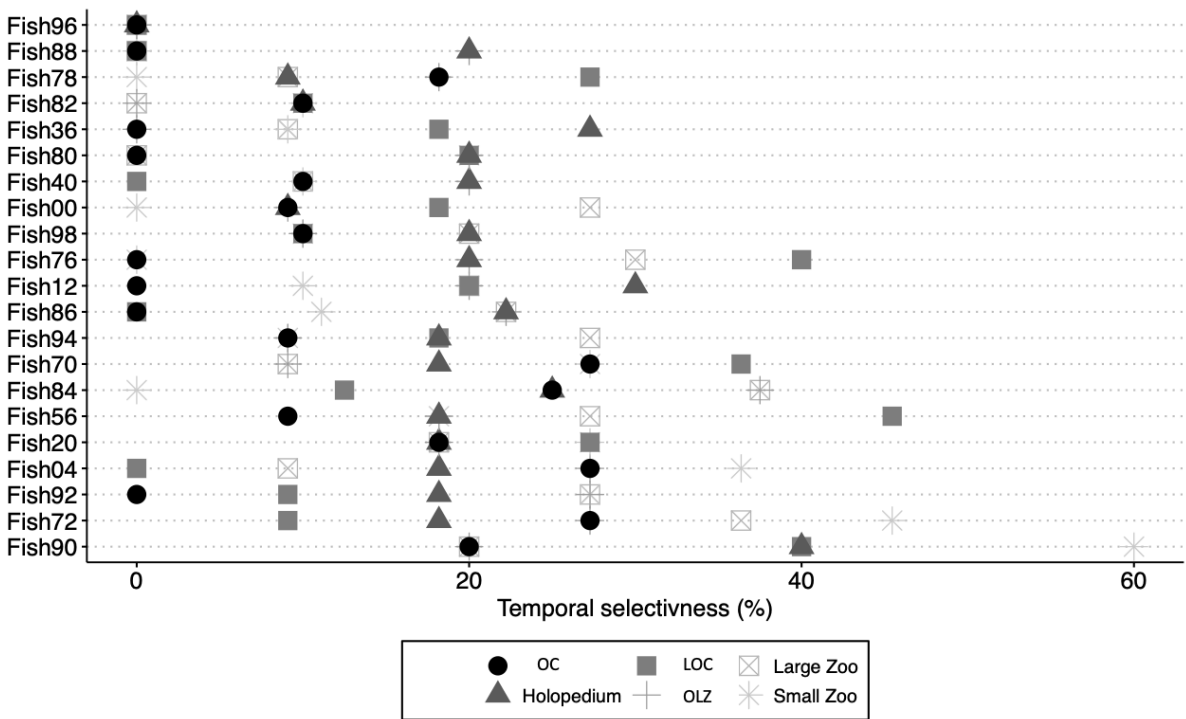
Table S9: Number of trajectories where Habitat Selection Analysis showed a positive and significant, negative and significant, or non-significant (i.e., “Neutral”) estimate for each tested variable. LOC: large other cladocerans (cladocerans other than *Holopedium* > 1 mm), OLZ: other large zooplankters (all zooplankton taxa > 1 mm excluding LOC), OC: other cladocerans (cladocerans other than *Holopedium*).

Parameter	Selectivity		
	Positive	Negative	Neutral
Resource			
LOC	35	33	126
OLZ	33	29	132
Holopedium	37	27	130
OC	22	43	129

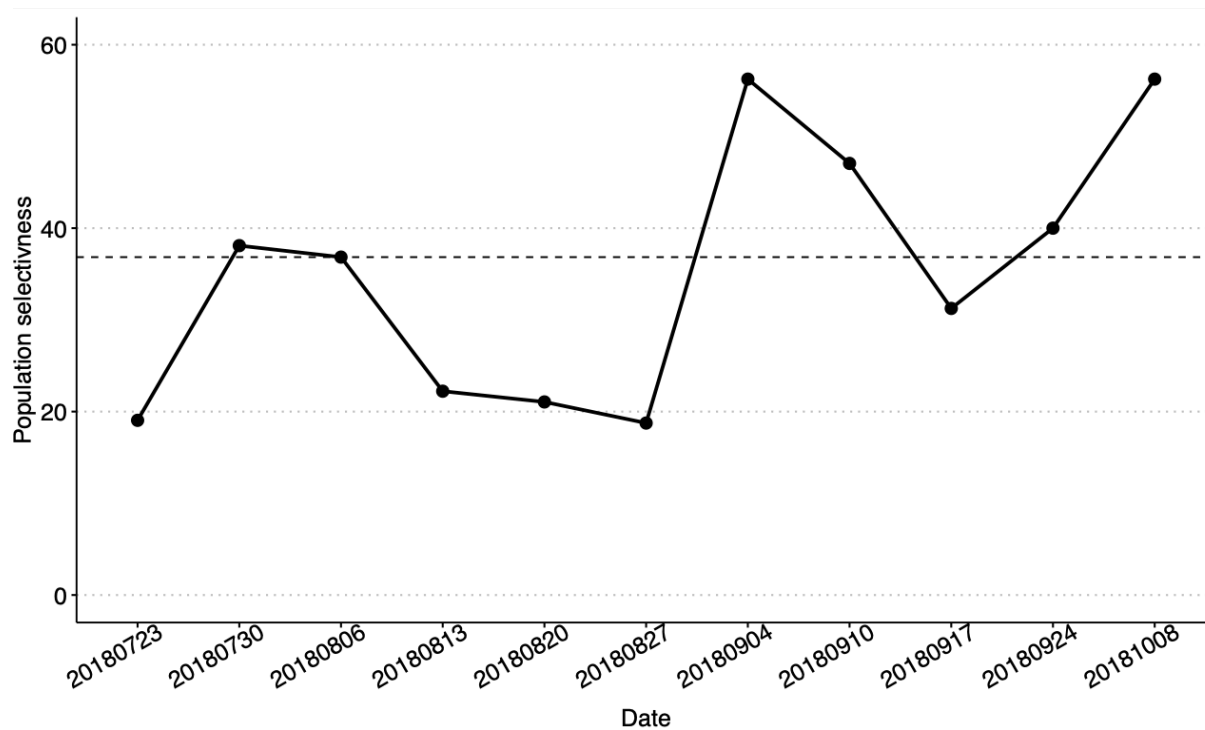
S10: Percent of weeks when each individual (rows) had a significant positive Habitat Selection Analysis coefficient for either the large or the small zooplankton category. The vertical line represents the median of fish selectivity for zooplankton.



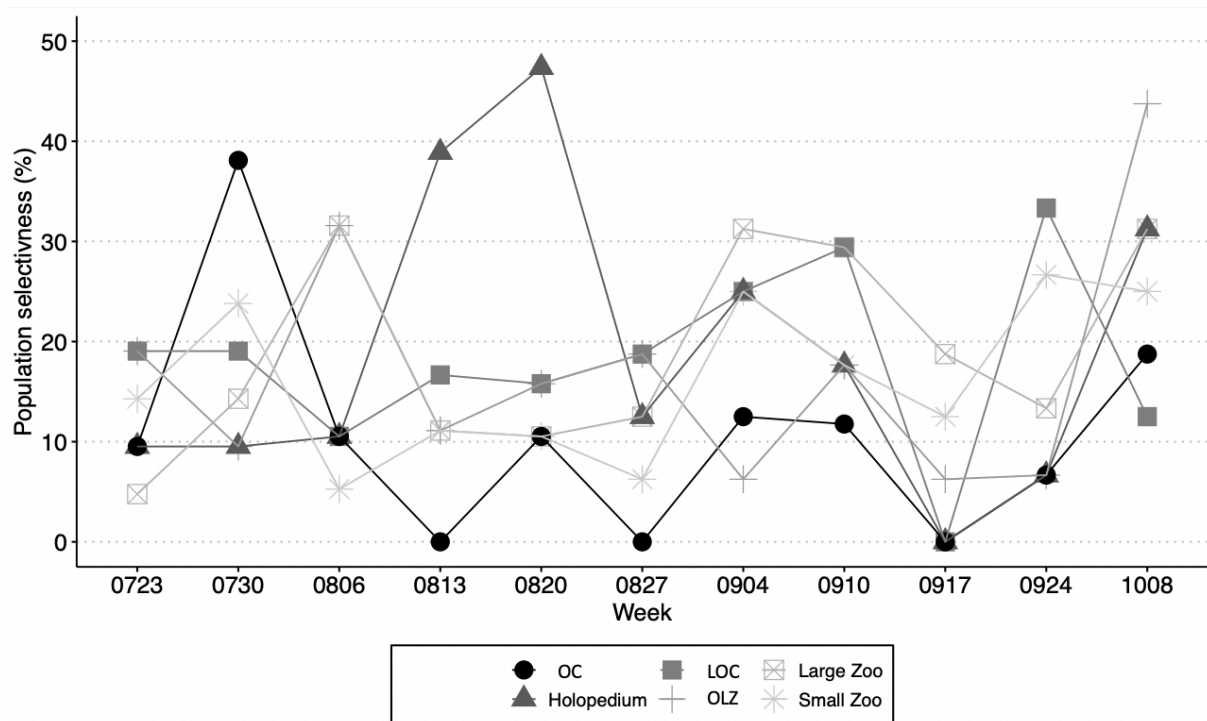
S11: Percent of weeks when each individual (rows) had a significant positive Habitat Selection Analysis coefficient for each zooplankton compartment variable. LOC: large other cladocerans (cladocerans other than *Holopedium* > 1 mm), OLZ: other large zooplankters (all zooplankton taxa > 1 mm excluding LOC), OC: other cladocerans (cladocerans other than *Holopedium*), Small zoo: small zooplankton (all zooplankters < 1 mm), Large zoo: large zooplankton (all zooplankters > 1 mm).



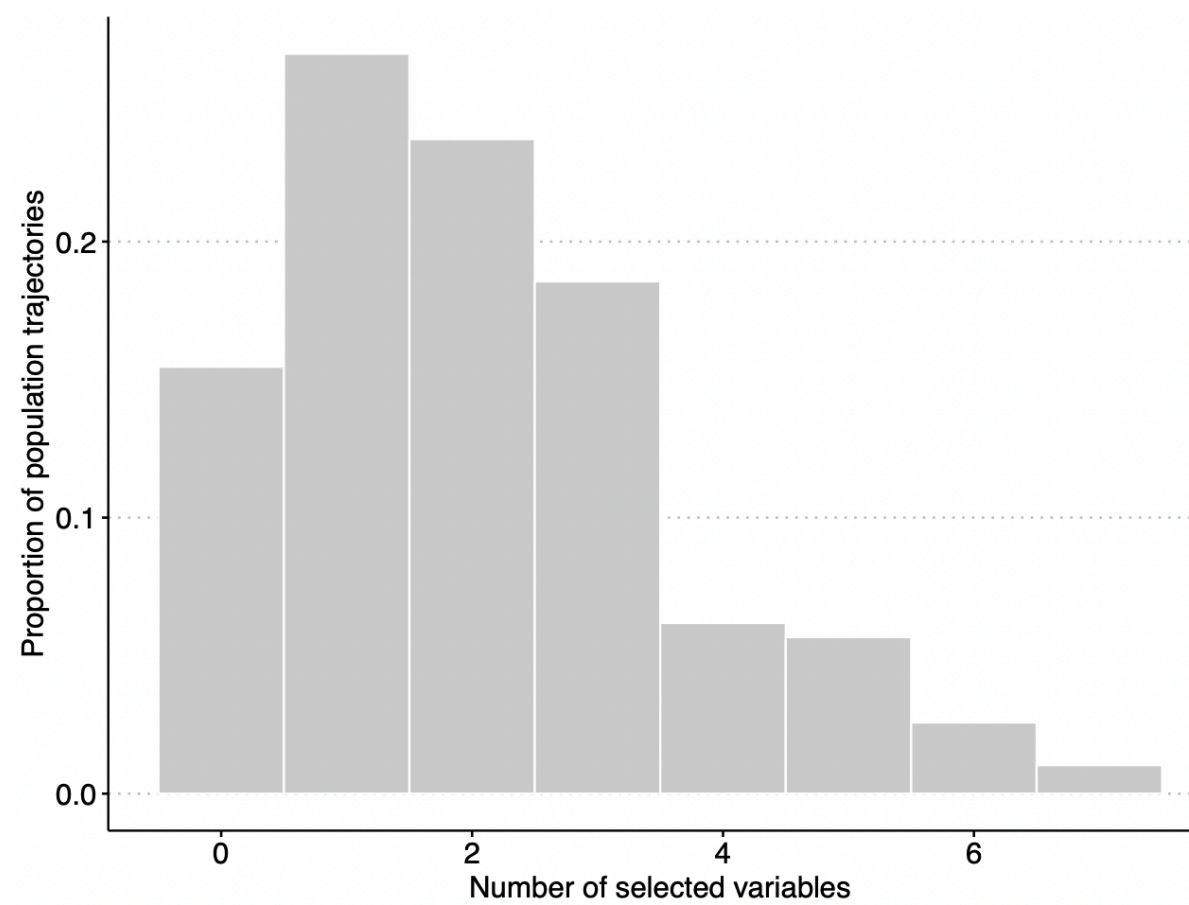
S12: Percent of individuals per week that had a significant positive Habitat Selection Analysis coefficient for either the large or the small zooplankton category. The dashed line represents the median of population selectiveness.



S13: Percent of individuals per week that had a significant positive Habitat Selection Analysis coefficient for each of the zooplankton compartment variables. LOC: large other cladocerans (cladocerans other than *Holopedium* > 1 mm), OLZ: other large zooplankters (all zooplankton taxa > 1 mm excluding LOC), OC: other cladocerans (cladocerans other than *Holopedium*), Small zoo: small zooplankton (all zooplankters < 1 mm), Large zoo: large zooplankton (all zooplankters > 1 mm).



S14: Distribution of the number of variables with non-zero estimates per trajectory.



CHAPTER III: Avoidance of planktivorous fish in the pelagic zone: expanding the zooplankton diel vertical migration paradigm to boreal lakes

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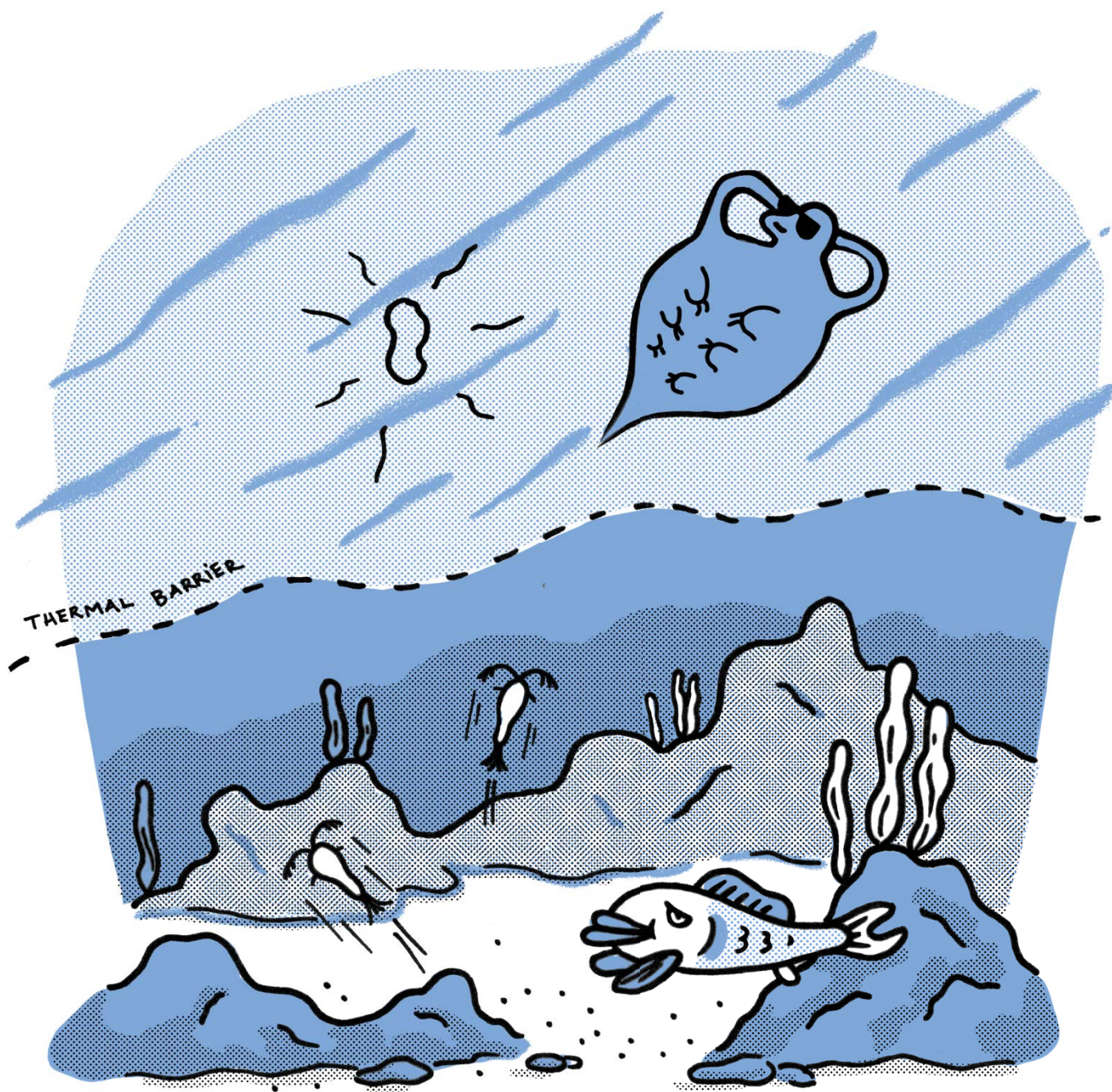
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Abstract

Zooplankton Diel Vertical Migration (DVM) plays a key role in many ecosystem functions and in trophic interactions. Following the actual paradigm, at least two main patterns are expected depending on the interplay of different factors such as predation, competition or resource availability. A large amount of research showed the role of fish predation in inducing a direct DVM in zooplankton, involving a movement toward deep waters during the day, to hide from the visual predators, and a return to the surface at night, to feed on phytoplankton. However, some cold-stenothermic fish species of boreal lakes are relatively unable to use the epilimnion during summer, due to its too warm temperatures. We tested the hypothesis that the surface could become a refuge to predation for zooplankton, leading to a “boreal anomaly” for zooplankton DVM. Using high-frequency in-situ imagery sampling to assess the vertical distribution of zooplankton and available data on vertical distribution of brook charr (*Salvelinus fontinalis*, a cold-stenothermic species), derived from acoustic telemetry, we studied the mechanisms behind the DVM patterns of different taxa, for both small and large zooplankters. Our results suggested that vulnerable taxa like *Daphnia* spp. performed a reverse DVM in relation to fish predation, or even stayed in the epilimnion during the whole 24h cycle. The DVM of zooplankters less vulnerable to predators was instead more influenced by temperature and invertebrate predation. In autumn, vulnerable zooplankton changed their DVM behavior as a consequence of the change of fish depth distribution, when the epilimnion cooled and become available to fish. This study highlighted the impact of the fish predation on zooplankton DVM in boreal lakes, improving our understanding of predator-prey relationships and the habitat coupling in these lakes.

Keywords: brook charr, thermal barrier, stratification, seasonal variations, behavioral defense, vulnerability.



Introduction

The paradigm of zooplankton diel vertical migration (DVM) has been proposed more than a century ago and since then, it has stimulated a great deal of research (Lampert 1989, Hays 2003, Ringelberg 2009, Bandara et al. 2021). Factors driving DVM and its adaptive value were scrutinized to evaluate their relative importance in the zooplankton biology (Ohman 1990, Hays 2003, Bandara et al. 2021). The current view considers that fish predation is one of the main factors influencing zooplankton DVM, by inducing a movement toward deep water layers during the day, to hide from visual predators (Gliwicz & Pijanowska 1988, Lampert 1989, Hays 2003, Pearre 2003, Bandara et al. 2021). During the night, zooplankton generally return to shallow layers in order to feed on phytoplankton and accelerate their growth in warmer water (Peters 1986, Ringelberg 2009, Bandara et al. 2021). However, in addition to this “direct DVM” pattern, some zooplankton taxa exhibit a “reverse DVM” with a migration toward deep layers during the night, to avoid invertebrate predators which go in the upper layers at night (Ohman et al. 1983, Lampert 1989, Bandara et al. 2021). Invertebrate and fish predation can have additive or interactive impacts on zooplankton community, depending on the size and taxon considered (e.g., invertebrate predation pressure on small zooplankters can be reduced when fish is present (i.e., interaction) or invertebrate and fish predation pressures can be additive; González & Tessier 1997, Fiksen et al. 2005). Still, both direct and reverse DVM allow a spatial decoupling of zooplankton and its predators when the predation risk is too high (during the day by fish and vice-versa by invertebrate predators) and a spatial match with its resources (e.g., food and temperature) when predation risk is reduced. In addition to predator avoidance, DVM has been also described as a trade-off made by zooplankton between thermal and food resources, when a deep-water food maximum occurs, uncoupling the two resources (Winder et al. 2004, Lampert 2005). Studies on zooplankton diel horizontal migration (DHM) in shallow lakes confirmed the “fish avoidance” hypothesis to explain zooplankton migrations (Burks et al. 2002, Sagrario & Balseiro 2010). However, these migration patterns should be less profitable where the use of the epilimnion by planktivorous fish is reduced or missing due to habitat constraints. In boreal lakes, when the main zooplankton fish predators are cold-stenothermic, such as salmonids, the epilimnion is too warm for them during summer and thus, becomes a refuge for zooplankton (Goyer et al. 2014, Littlefair et al. 2021, Leroux et al. 2022a).

Brook charr (*Salvelinus fontinalis*), a cold-stenothermic salmonid fish, is found in many boreal lakes of the Canadian Shield. Its main prey are zooplankton in the pelagic area and zoobenthos in littoral area (Magnan 1988, Bourke et al. 1999). Previous studies suggested that brook charr cannot access the

epilimnion when it exceeds a threshold of 22°C, thus nearly entirely hindering the access to the large - and warm - shallow area of the lake during summer (Bourke et al. 1996; Bertolo et al. 2011; Goyer et al. 2014). During this period, pelagic zooplankton performing a typical direct DVM between the epilimnion and the deeper layers should then become, paradoxically, heavily exposed to predation by brook charr. The main objective of this study was thus to show that vulnerable zooplankton stayed in the epilimnion during the day and migrate in the metalimnion during the night in summer (i.e., reverse DVM) diverging from the current paradigm developed for temperate lakes (i.e., direct DVM as a response of fish predation). Indeed, it has been shown that chlorophyll peaks (deep chlorophyll maximum, or DCM; Leach et al. 2018) can be found in the metalimnion of oligotrophic boreal lakes and thus relatively transparent (Gignac-Brassard et al. 2022). In contrast, vulnerable zooplankters should initiate a typical direct DVM in autumn, when the thermal barrier preventing fish to move in surface layers disappeared, thus allowing these latter to predate freely in the epilimnion during the day.

Based on above mentioned considerations, we first tested the hypothesis that zooplankton vertical distribution patterns in summer will differ among zooplankton taxa or size classes (a proxy of their vulnerability to size-selective predators such as fish), with more vulnerable individuals avoiding the layers of the water column where fish are present. More specifically, we explicitly tested the hypothesis that fish vertical distribution explained most of the vulnerable zooplankton vertical distribution by using 3D acoustic tracking of fish. Small zooplankters, which are less conspicuous to fish, should not avoid depths where fish are present (Burks et al. 2002, Tavşanoğlu et al. 2015). This should lead to patterns related to fish avoidance for vulnerable categories of zooplankton (i.e., “surface” reverse DMV between epilimnion and metalimnion) and DVM patterns related to resource variables and invertebrate predation for others (i.e., “deep” reverse DVM between metalimnion and hypolimnion).

Second, we tested the hypothesis that vulnerable zooplankters will perform a direct DVM only in autumn, when the disappearance of the thermal barrier allows fish to use surface layers. In contrast, small zooplankters should continue to make a reverse migration to avoid invertebrates also going shallower during the night in autumn. This would assume that *Chaoborus* sp. direct DVM would not change seasonally, since it feeds on zooplankton in the water column during the night and hide from fish near the bottom of the lake during the day (Dawidowicz et al. 1990).

Finally, we tested the relative importance of invertebrate predation, temperature and phytoplankton abundance in driving the zooplankton migration after taking into account the potential effect of fish predation risk. We expect that during summer, large zooplankters (i.e., > 1 mm), especially daphnids, would remain in the epilimnion to stay in warm waters while avoiding fish and exhibit a reverse DVM to feed on deeper phytoplankton at night. On the other hand, we expect that zooplankters with morphological defenses (like jelly capsules of *Holopedium glacialis*) or behavioral responses (such as copepods fleeing from predators) exhibit a direct DVM linked to a tradeoff between feeding in deep-water phytoplankton maximum during the day and avoid competition with other migrating taxa otherwise (Gignac-Brassard et al. 2022). We also expect that small-bodied zooplankters show a reverse DVM pattern to feed and select warmer water during the day and avoid *Chaoborus* sp. predation at night

Zooplankton DVM is of utmost importance for many ecosystem functions in both lakes and oceans (Pearre 2003, Dawidowicz & Pijanowska 2018). It creates daily fluxes of matter and energy from the surface to the depths and connects several trophic networks (Pearre 2003, Bollens et al. 2011, Dawidowicz & Pijanowska 2018). It is a well-known phenomenon but there are still uncertainties about the factors driving it and the plasticity of this behavior in zooplankton communities with different predation regimes (González & Tessier 1997, Fiksen et al. 2005, Bandara et al. 2021). Here, we use state-of-the art field technology to unravel the specificity of DVM in boreal lakes with cold-stenothermic predators to expand the current DVM paradigm. To our knowledge, this is one of the first studies to explicitly analyze the role of stenothermic fish on DVM in these ecosystems (but see Gignac-Brassard et al. 2022).

Methods

Study site

The study was conducted in 2018 in Lake Ledoux, Mastigouche Wildlife Reserve, Quebec, Canada (46.802381, -73.277136), a small oligotrophic boreal lake for which a large amount of data on brook charr habitat use is available (e.g., Bourke et al. 1996, Dynes et al. 1999, Bertolo et al. 2011, Goyer et al. 2014). Brook charr is the only fish species in the lake and has two main prey sources, zoobenthos and zooplankton (Magnan 1988, Lacasse & Magnan 1992, Rodríguez et al. 1993, Bourke et al. 1999). Given that brook charr avoid temperatures above 22°C (Bertolo et al. 2011, Goyer et al. 2014), it encounters thermal constraints to feed in the epilimnion during the summer stratification period (based on recent high-frequency hydroacoustic telemetry surveys; Leroux et al. 2022a, P. Magnan, unpub. data). Thus, fish individuals make

forays in the epilimnion but spend most of their time in the 5–6 m depth layer, which corresponds to their preferential temperature in the metalimnion (Bertolo et al. 2011, Goyer et al. 2014, Smith & Ridgway 2019). During this period, zoobenthic preys in the littoral zone were largely inaccessible to fish which had to rely on zooplanktonic resources.

Zooplankton sampling

In order to detect zooplankton diel patterns, we made vertical profiles at a station in the deepest area of the lake (14 m c.a.) with an Underwater Vision Profiler (UVP; Hydroptic, L'Isle-Jourdain, France) at both midnight and noon (i.e. 12:00 pm and 12:00 am at solar time). This instrument allowed to take up to 11 pictures per second and was modified to better detect small freshwater zooplankton by reducing the focal length, improving the resolution while reducing the sampling volume (M. Picheral, LOV oceanographic observatory, Villefranche sur Mer, France, pers. comm.). Thus, the UVP was able to take identifiable pictures of zooplankton individuals larger than 262 μm . Profiles were repeated weekly from 23 July to 8 October to account for seasonal variations. Due to weather conditions, samples on 6th August (night) and 1st October (day and night) were not performed. The UVP took pictures of zooplankton from the surface up to 2 m from the bottom to reduce the risk of interference by bottom substrate and/or sediment resuspension (i.e., from 0.5 m to 12 m depth).

The protocols of image processing, semi-automatic classifications and validations are detailed in Leroux et al. (2022b). In summary, UVP images were cut into zooplankton individual vignettes, which were identified and measured with Zooprocess software and Ecotaxa platform (Picheral et al. 2017). To minimize the incertitude associated with image resolution, vignettes were not identified at the lowest possible taxonomic level but rather pooled into coarse groups: copepods, holopediidae (*Holopedium glacialis*), other cladocerans, chaoboridae, colonial rotifers (large colonies of *Conochilus* sp., non-colonial individuals being too small to be counted by UVP), and non-zooplanktonic categories (e.g., volvocales, aquatic plant, arachnidae, and detritus). “Other cladocerans” (OC) included mainly bosminidae and daphnidae, the latter being largely dominant in large size classes. Debris of submerged aquatic vegetation (SAV) were also considered whereas an “Others” category was created for living-particles that could not be identified (too small or blurry) (Leroux et al. 2022b). Equivalent Spherical Diameter (ESD) was calculated for each zooplankton vignette from its area (Eq. 1):

$$(1) \ ESD = 2 \times \sqrt{\frac{A \times C^2}{\pi}}$$

where A is the area of the particle and C is the conversion factor to transform pixels to millimeters (C = 0.06).

Aggregation of zooplankton data

UVP depth measurement resolution is 0.1 m but to avoid biases due to small sampling volumes (i.e., zero-inflated distribution of abundances), abundances were averaged every 0.5 m depth. To test our hypotheses, we first summed average abundances of zooplankters larger than 1 mm (i.e., “*Large*”) and those smaller than 1 mm (i.e., “*Small*”). Second, a PCA was made with abundances of each zooplankton taxa and size classes (e.g., “*Small copepods*” or “*Large Holopedium*”) (Figure 1). This approach allowed to discriminate which groups of zooplankton covaried (Figure 1). Thus, we considered eight categories among the zooplankton community in addition to *Total*, *Large* and *Small zooplankton* (i.e., *Small other cladocerans*, *Large other cladocerans*, *Small copepods*, *Large copepods*, *Small Holopedium*, *Large Holopedium*, colonial *Rotifers* and *Others*). Once the abundances for each of these 11 categories were calculated for each 0.5 m depth at each sampling occurrence, environmental variables were associated to each category value.

Measurement of environmental variables

Tagged fish were tracked by 23 acoustic receivers (HR2-180k-100; VEMCO inc., Halifax) installed at different locations within the lake (see S3 in Leroux et al. 2022a). A total of 21 fish equipped with acoustic transmitter (V9TP-2x-180k-xxm; VEMCO inc., Halifax) were tracked during this study. The minimum time between two recordings of a single fish is 10-14 s (i.e., delay between two consecutive tag signal transmissions) but gaps could appear in the data at some occasions due to fish not properly positioned by the receivers. Depth distribution of fish was then extracted for each zooplankton profile with all positioning data from 2 h before to 2 h after the zooplankton sampling (4 h period) . Proportion of water column occupancy by fish was extracted for each 0.5 m depth to match our zooplankton database. When fish occupancy in a 0.5 m-layer was less than 1% of the total distribution, this layer was considered fishless.

The main invertebrate predator in the lake was *Chaoborus* sp. (Gignac-Brassard et al. 2022) and its depth distribution was measured with the UVP simultaneously with zooplankton abundances. *Chaoborus* sp. abundances were also averaged for each 0.5 m-layer.

Vertical profiles of fluorescence and temperature (fluoroprobe, bbe Moldaenke) were recorded weekly at the sampling station in parallel to UVP profiles. The fluorescence profiles were converted by the instrument into phytoplankton concentration (PC). Temperature and PC were also average for each 0.5 m-layer to match zooplankton data resolution.

Dissolved oxygen concentrations were monitored in epilimnion, metalimnion and hypolimnion with four probes (PME miniDOT logger) at 1.5, 3, 5 and 8 m depth. Oxygen was monitored every minute during the study period and no oxygen limitation was detected for both zooplankters and fish, confirming that oxygen was not limiting the movements of animals (see Figure S1).

Statistical analyses

We tested our three working hypotheses in three steps. First, we build four models for each zooplankton category with the abundance as response variable and Depth (*Model 1*), Depth in interaction with Period (i.e., day or night; *Model 2*), Depth in interaction with Season (*Model 3*) or Depth in interaction with both Season and Period (*Model 4*; Figure 2A) as explanatory variables. We considered two levels for the Season variable: summer (i.e., before 10 September), where epilimnion temperature was above 20°C, and autumn (i.e., from 10 September), where epilimnion temperature was below 20°C (Figure 3A; Leroux et al. 2022b). The date of sampling was included in all models as random effect. For each zooplankton category, the selection of the best model among the four models would inform us about variation of its depth distribution (Figure 2A). More specifically, we hypothesized that (i) Depth distribution never change during the sampling period (selection of *Model 1*) or (ii) zooplankton make a DVM (selection of *Model 2*) or (iii) zooplankton do not make a DVM but its depth distribution change between summer and autumn (selection of *Model 3*) or finally, (iv) zooplankton DVM is modulated throughout the seasons (selection of *Model 4*). We also produced an alternative version of *Models 3* and *4* by replacing Season with fish presence/absence data, to test the hypothesis that seasonal variation in zooplankton depth distribution was explained by seasonal variation in vertical distribution of fish in the water column (*Model 3bis* and *Model 4bis* respectively, Figure 2A). The Akaike Information Criteria corrected for small samples was calculated (AICc). The $\Delta AICc$ ($AICc_{model} - AICc_{min}$), and the degrees of freedom (as an index of the number of

variables used in a model) were used to select the best and most parsimonious model (Burnham & Anderson 2004). The four models were ranked using Δ_{AICc} , i.e., the difference in AICc between a candidate model and the model with the lowest (best) AICc. Models with a $\Delta_{AICc} < 2$ were considered to have substantial support (Burnham & Anderson 2004) and the most parsimonious (i.e., minimum degrees of freedom) was selected. Depth and interactions between Depth and other variables were modeled using a generalized additive model (GAM, Wood 2017) to take into account the non-linearity of the relationship between zooplankton abundances and depth (see S2). Moreover, the number of basis functions was set to five to reduce overfitting but keeping high quality predictions. The analyses were done with the *mgcv* package in the R software (R Core Team 2021, Wood 2015). The selected model (among *Model 1, 2, 3* and *4*) will be thereafter referred as “*DVM model*” for each of the zooplankton categories.

Second, a Fish presence/absence variable was added to the selected *DVM model* for each zooplankton category, to assess if the model could have more support (lower AICc) than the *DVM model* alone in explaining the depth distribution of zooplankton (Figure 2B). If the model with Fish presence/absence better explained zooplankton depth distribution, it was selected for further analyses, otherwise, the *DVM model* was kept.

Finally, the last three covariables (i.e., temperature, *Chaoborus* sp. and PC) were added simultaneously to the selected model (*DVM model* or *DVM model* with Fish presence/absence depending on the selected model in each zooplankton categories; Figure 2C). To evaluate the relative importance of these three covariables, the *dredge()* function of the MuMIn package (Wood 2015) was used to make a model selection among all combinations among the selected *DVM model* (+ Fish presence/absence when appropriate) and the three covariables. All best models were pooled to get a cumulative weight up to 95% and averaged coefficients for the covariables. These averaged coefficients were considered different from zero when their 95% confidence interval calculated with all models gathering 95% weight excluded zero.

Model predictions were used to visualize theoretical depth distribution of zooplankton according to the Fish presence/absence. Depth distribution predictions were made on the 0.5-8.5 depth interval because of too many zero abundances below 8.5 m depth, which made the predictions less robust. Two theoretical situations were explored with model predictions, (i) setting Fish as being totally absent from the water column and (ii) mimicking the real fish distribution in the Lake Ledoux. Mimicking the real fish distribution was made by setting Fish as present below 4 m depth during summer and as present at

every depth during autumn. Furthermore, the predictions imitating real fish distribution were graphically compared to predictions made by *DVM model* without fish to evaluate the importance of the fish variable to fit observed values when the variable was selected.

Diurnal deficit

Zooplankton absolute abundances can be biased depending on the period of sampling (Doubek et al. 2020). A diurnal deficit (i.e., less abundant zooplankton during the day compared to the night; DD) is in fact often observed in zooplankton profiles and can be the result of burrowing behaviors during the day or horizontal movements of some zooplankton taxa (De Stasio 1993, Doubek et al. 2020). Even if we sampled most of the water column and our models always considered the relative depth distribution of zooplankton and not the abundance *per se*, the importance of this diurnal deficit was investigated for each zooplankton category, following Doubek et al. (2020) equations:

$$Diurnal\ Deficit = \frac{(OD_N - OD_D)}{OD_N} \times 100 \quad \text{if } OD_N > OD_D$$

$$Diurnal\ Deficit = \frac{-(OD_D - OD_N)}{OD_D} \times 100 \quad \text{if } OD_N < OD_D$$

where OD_N and OD_D are the observed densities at night and during the day respectively. The DD index was calculated for each sampling period where day and night samplings occurred (i.e., ten dates).

Results

Lake environmental variations

Lake Ledoux was stratified during summer with an epilimnion temperature higher than 20°C from 25 July to 06 September (Figure 3A). From 10 September to 8 October, the thermocline deepened whereas surface temperature decreased to 10°C. From 25 July to 06 September, fish aggregated in the metalimnion, between 4.5 and 6.5 m (Figure 3B). Individuals rarely went into the 0-3 m layer during this period (less than 2% of occurrences in average). In autumn, while they also continued to occur in the metalimnion, they went clearly more frequently in shallow waters (36% of occurrences in average). Phytoplankton concentration (PC) peaked in the metalimnion or even in the hypolimnion during summer (Figure 3C).

In autumn, PC was more homogeneous in the water column, with higher concentrations at the surface compared to summer, especially the 8 October (Figure 3C). *Chaoborus* sp. distribution pattern in the water column was less clear even if we noticed a higher abundance at night after 20 August (Figure 4D).

DVM patterns

Among all zooplankton categories, eight had their abundance depth distribution better explained by the *Model 4* which involved a DVM (either direct or reverse) modulated by season (Table 1, Table S3). The other three categories, i.e., *Total zooplankton*, colonial *Rotifers* and *Others*, were better explained by *Models 1, 3 and 3*, respectively (Table 1). These results suggest that (i) *Total zooplankton* was unequally distributed in the water column but that this distribution did not differ from day to night neither from summer to autumn (Figure 4A); and (ii) that the categories colonial *Rotifers* (Figure 4C) and *Others* were also unequally distributed in the water column but their depth distribution did not differ between noon and midnight. In contrast, the selection of *Model 3* meant that (iii) the depth distribution of these two zooplankton categories changed between the two seasons. Colonial *Rotifers* seemed to stay in the epilimnion day and night, even if they went deeper in autumn (Figure 4C). As for the *Small other cladocerans* (explained by *Model 4*), they were shallower during the day compared to night in summer but their depth distribution was more homogeneous between the epilimnion and the metalimnion in autumn (Figure 4B). Depth distributions for zooplankton categories that are not presented in Figure 4 are presented in Figure S4. *Small zooplankton* is relatively evenly distributed along the water column whereas *Large zooplankton* is more concentrated in the epilimnion, especially the *Large other cladocerans*. *Holopedium* sp. performed a direct DVM during summer but the signal faded in autumn. *Large copepods* performed a reverse DVM, especially during summer but this behavior was more confused for *Small copepods*. As for the *Others* category, they were more concentrated in the metalimnion and the hypolimnion. *Small* and *Large other cladocerans* were the only zooplankton categories for which depth distribution was better explained when replacing the Season term by fish presence/absence data (*Model 4bis*, Table S3).

Fish influence

When fish presence/absence data were added to the selected models, only the depth distribution of *Other cladocerans* (small or large) was better explained compared to all other zooplankton categories (Table 1). Based on AICc and degrees of freedom, models including interactions between Depth and Season, Period and Fish presence/absence were selected compared to *Model 4bis* (Table S3). The models

predict that *Large other cladocerans* vertical distribution would be concentrated in the epilimnion during both day and night in summer, when there is fish in the metalimnion, whereas it would be more evenly distributed during the day in absence of fish (Figure 5A). There are no strong differences in the model predictions between summer and autumn when fish are absent (Figure 5A). However, in autumn, *Large other cladocerans* peak in the epilimnion during the day and in the metalimnion during the night when fish are present (Figure 5A). Peak of *Small other cladocerans* abundances were slightly more important during the day and 2 m lower during the night compared to the day in summer, when fish are present in the metalimnion, compared to when fish are absent (Figure 5B). In autumn, the models for *Small other cladocerans* predict a peak in the epilimnion during the day and in the metalimnion during the night, when there is no fish, whereas they tend to remain in the metalimnion, when fish are set as present (Figure 5B).

Model predictions for both *Small* and *Large other cladocerans* better fitted the observed data during the day, when fish are present, whereas they better fitted the observed data at night when fish are absent (Figure 5). When there was no influence of fish on zooplankton depth distribution (i.e., all zooplankton categories except for the *Other cladocerans*), model predictions were similar whether we made a prediction with *Model 4* with or without fish presence/absence data (Figure S5).

Relationships with temperature, invertebrate predation and food resources

Adding temperature as a covariable in the models improved the predictions of abundances for several taxa (Table 1). After taking into account the effects of fish, the abundance of *Small zooplankton* together with both *Small* and *Large other cladocerans* showed a positive relationship with temperature whereas *Small Holopedium* and the *Others* categories were negatively related to temperature. The abundance of *Chaoborus* sp. was almost always negatively related, albeit not always significantly, to zooplankton abundance, especially that of *Small zooplankton*, *Small copepods* and *Small other cladocerans* (Table 1). Nonetheless, based on the confidence intervals, no coefficient for the effect of *Chaoborus* sp. was different from zero when specific taxa (small or large) were considered. PC coefficient was negative for the *Total zooplankton* category while it was positive when *Small zooplankton* alone were considered (Table 1). Apart from these categories, no effect of PC was detected to explain zooplankton abundances, as for the *Chaoborus* sp. effect.

Diurnal deficit

We found no diurnal deficit when *Total* or *Small zooplankton* were considered (Figure 6). The analysis of the different categories of small zooplankton confirmed that no trend occurred in the differences of abundances between day and night. However, DD was higher for large zooplankters, especially for *Large other cladocerans* which exhibit a positive diurnal deficit (i.e., more abundant at night; Figure 6). Finally, while almost no deficit was observed for the *Others* category, colonial *Rotifers* were the only category that was often less abundant during the night compared to the day (i.e., negative DD; Figure 6).

Discussion

Among the 11 categories of zooplankton analyzed, eight showed a DVM (either direct or reverse) pattern. In contrast, *Total zooplankton* did not show variations (neither seasonal nor nycthemeral) of its depth distribution while colonial *Rotifers* and *Others* showed only seasonal variations. For those exhibiting a DVM (either direct or reverse), their behavior changed over seasons. Seasonal variations in DMV of *Other cladocerans* (large or small) was mostly related to the seasonal variation of brook charr depth distribution. When fish were able to feed in the epilimnion in autumn, *Other cladocerans* went deeper, especially during the day. *Large other cladocerans* were not confined in the epilimnion during the fall compared to the summer. By coupling a high-resolution zooplankton sampling with taxonomic information and fish tracking, this study highlighted the importance of fish thermal tolerance and its impact on zooplankton behavior in boreal lakes.

DVM patterns and fish influence

Whereas most zooplankton categories showed DVM signals (either direct or reverse), no DVM could be detected when pooling all zooplankton together (i.e., *Total zooplankton*). This result and the type of DVM (direct or reverse) found here suggested that the zooplankton community as a whole exhibited a partial DVM patterns in Lake Ledoux, meaning that only some zooplankton groups were migrating, and not necessarily in the same direction. When considering the *Others* category, as expected, no migration pattern was found either. As *Total zooplankton*, *Others* category grouped different taxa (mostly small cladocerans and copepods; Leroux et al. 2022b) which are likely exposed to different constraints, thus blurring the potential patterns. This suggests that to highlight patterns of DVM, a minimum of taxonomic

information is needed. Furthermore, migration patterns were different for small and large individuals of the same category (e.g., *Copepods*, *Other cladocerans*), suggesting possible intra-specific plasticity depending on body size for the DVM behavior and, more likely, potential inter-specific differences (e.g., calanoids or cyclopoids within the *Copepods* category) (Folt & Burns 1999, Hays 2003).

The most important factor influencing DVM in zooplankton communities is predation and partial migrations is often related to different levels of vulnerability linked to body size or pigmentation (i.e., conspicuousness), and defenses (physical or behavioral) (Hays 2003, Bandara et al. 2021). For example, copepods are able to flee predator fish with “jumps” (Buskey et al. 2002) and *Holopedium* sp. is embedded in a gelatinous capsule making it less vulnerable to invertebrate predation and distasteful to fish (Detmer et al. 2017). Interestingly, among large zooplankters, while *Large Holopedium* or *Large copepods* were abundant in the metalimnion during summer (i.e., the layer where fish were present), *Large other cladocerans* remained in epilimnion (i.e., the layer without fish during summer). This apparent avoidance of brook charr by the *Large other cladocerans* category was confirmed by the selection of the Fish variable to model variations in the depth distribution of this category. On the other hand, the depth distribution of the *Large Holopedium* and *Large copepods* categories were unrelated to fish presence, as expected, but we could not make strong conclusions about the variables selected, even if invertebrate avoidance seemed stronger. Interestingly, the same DVM patterns emerged considering the same taxa in small categories, with *Small other cladocerans* exhibiting reverse DVM related to fish distribution even if their small body size made them less conspicuous for fish (Confer et al. 1978, Magnan 1988).

The *Large other cladocerans* category, composed mostly by large *Daphnia* spp., did not exhibit a migration pattern that could be explained by the actual zooplankton DVM paradigm (Hays 2003, Bandara et al. 2021). Based on this latter observation, we expected a direct DVM (i.e., dark hypolimnion during the day and epilimnion at night). In contrast, by taking into account the thermal constraints of the brook charr during summer, we can formulate as alternative prediction a reverse DVM during summer, with individuals matching the chlorophyll maximum (i.e., between 5 and 10 m during summer) and returning to refuge (i.e., warm epilimnion) during the day (as for *Small other cladocerans*). Instead, the selection of epilimnion during both day and night suggest that the *Large other cladocerans* category avoided fish during the whole 24h cycle. By staying in the epilimnion during the night, they selected temperature above 20°C, probably to increase their growth rate and become mature more rapidly despite that their access to the phytoplankton peak was limited because of predators (Peters 1986, Brown et al. 2004, Winder et al. 2004).

This strategy might be an advantage in the food-poor epilimnion given that large cladocerans are known to be better competitors for food than smaller ones, given their higher tolerance to low levels of food (Gliwicz 1990). The nearly absence of the *Large other cladocerans* category in the hypolimnion supports the conclusion of a trade-off between predation avoidance and food resource since phytoplankton was more abundant in deeper layers during the summer. However, one part of their behavior still remained unexplained due to a relatively high diurnal deficit which could be an effect of horizontal migration, with a possible lower selection for pelagic toward littoral areas during the day, to avoid fish predation (Burks et al. 2002). The nearly complete lack of *Large other cladocerans* in the hypolimnetic waters sampled in this study (which is not the case for other taxa performing a direct DVM in Lake Ledoux such as *Chaoborus* sp.) suggests that the DD is not due to a massive use of layers deeper than 12m.

In autumn, when fish were able to feed in the epilimnion (Leroux et al. 2022b), both *Large* and *Small other cladocerans* categories clearly used deeper layers, especially during the day, probably to avoid fish predation. However, they did not completely avoid the epilimnion, maybe due to a shift of fish towards littoral areas, where zooplankton and benthic resources were free of fish predation during summer (Bourke et al. 1999, Leroux et al. 2022a). Both *Large* and *Small Holopedium* also exhibited a clear shift in their depth distribution between seasons, aggregating closer to the surface in autumn compared to summer. This might be explained by a competitive exclusion performed by *Other cladocerans* going deeper in autumn (Tessier 1986). DVM seasonal variation for other zooplankton categories were more difficult to interpret, with environmental covariables not explaining much of the variance of their abundances. Whereas this goes beyond the scope of this work, a further step in the analysis would be to explicitly model all depth distributions of each category simultaneously to better understand the potential relationships (e.g., predation and competition) among them.

Relationships with temperature, invertebrate predation and food resources

Temperature, *Chaoborus* sp. and phytoplankton concentration (PC) added only little extra information to explain zooplankton abundances. While model coefficients for *Chaoborus* sp. were negatives, especially for small zooplankton categories (e.g., *Small other cladocerans* or *Small copepods*), it was considered different from zero only for the *Total* and the *Small zooplankton* categories. This is in contradiction with our predictions of stronger negative relationships with *Chaoborus* sp. since this invertebrate predator is relatively abundant in Lake Ledoux, compared to other lakes in the same area (Gignac-Brassard et al. 2022). Nevertheless, our results suggested a stronger impact of *Chaoborus* sp. on

smaller than larger taxa, as it should be expected, since *Chaoborus* sp. is more gape-limited than fish (Drouin et al. 2009). In contrast, we suggest that the reverse DVM observed for the *Small other cladocerans* category is most likely due to fish presence in the metalimnion during summer which contradicts the paradigm that reverse DVM is almost always linked to invertebrate avoidance (Ohman et al. 1983, Lampert 1989, Bandara et al. 2021). However, *Chaoborus* sp. might play a role in this reverse DVM, exerting a predation pressure in the epilimnion during the night. PC did not add much more to our models. Since all these variables were structured vertically in the water column, it was obvious that their influence on the depth distribution would be difficult to disentangle from a simple depth selection. This could be the reason why no other relationships with the depth distribution were found with any of the three covariables (PC, temperature, *Chaoborus* sp.) or with the Fish variable. However, when adding one of these vertically structured variables in the models improved the support of a DVM model, this can be considered a robust result because of the above-mentioned effect of the variable Depth in capturing most of the variation due to any of the vertically-structured variables. Thus, the positive relationship between the relative abundance of both *Large* and *Small other cladocerans* categories with temperature suggested that these categories preferred warmer layers while at the same time avoiding fish predation. This was detected even though the fish drove the zooplankton in the warm epilimnion and therefore the temperature effect should have been partially included in the fish effect.

In conclusion, this study succeeded to highlight several patterns of DVM in a relatively small boreal lake, with some being strongly influenced by fish depth distribution. The effect of fish on the depth distribution of the *Other cladocerans* category changed between seasons and from day to night, as shown by the improved predictions of observed values with fish during the day and without fish during the night. We could not demonstrate an effect of fish on other taxa depth distribution, especially for large individuals (i.e., *Holopedium* sp., copepods), whereas we expected that fish could feed on at least *Holopedium* sp. (Leroux et al. 2022a). This could reflect a less effective predation on these taxa, that would be insufficient to induce a behavioral response such a migration (Leroux et al. 2022b). In boreal lakes, we showed that a characteristic DVM pattern occurred when vulnerable zooplankton was avoiding cold-stenotherm predators. Indeed, the epilimnion becomes a refuge area for zooplankters when the stratification creates a thermal barrier almost impermeable to fish. This induces a reverse migration of the most vulnerable taxa but close to the surface instead of going in hypolimnion at night. In the context on climate change, thermal stratification and thus thermal barriers can occur sooner and for a longer period, with a possibly deeper thermocline (Keller 2007). To improve our understanding of zooplankton migration behavior and its impact

on trophic networks in warming systems, more studies have to focus on specific ecosystems like boreal lakes. These lakes are particularly sensitive to warming, and a stratification lasting longer could force some zooplankters to remain in the epilimnion, far from deeper phytoplankton-rich layers, uncoupling phytoplankton and zooplankton, but also uncoupling the zooplankton with stenothermic fish. These fish could be prejudiced by the arrival of new fish species extending their ranges to northern regions because of climate change, unbalancing existing trophic networks.

Acknowledgments

We thank those who helped in the field and laboratory, especially Arthur de Grandpré, Geoffrey Marselli, Arianne Merineau, Vickie Lapointe, Alexandre East, and Maxime Clermont. We are grateful to Yannick Huot from Sherbrooke University, who loaned us the UVP, and Marc Picheral for his help with UVP and Ecotaxa. We thank Chantal Fournier, Nathalie Godbout, Pierre-André Bordeleau, and the Information Technology Department of the Université du Québec à Trois-Rivières for the support provided in their field. All authors collected data for this study, RL wrote the manuscript and conducted analyses. All authors provided revisions and comments on the analyses and the manuscript writing. This work was supported by grants from Natural Sciences and Engineering Research Council of Canada (NSERC) to AB and PM and the Canada Research Chair Program to PM. RL was supported by an ÉcoLac NSERC-CREATE doctoral fellowship.

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Table

Table 1: Diel vertical migration (DVM) models selected based on AICc and degrees of freedom for each zooplankton category, with their respective R^2 . Sign “+” is present in the Fish column if fish occurrence increased the support of the DVM model. Average coefficient for each covariables (Temperature, Chaoborus and Phytoplankton Concentration - PC) from models gathering 95% weight are represented in bold when the 95% confidence interval excluded zero. The R^2 for the best model retaining or not the different covariables is added.

	DVM model	R^2	Fish	Temperature	Chaoborus	PC	R^2 of the best model
Total zooplankton	Mod 1	0.41		- 2.6 10^{-3}	- 0.74	- 4.6 10^{-3}	0.48
Small zooplankton	Mod 4	0.26		6.0 10^{-4}	- 0.61	3.8 10^{-4}	0.32
Large zooplankton	Mod 4	0.49		- 7.5 10^{-4}	- 6.4 10^{-2}	- 3.2 10^{-4}	0.49
Small OC	Mod 4	0.52	+	2.0 10^{-3}	- 9.9 10^{-2}	2.8 10^{-3}	0.63
Small Holopedium	Mod 4	0.41		- 7.3 10^{-4}	- 7.3 10^{-4}	- 6.7 10^{-5}	0.42
Small Copepod	Mod 4	0.26		- 1.2 10^{-3}	- 9.8 10^{-2}	- 2.2 10^{-3}	0.28
Large OC	Mod 4	0.30	+	7.2 10^{-4}	7.7 10^{-4}	1.6 10^{-5}	0.42
Large Holopedium	Mod 4	0.49		3.0 10^{-5}	- 2.5 10^{-2}	4.0 10^{-4}	0.49
Large Copepod	Mod 4	0.37		- 1.8 10^{-4}	- 2.2 10^{-3}	2.1 10^{-5}	0.37
Rotifer	Mod 3	0.39		7.4 10^{-4}	- 6.7 10^{-2}	4.9 10^{-5}	0.39
Others	Mod 3	0.66		- 5.3 10^{-3}	- 5.5 10^{-2}	- 1.6 10^{-3}	0.67

Figures

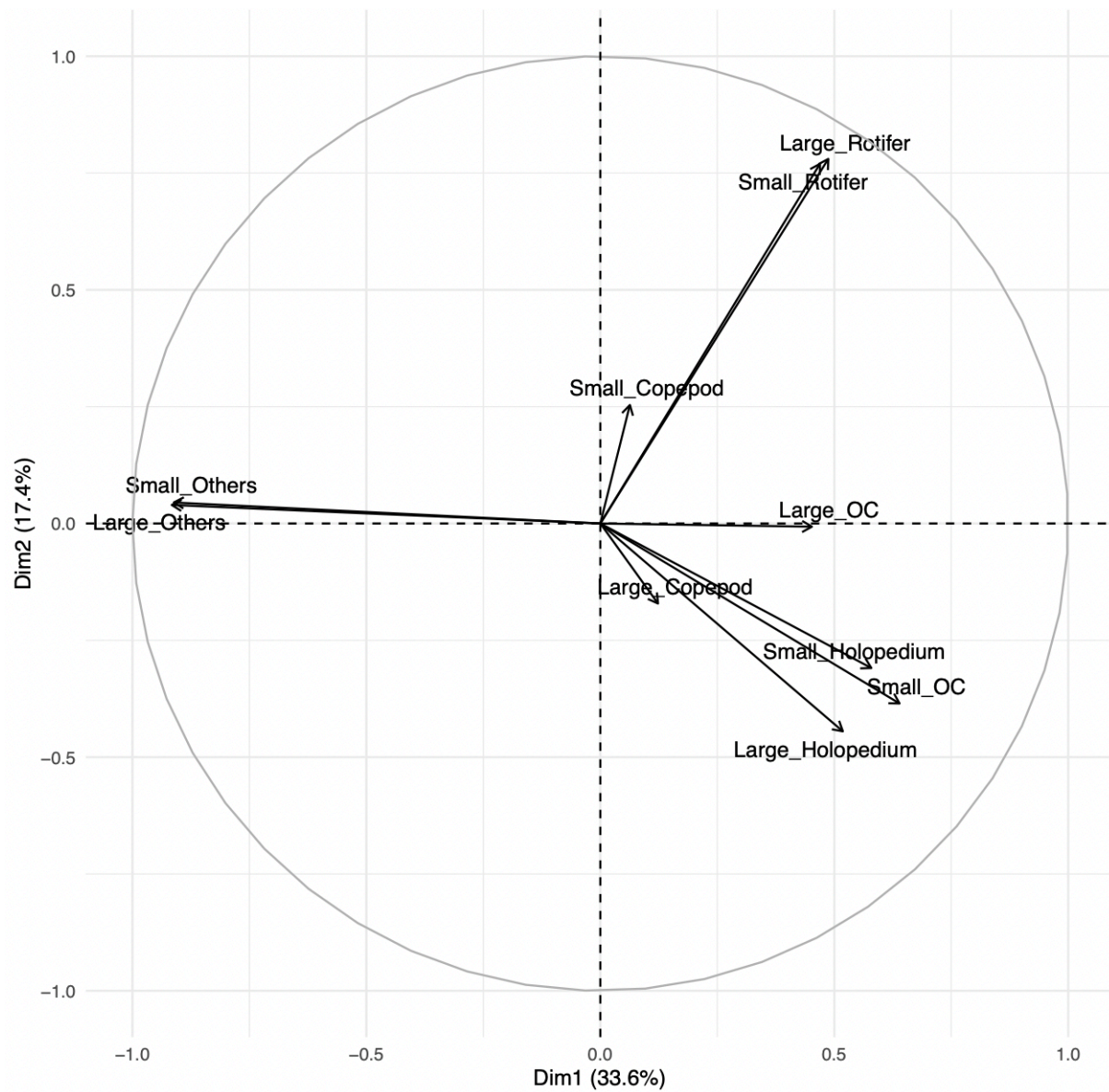


Figure 1: PCA made on abundances of the 10 zooplankton categories, separating taxa and size classes. *Total*, *Large* and *Small* zooplankton categories were not included in this analysis.

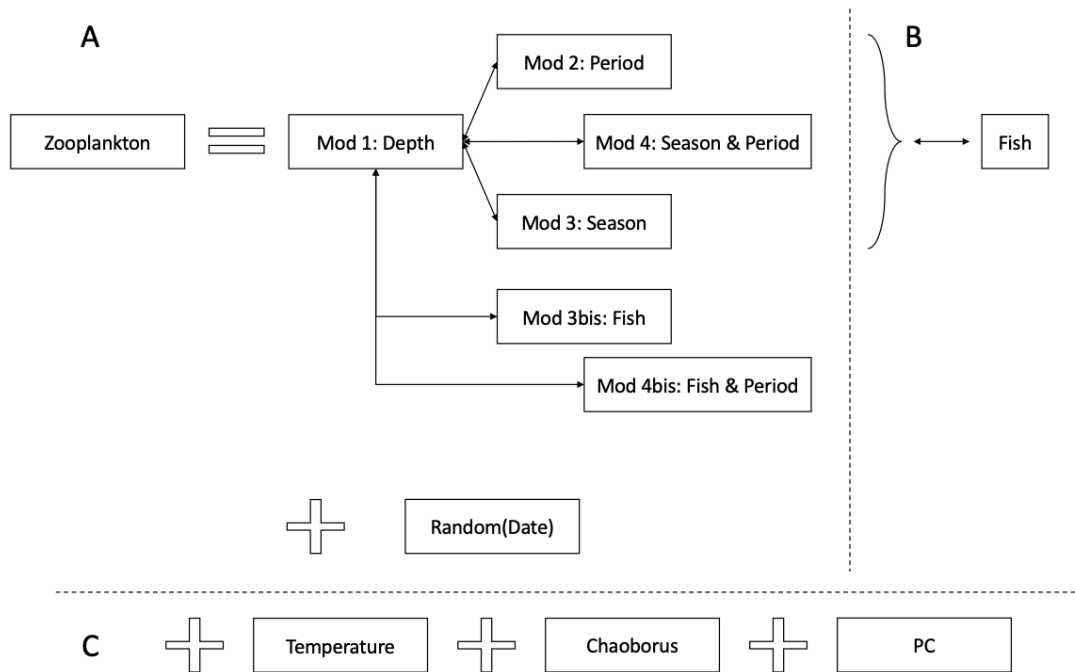


Figure 2: Conceptual diagram to represent the process of model building beginning with DVM models (A), then adding fish presence/absence data (B) and adding the contribution of the last three covariables (C). PC: Phytoplankton concentration.

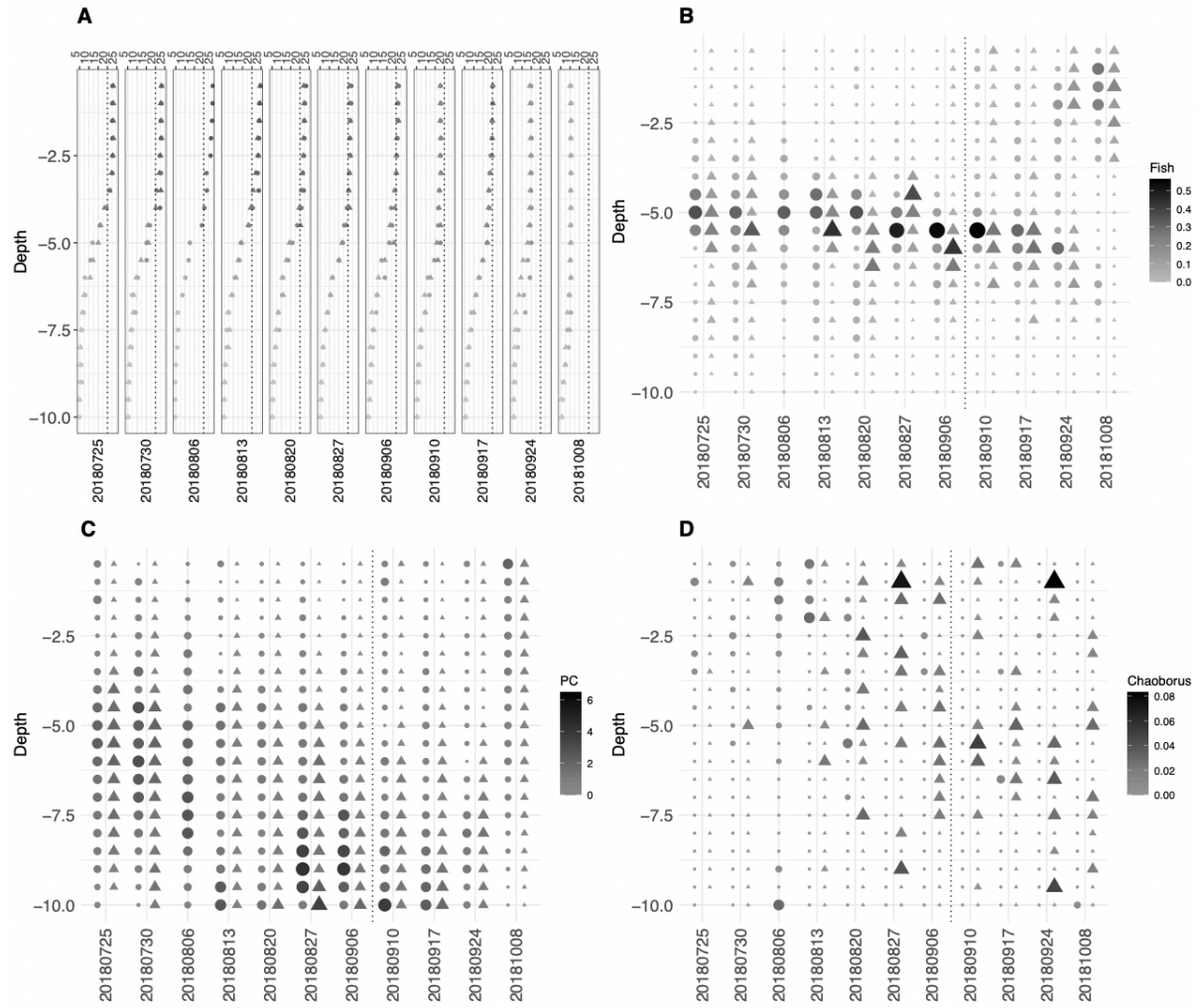


Figure 3: Representation of seasonal variations of environmental covariables in the water column. Grey levels represent the Temperatures in $^{\circ}\text{C}$ (A), Fish in frequency of occurrence (B), Phytoplankton concentration (PC) in $\mu\text{g.L}^{-1}$ (C) and *Chaoborus* sp. abundances in $\text{individuals.L}^{-1}$ (D). Triangles and circles represent values during night and day respectively.

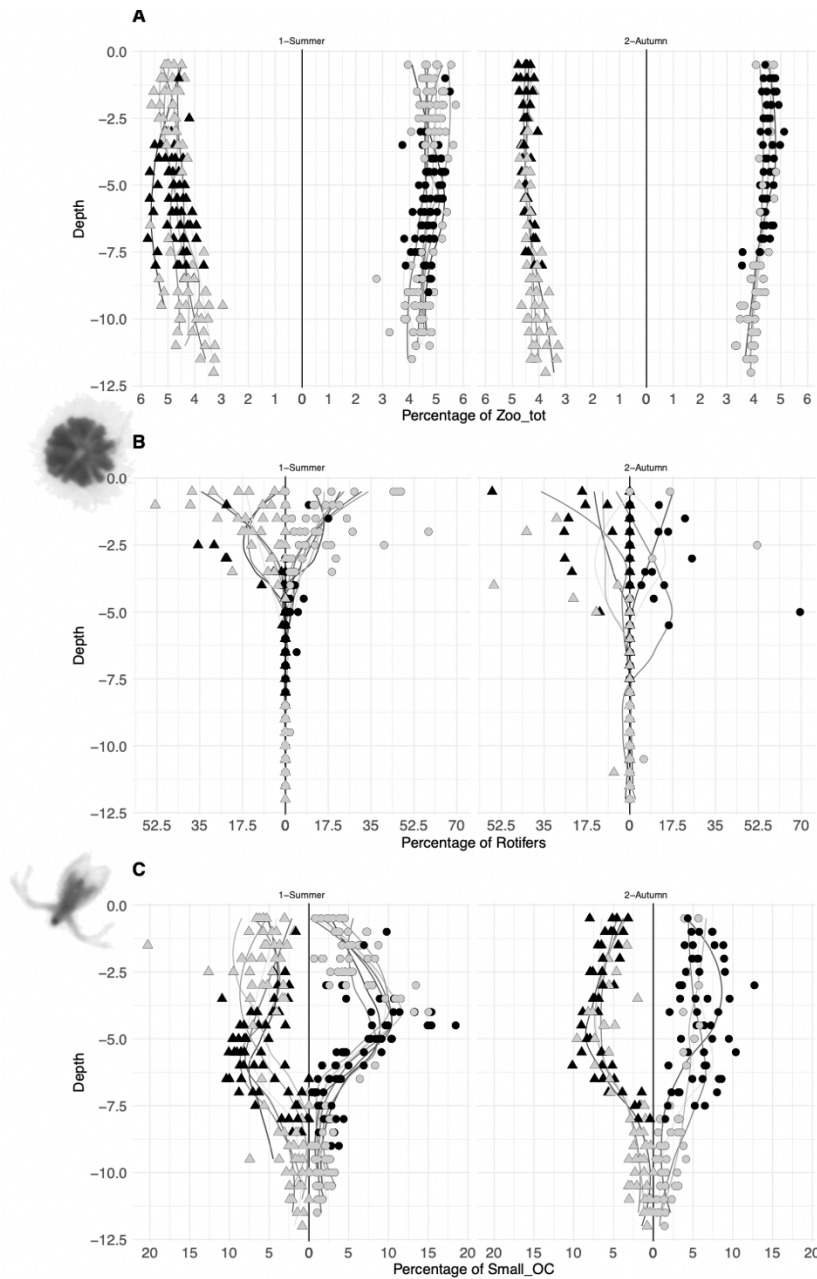


Figure 4: Depth distribution of Total zooplankton (A), Small Other Cladocerans (small OC, B) and Rotifers (C) during day (circles) and night (triangles). Samples from 25 July to 06 September in the left panel (1-Summer) and those from 10 September to 9 October in the right panel (2-Autumn). Grey symbols represent zooplankton measurement at depth free of fish and black symbols, those with fish presence. Curves represent smoothed depth distribution for each week, day and night.

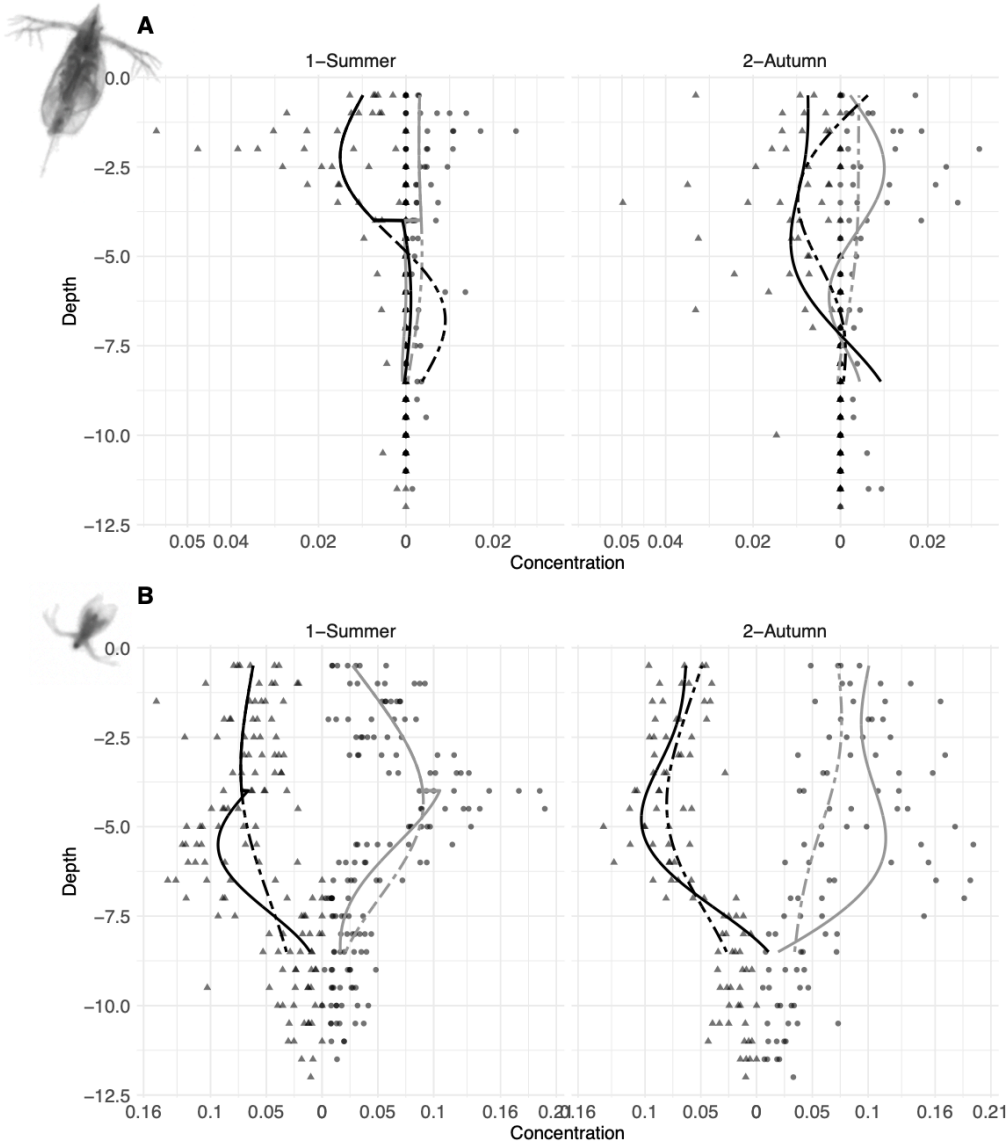


Figure 5: Model predictions (with *Model 4 + Fish*) for the depth distribution of *Large other cladocerans* (large OC, A) and *Small OC* (B) during day (grey curves) and night (black curves) in summer (left panels) and autumn (right panels). Predictions were made with the fish presence/absence variable as always absent (dashed lines) and as present for depth below 4 m in summer and all depth in autumn (solid lines). In both cases, fish was set to absent in the surface layer which imply an overlap of the dashed and the solid lines in the epilimnion. Observed values of zooplankton depth distribution were added with circles (day measurements) and triangles (night measurements) in grey or black if the point of measurement was free of fish or with fish respectively.

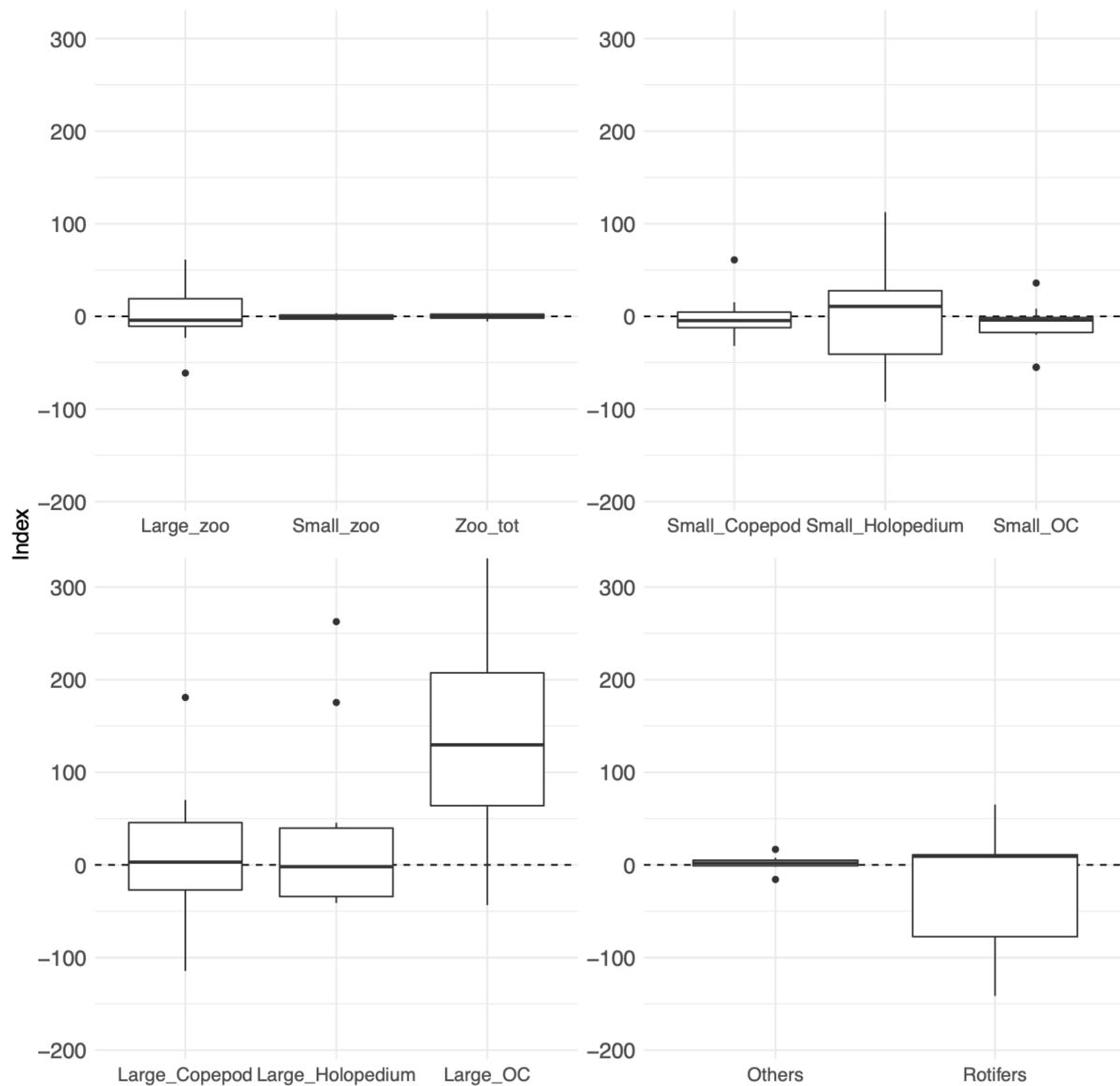
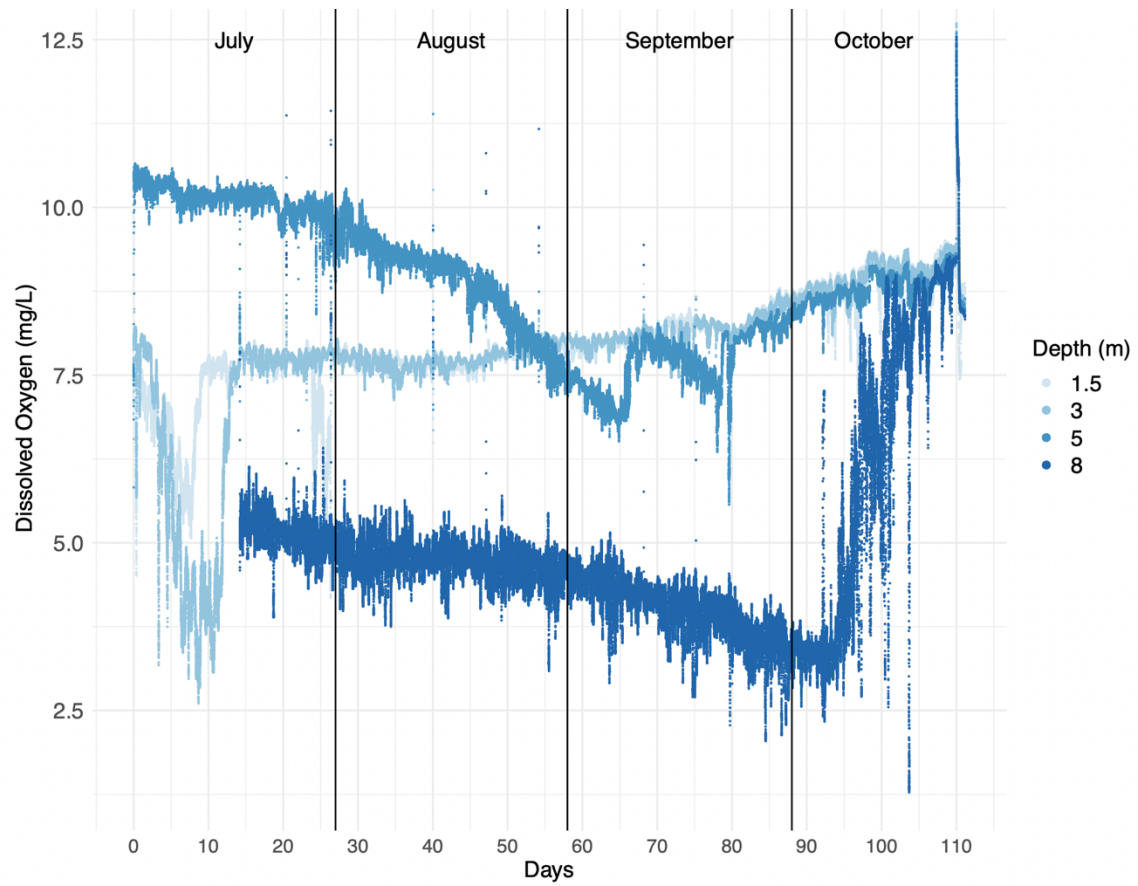


Figure 6: Boxplot of Doubek indexes calculated for each zooplankton category and date where night and day sampling were available (10 dates). Positive values indicate a diurnal deficit for the category considered whereas negative values suggest a nocturnal deficit.

Supplementary

S1: Seasonal variations of dissolved oxygen concentrations at the four depth with probe recordings. Recordings started on the 4 July.



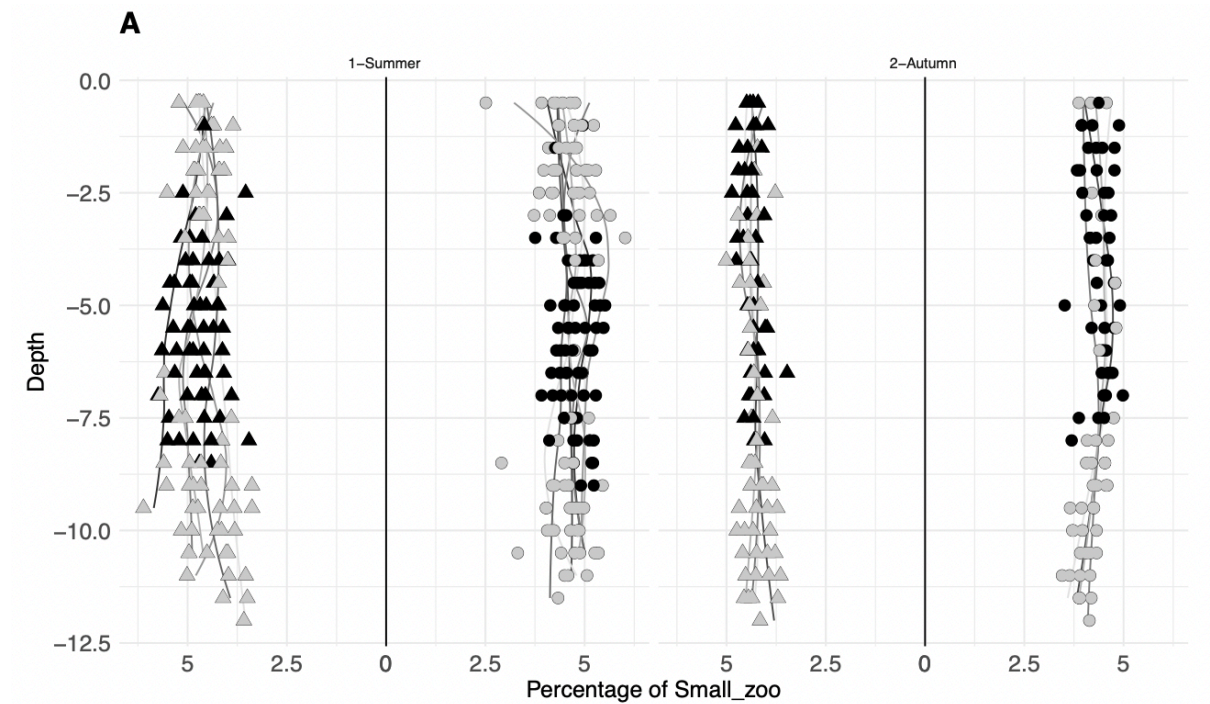
S2: Example of R code to run the DVM models using GAMs with the **mgcv** package

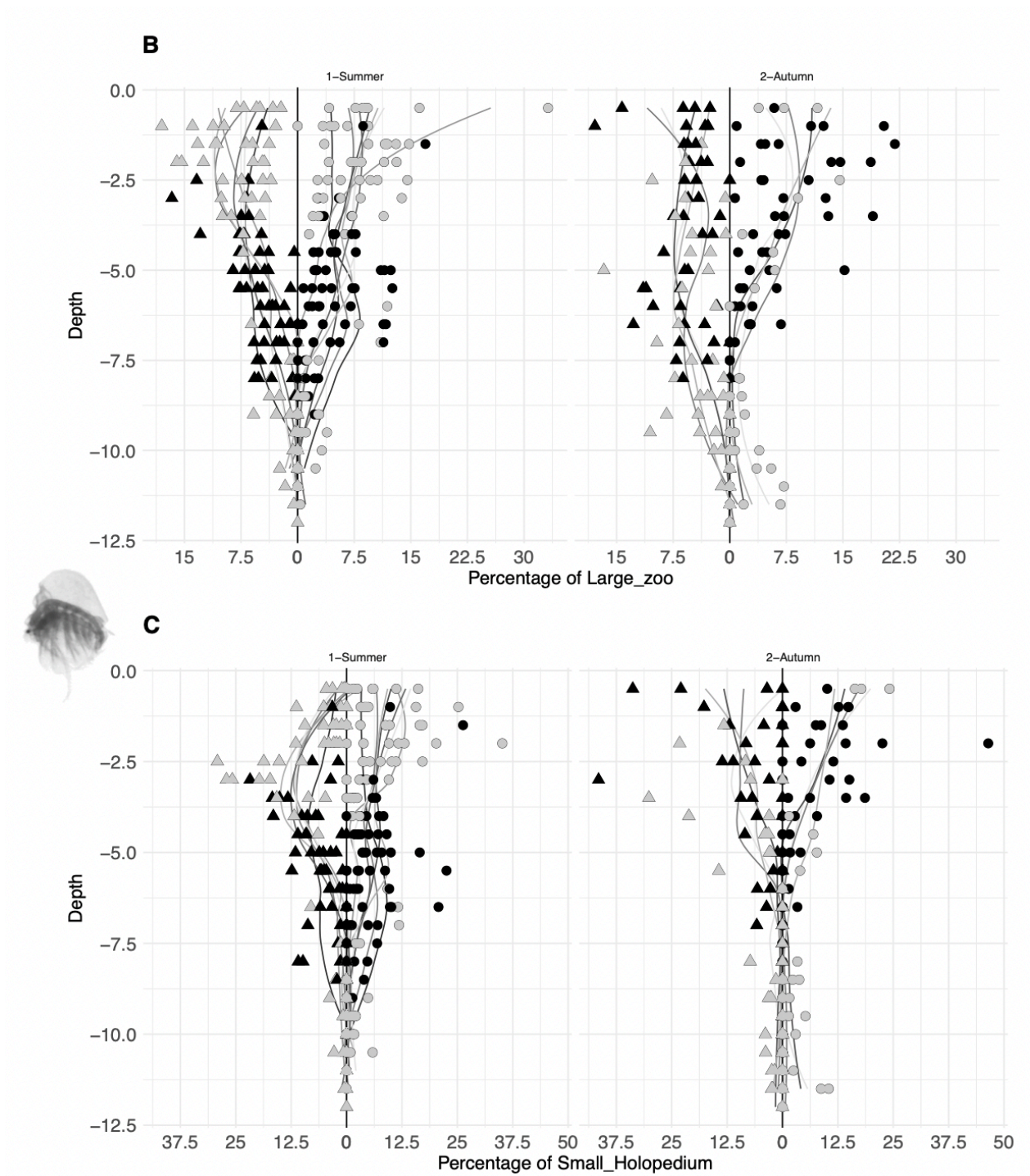
```
mod1 <- gam( Zooplankton ~ s(Depth,k=5,fx=TRUE)+  
              s(Date,bs="re"),  
              data=Table,method="ML")  
  
mod2 <- gam( Zooplankton ~ s(Depth,k=5,fx=TRUE,by=Period)+  
              s(Date,bs="re"),  
              data=Table,method="ML")  
  
mod3 <- gam( Zooplankton ~ s(Depth,k=5,fx=TRUE,by=Season)+  
              s(Date,bs="re"),  
              data=Table,method="ML")  
  
mod3bis <- gam( Zooplankton ~ s(Depth,k=5,fx=TRUE,by=Fish)+  
                s(Date,bs="re"),  
                data=Table,method="ML")  
  
mod4 <- gam( Zooplankton ~ s(Depth,k=5,fx=TRUE,by=interaction(Season,Period))+  
              s(Date,bs="re"),  
              data=Table,method="ML")  
  
mod4bis <- gam( Zooplankton ~ s(Depth,k=5,fx=TRUE,by=interaction(Fish,Period))+  
                s(Date,bs="re"),  
                data=Table,method="ML")
```

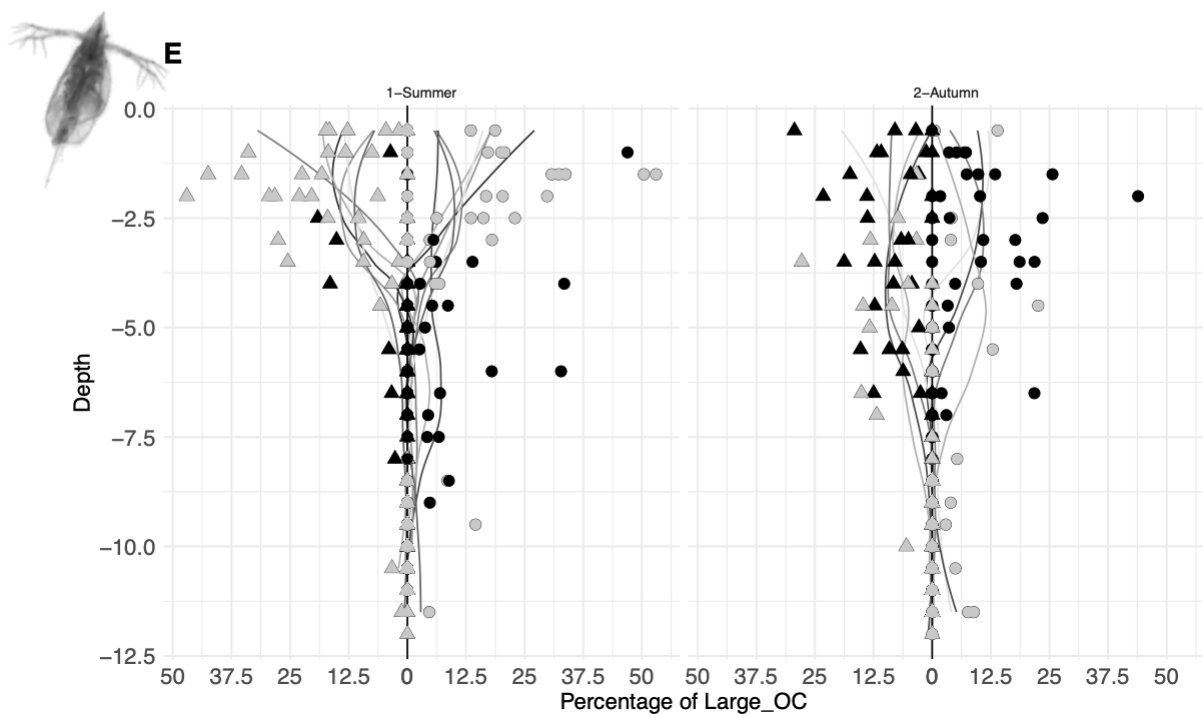
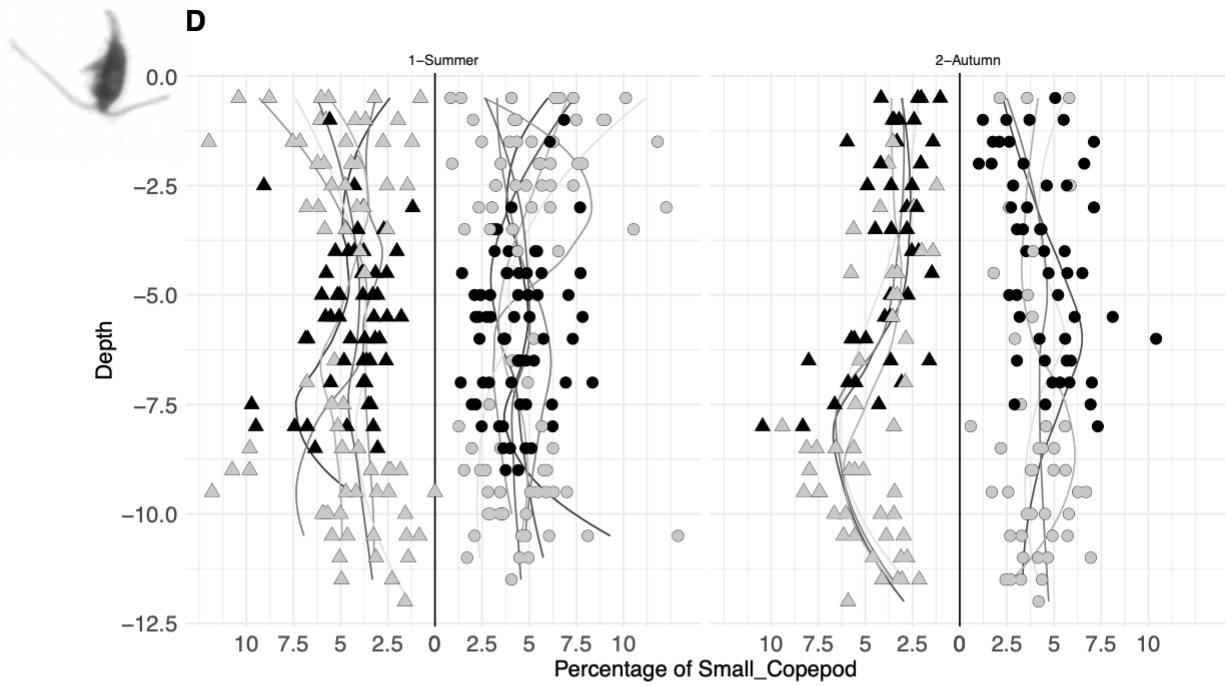
S3: Table with Δ_{AICc} and degrees of freedom (df) for each DVM models. In bold, Δ_{AICc} and df for the most parsimonious model.

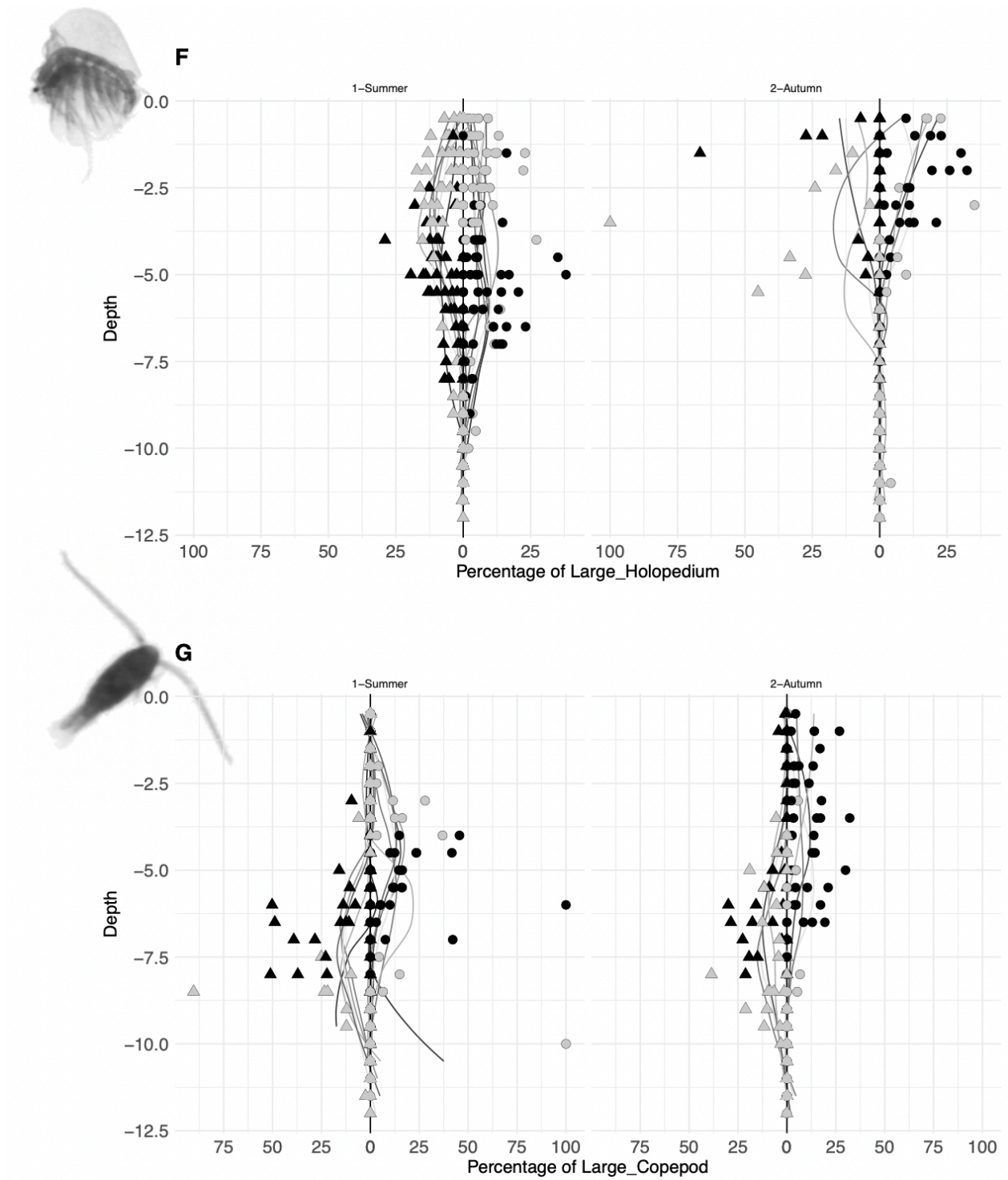
	Mod1		Mod2		Mod3		Mod3bis		Mod4		Mod4bis	
	Δ_{AICc}	df	Δ_{AICc}	df	Δ_{AICc}	df	Δ_{AICc}	df	Δ_{AICc}	df	Δ_{AICc}	df
Total zooplankton	0	14.6	4.6	18.6	7.5	18.6	5.7	18.5	8.6	26.5	12.4	26.6
Small zooplankton	9.4	15.0	11.3	19.0	8.5	19.0	15.5	18.9	0	27.0	26.4	26.9
Large zooplankton	25.2	14.6	25.4	18.7	16.8	18.6	28.9	18.4	0	26.7	30.2	26.4
Small OC	22.2	15.0	17.5	19.1	19.7	19.1	14.3	19.0	6.7	27.1	0	27.1
Small Holopedium	47.9	14.7	24.1	18.8	33.2	18.7	53.3	18.7	0	26.8	25.6	26.9
Small Copepods	46.2	14.6	35.8	18.7	9.5	18.7	38.7	18.7	0	26.8	32.4	26.8
Large OC	26.4	13.8	14.5	17.9	24.0	17.9	30.2	17.9	0.1	26.0	0	25.8
Large Holopedium	59.8	15.5	52.9	19.5	17.7	19.5	65.4	19.5	0	27.4	60.3	27.5
Large Copepods	82.6	14.6	26.1	18.8	81.8	18.6	87.4	18.6	0	26.8	30.6	26.7
Rotifers	56.7	14.3	62.2	18.3	0	18.5	40.4	18.0	7.8	26.5	53.1	25.1
Others	23.0	14.6	17.6	18.6	0	18.7	14.5	18.6	3.3	26.7	13.8	26.7

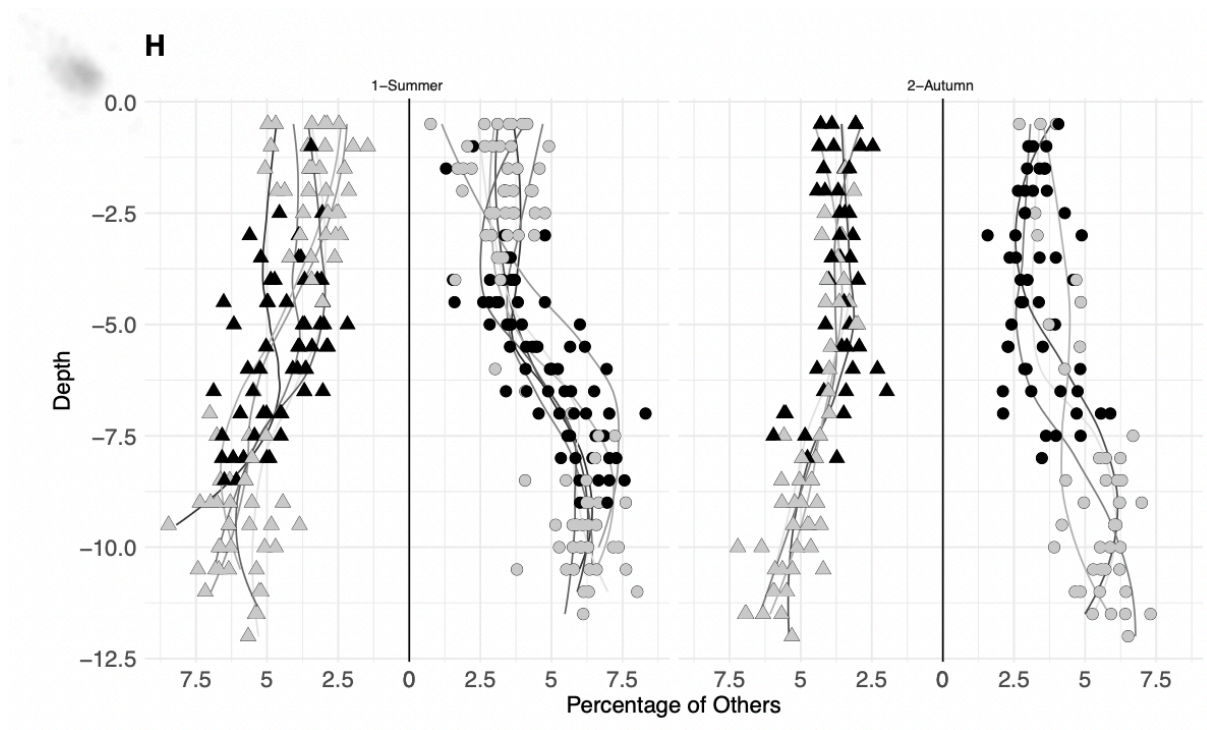
S4: Depth distribution of small zooplankton (A), large zooplankton (B), small holopedium (C), small copepods (D), large Other Cladocerans (Large OC, E), large holopedium (F), large copepods (G) and others (H) during day (circles) and night (triangles). Samples from 25 July to 06 September and in the left panel (1-Summer) and those from 10 September to 9 October are in the right panel (2-Autumn). Grey symbols represent zooplankton measurement at depth free of fish and black symbols, those with fish presence. Curves represent smoothed depth distribution for each week, day and night.



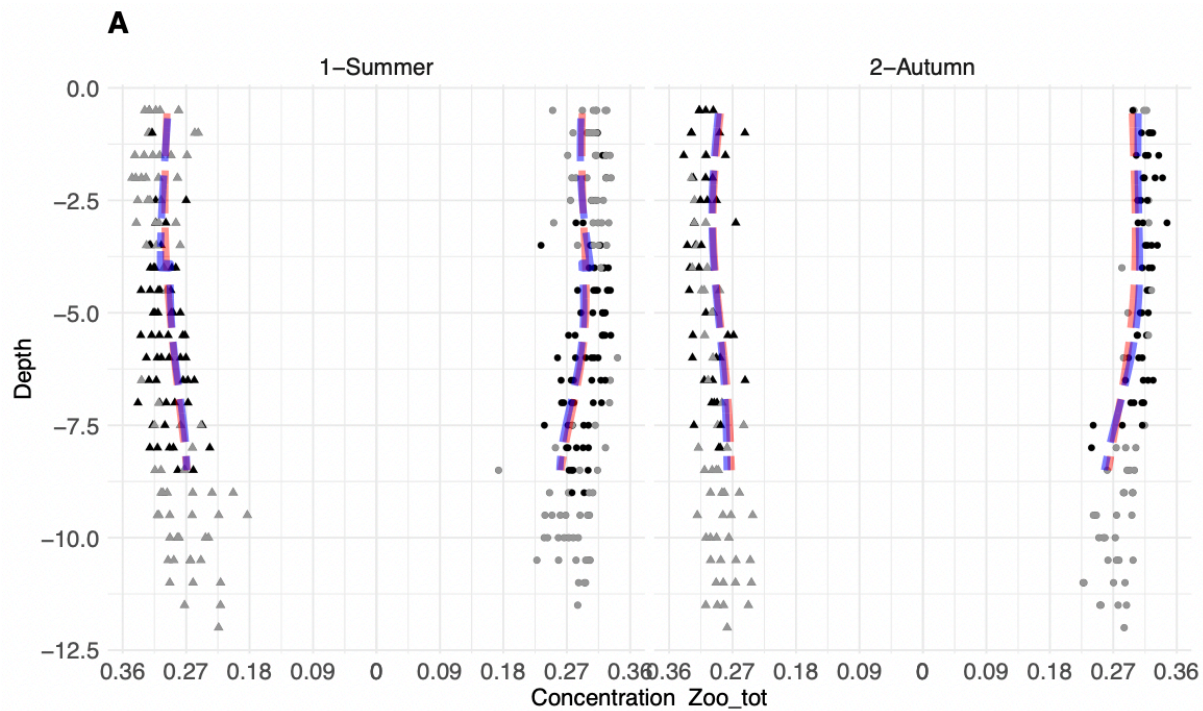


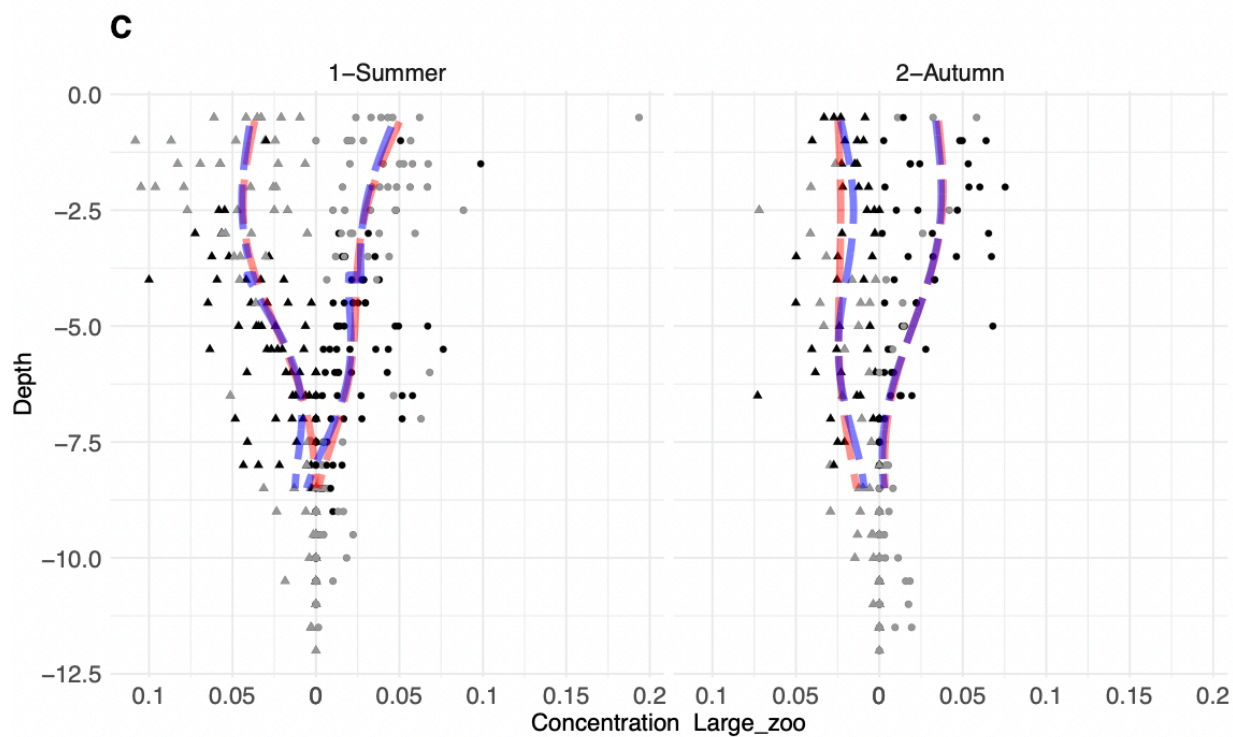
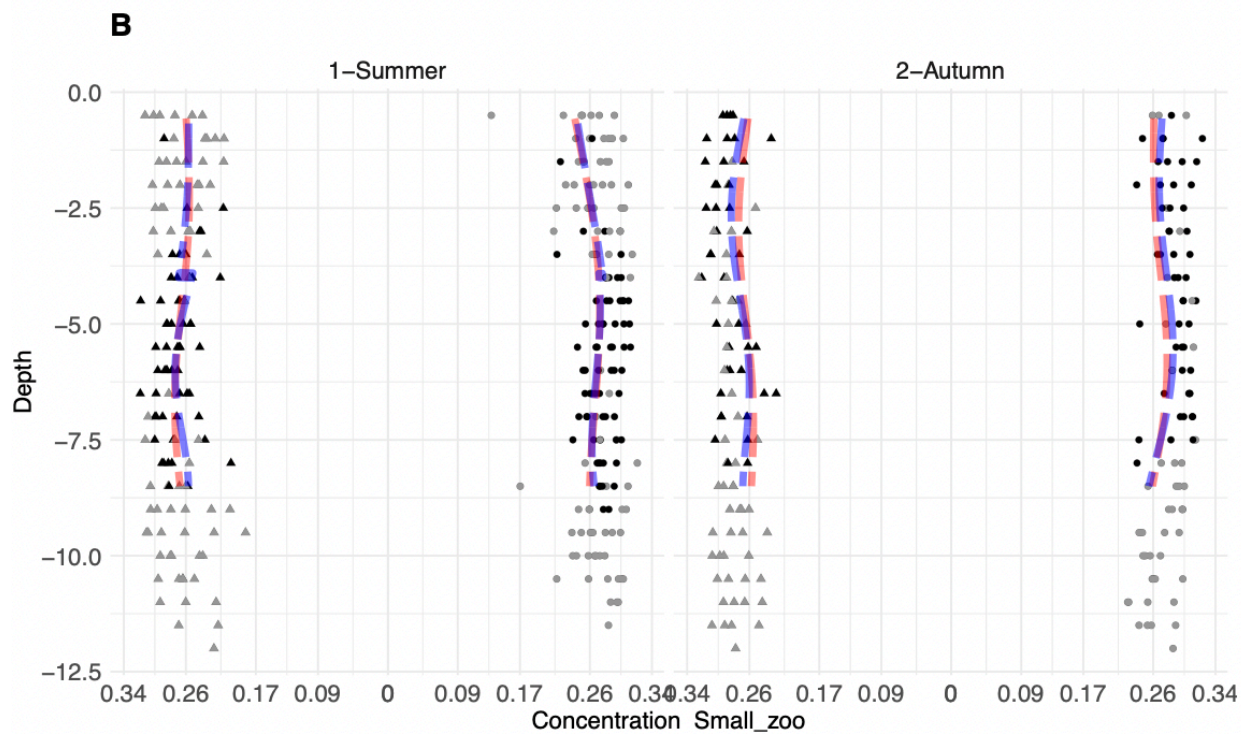


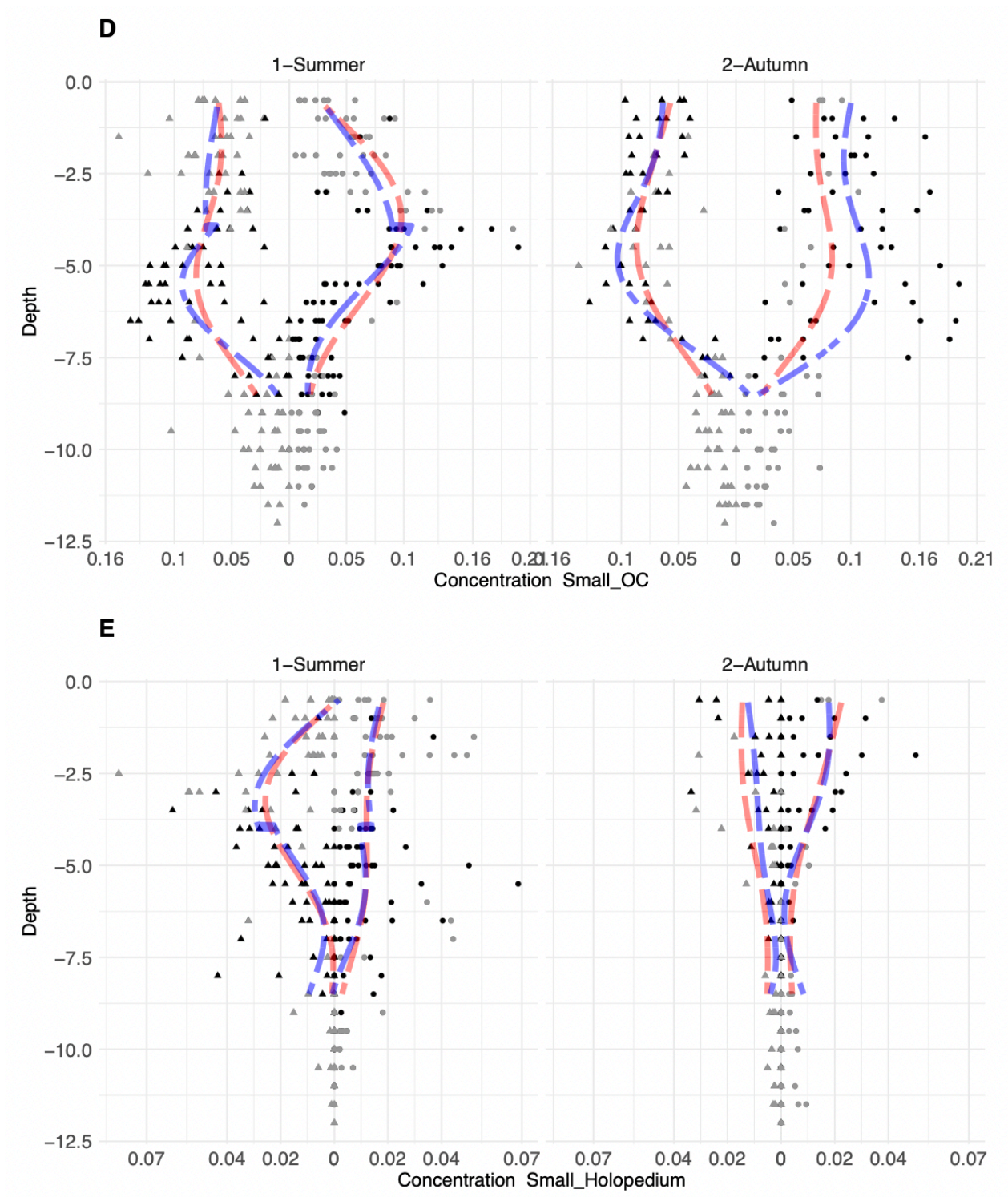


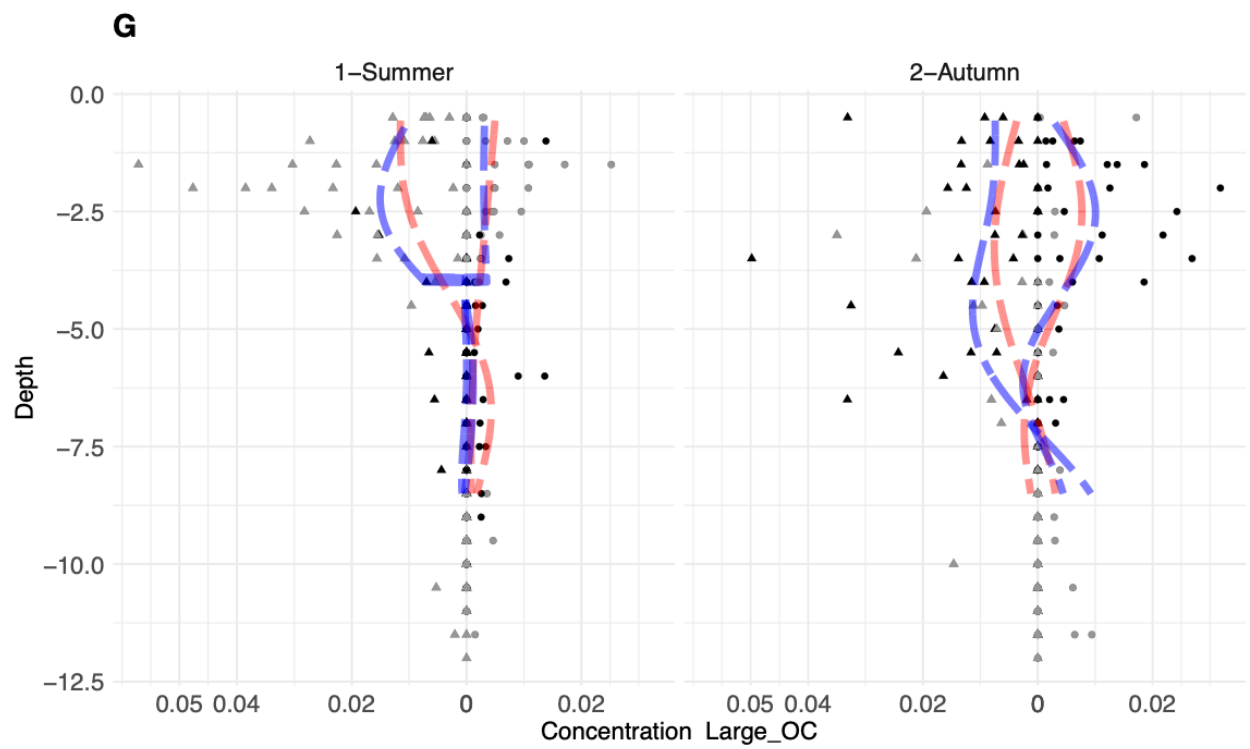
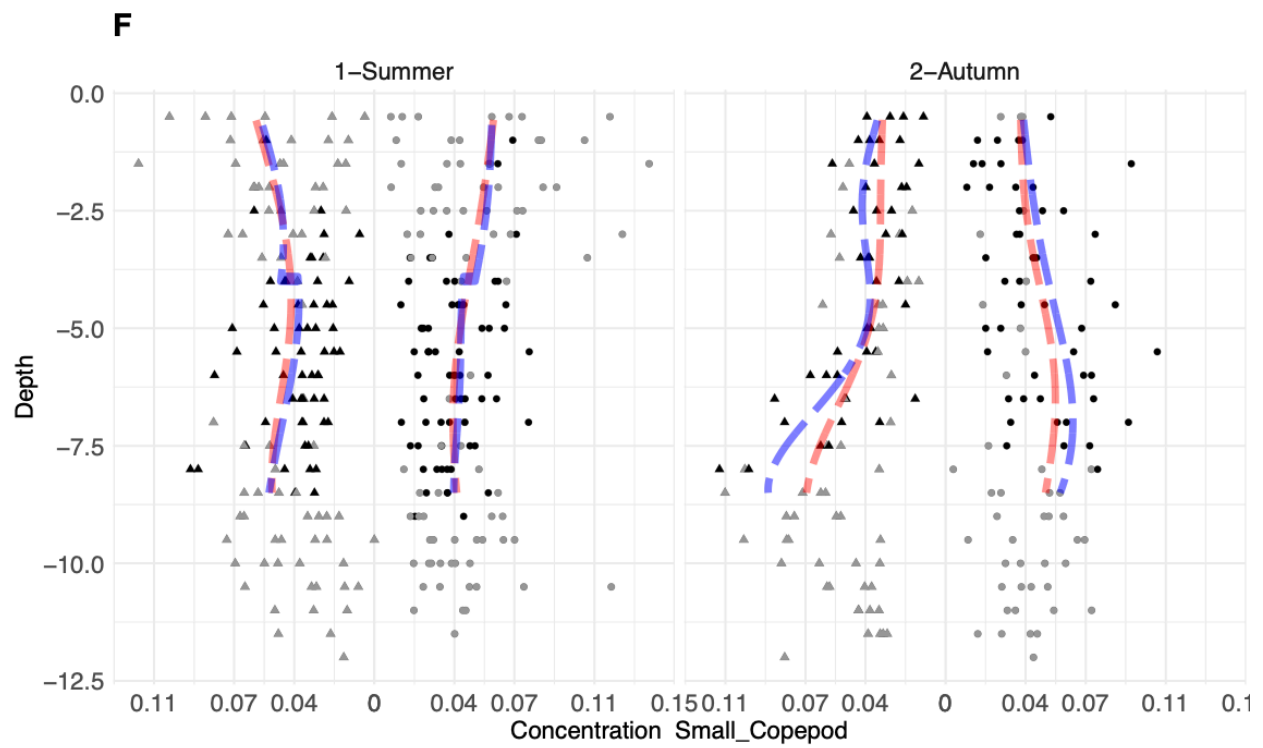


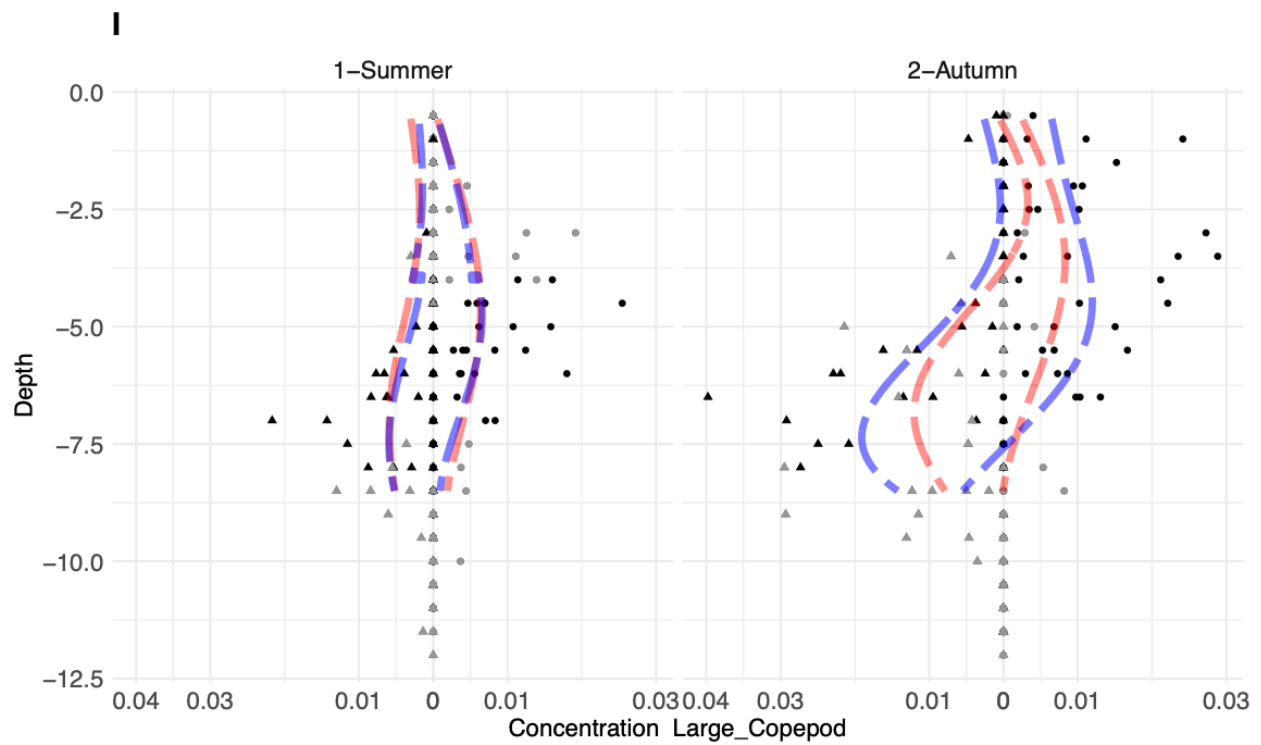
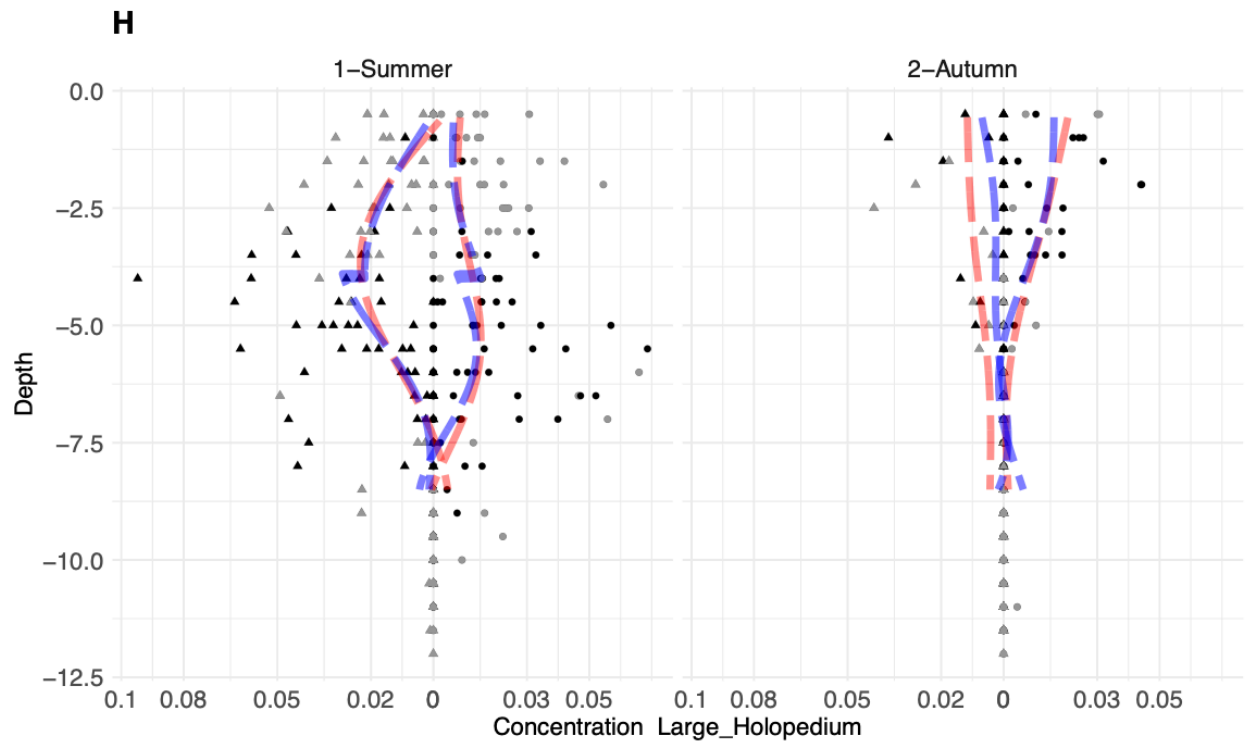
S5: Model predictions (with model mod4 + fish in blue and with mod4 in red) for the depth distribution of total zooplankton (A), small zooplankton (B), large zooplankton (C), small other cladocerans (D), small holopedium (E), small copepods (F), large other cladocerans (G), large holopedium (H), large copepods (I), others (J) and rotifers (K) in summer (left panels) and autumn (right panels) during day (curves to the right of the panel) and night (curves to the left of the panel). Predictions for mod4 + fish were made setting the fish variable as absent from the surface to 4m and as present from 4 m depth in summer to fit actual fish distribution. In summer, predictions were made setting fish as present for all depth. Observed values of zooplankton depth distribution were added with circles (day measurements) and triangles (night measurements) in grey or black if the point of measurement was free of fish or with fish respectively.

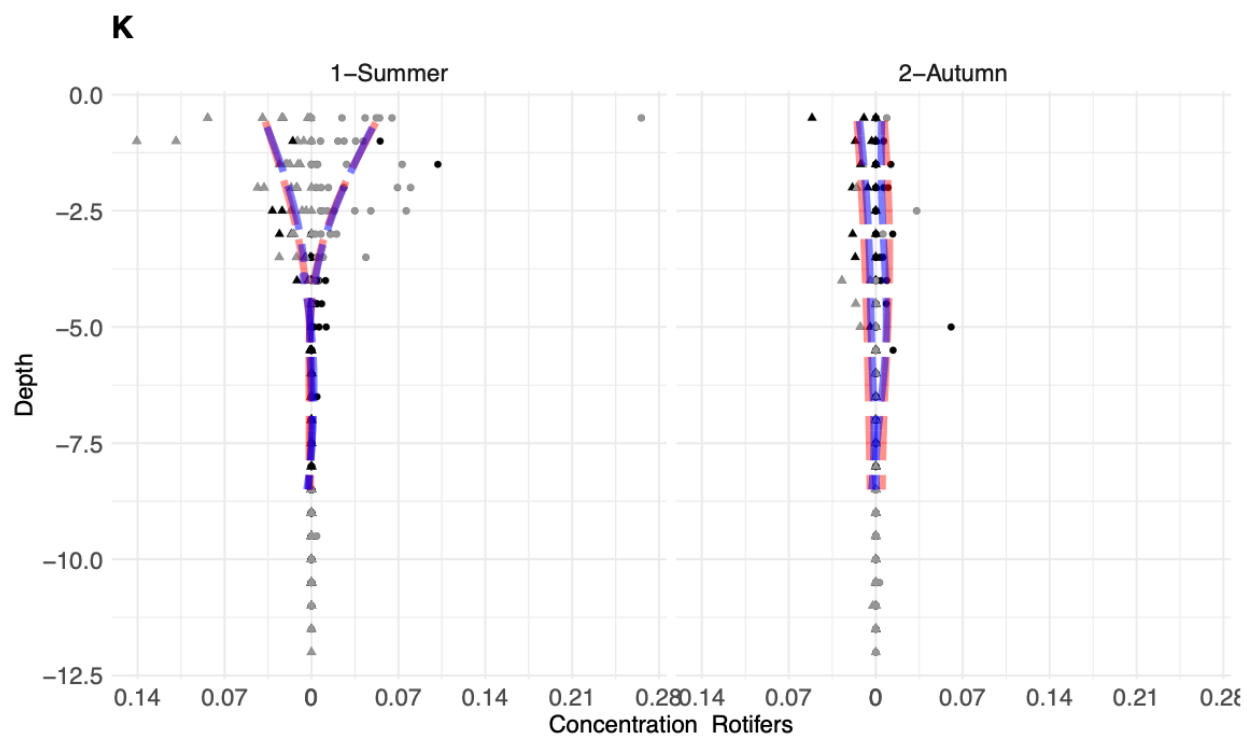
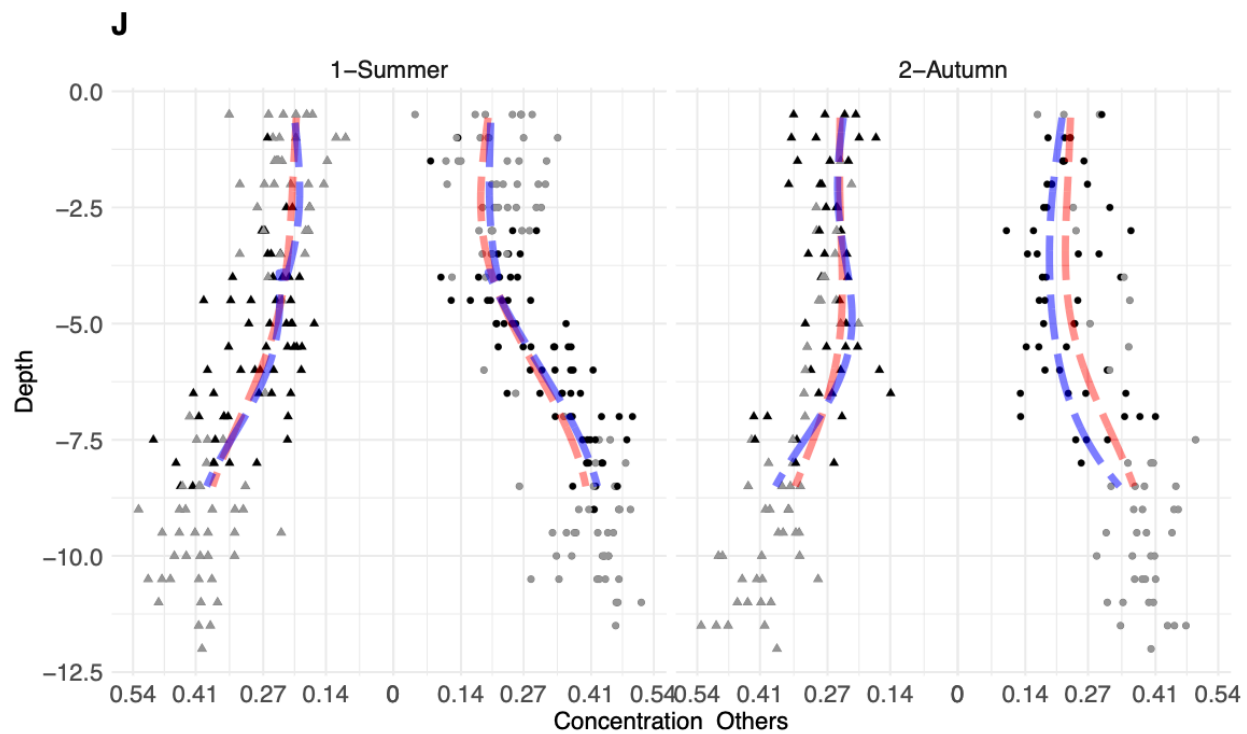












DISCUSSION

Synthesis and scientific contribution of the thesis

This thesis represents my research on the habitat coupling by predator and its impact on the ecosystem functioning of a small boreal lake. This is a typical system of Canadian Shield lakes and our findings thus brought valuable insights about the functioning of these ecosystems and the trophic relationships within them. I aimed to highlight that even at a small spatial scale (i.e., a few meters), a predator was involved in the connection between different trophic networks (i.e., littoral and pelagic areas). I especially aimed to show how the occurrence of a thermal barrier, disrupting the access of some habitats by the predators and therefore modifying the levels of predation risk among habitats, could change the structure and behavior of prey populations. I adopted two levels of analysis in the thesis. First, I analyzed habitat coupling from the prey perspective to emphasize the impact of variable fish predation risk on zooplankton size structure and composition. Second, I adopted the fish perspective to demonstrate how the predator selected its habitats according to prey availability but also seasonal thermal constraints. These two approaches confirmed that predator-prey relationships were not as linear as expected but rather, related to fish preferences, zooplankton vulnerability, interactions with other trophic compartments (i.e., phytoplankton) and local environmental conditions (i.e., summer thermal stratification). Finally, in order to show how the predation-induced habitat coupling could have effects on some ecosystem functions at very fine scales, I focused on the impact of fish depth distribution on zooplankton migration behavior. I showed that unusual migration patterns occurred when fish were thermally constrained and avoided the epilimnion despite the favorable light environment for a visual predator. By showing the great influence that fish could have on the ecosystem when the habitat coupling was broken or restored, this thesis provides a clear evidence of the effect of predation-driven impact on trophic networks structures and ecosystem functioning.

In Chapter I, my objective was to highlight the within-lake diversity of habitats and trophic networks in relation to predator-induced habitat coupling. I extensively sampled the zooplankton community in several habitats experiencing a gradient of fish predation risk ranging from almost no fish to a continuous fish presence. I studied the size structure of the zooplankton community according to this gradient using the Normalized Size Spectra analytical framework (NSS; Sprules & Munawar 1986, Sprules & Barth 2016). I found that distinct communities inhabited pelagic and littoral areas (i.e., accessible by fish or not during summer). Specifically, more abundant zooplankton was found in the shallow western basin, where fish

were absent during summer, suggesting a lower impact of predation on the zooplankton community. In the deeper basin, an unexpectedly high proportion of large zooplankters was found, especially jelly taxa (i.e., *Holopedium glacialis*). Since fish are more likely to feed on large individuals, this suggests that fish might be less important than *Chaoborus* sp. (gape-limited predators feeding on smaller zooplankters, without capsule) in the actual predation pressure. Although spatial differences in the zooplankton community might be related to confounding variables other than the fish predation risk (e.g., *Chaoborus* sp. or primary productivity), the seasonal differences from summer to autumn suggest that fish predation risk was indeed the central factor involved in shaping the structure of the zooplankton community. NSS parameters (i.e., proxies of the size structure of the communities, Sprules & Barth, 2016) changed abruptly when fish were able to go in the littoral areas in mid-September, as opposed to a gradual variation in line with temperature decrease: both littoral and pelagic communities showed a decreasing proportion of large individuals while their overall abundances remained constant over time. This suggested an increased impact of fish predation on both zooplankton communities in autumn, demonstrating the restoration of the habitat coupling by fish between pelagic and littoral areas at this time of the year. As a matter of fact, changes in zooplankton size structure in the pelagic community in autumn (i.e., unexpected decrease of large zooplankton proportion) also suggested a restoration of habitat coupling in this basin (i.e., between epilimnion and the rest of the water column), although we did not expect to find such a strong signal with the first chapter.

In Chapter II, I integrated fish movements to further understand the functioning of predator-prey relationships. Here, I coupled high frequency zooplankton sampling and fish positioning to map their distributions in addition to environmental variables. My objective was to demonstrate the seasonal variability of resource and habitat selection by fish depending on the thermal constraints. Furthermore, I investigated whether inter-individual differences in fish could affect predator-prey relationships. First, I showed unequivocally that a thermal barrier effectively prevents the brook charr from accessing the epilimnion or littoral areas during summer. Mapping zooplankton showed that large conspicuous zooplankters were concentrated in the epilimnion (Figure 2) whereas almost none of the tracked fish occupied surface waters (i.e., depth < 3m) or the shallow western basin during summer. This suggests that fish were unable to make a coupling between epilimnion and deeper waters in the summer. However, the majority of fish were in the metalimnion and close to littoral areas suggesting a trade-off between thermoregulation and short feeding forays (Bertolo et al. 2011, Goyer et al. 2014, Pepino et al. 2015). The selectivity of zooplankton prey by some individuals supports this conclusion even if I found strong

inter-individual variations, with individuals never selecting zooplankton patches to some individuals selecting patches almost all the time. I also found inter-individual variations of the selected size or taxa of zooplankton prey. Both zooplankton vulnerable to fish predation (e.g., large zooplankton, daphnids) and zooplankton with defenses (e.g., small zooplankton, *Holopedium glacialis*) were selected by the individuals. This was not detected in Chapter I, where we found for example NSS non-linearities due to *Holopedium glacialis* abundances. This could be explained by a weak impact of fish on zooplankton during summer, due to their thermoregulatory needs. Finally, in autumn, a radical change took place in the habitat selection by fish, with an increasing occupation of epilimnion and littoral areas. On one hand, this suggests that brook charr fed on newly accessible zoobenthic invertebrates. On the other hand, the disappearance of the thermal barrier at this time of the year is concomitant with an increased predation of zooplankton by fish. This shows a restoration of the habitat coupling made by brook charr which connects both pelagic and littoral trophic networks in autumn. This chapter therefore strengthens the conclusions of chapter I about an indirect effect of fish predation on zooplankton communities in summer.

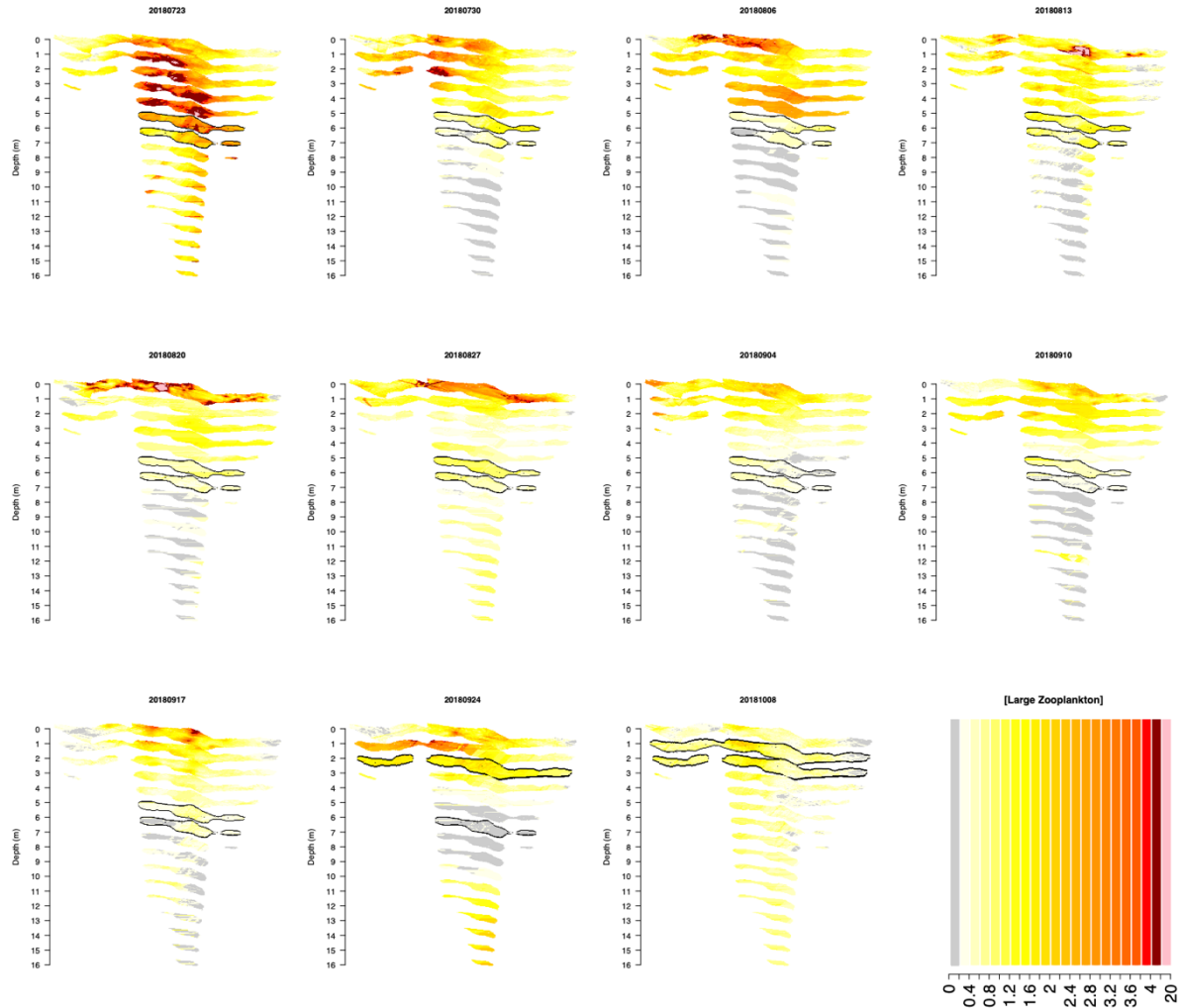


Figure 2: Spatial 3D distribution of the large zooplankton (i.e., > 1mm; in $\mu\text{L/L}$) during the sampling period. The layers depth circled in black represent the two most used layers by fish at each week.

In Chapter III, I investigated the consequences of the exclusion of fish from the epilimnion on prey behavior. My objective was to highlight that vulnerable zooplankters (i.e., more predated, large zooplankton) adapted their migratory behaviors in a unique way, as a response to the predator restricted depth distribution. Although we expected a reverse migration of these zooplankters, especially daphnids, we found diverse migration patterns depending on the taxa. Only few taxa significantly avoided fish isobaths occupied by fish during summer (i.e., stayed in the epilimnion). Interestingly, this was also true during the night albeit fish predation risk was low and the phytoplankton maximum density was deeper, possibly as a consequence of a selection for the thermal resource. This suggests that fish predation

pressure was strong enough to repel these zooplankton taxa into the surface layer (at the detriment of an access to phytoplanktonic resources), which was not true for other taxa like copepods or *Holopedium glacialis*. However, migration occurred in these latter taxa as well. The results show a weak evidence that predation pressure exerted by *Chaoborus* sp. influenced the migration in these other taxa. Interestingly, I demonstrated in Chapter II that some fish were targeting small zooplankters or *Holopedium glacialis*. I showed here that this selectivity by some fish was not sufficient to induce a behavioral response from these zooplankton categories. This support the results of Chapter I showing that *Holopedium glacialis* was not affected and even favored (i.e., secondary structures) by fish presence in the deep basin, even when their depth distribution was similar to the one of the fish. One could argue that during summer, fish are excluded from warm areas, where prey can find refuge (vulnerable zooplankters but also zoobenthic invertebrates). The small zooplankton, copepods or *Holopedium glacialis* are either not conspicuous in the environment or possess defenses (e.g., jelly capsule or escape jumps), and therefore are not strongly affected by fish predation during summer. This could lead to a lack of food for the brook charr during summer in these boreal lakes. In contrast during autumn, once the habitat coupling is restored, brook charr can go in the littoral areas, rich in zoobenthic prey, but also select areas where a large number of zooplankton is available, after a summer of scarcity.

In this dissertation, I was able to show that the predator-prey relationships and the ecosystem functioning within a small lake could be considerably contrasted through time and space. The habitat coupling induced by highly mobile predators is responsible for the connection of relatively distinct trophic networks. In the absence of predator-induced coupling, I observed variations of the trophic networks structure with potential consequences on some ecosystem functions. I highlighted the mechanisms involved in the habitat coupling by brook charr, specifically how a thermal barrier affected the movements and behavior of the fish and thus the fluctuations of predation risks for prey. Finally, I explored the potential cascading effects of the predation and the habitat coupling on the ecosystem functioning. I showed that fish and zooplankton could be decoupled, as well as zooplankton and phytoplankton. The disruption of the predator-induced habitat coupling could therefore have impacts on the habitat coupling generated by other organisms (e.g., zooplankton migration, phytoplankton). By impacting migratory behaviors in some zooplankton taxa, fish could have an impact on the phytoplankton grazing or on the export of matter and energy in deep waters (Steinberg et al. 2000, 2002, Steinberg & Landry 2017). Such a holistic understanding of habitat-coupling effects throughout the entire trophic networks of interconnected ecosystems is absolutely central to better predict the impact of potential future

interruptions of habitat coupling on various ecosystems. As my thesis points out, this is especially relevant in aquatic systems where climate change will induce warmer and longer summers, with more frequent heat waves, disruption of seasonal timing or strengthening of thermal stratification (Keller 2007).

Research perspectives

This thesis brought many answers in the field of predator-prey relationships, especially between cold-stenothermic fish and zooplankton. This was an ambitious project where I combined various methods with high-frequency sampling and fine spatial scales. This allowed to understand comprehensively the functioning of the trophic networks and the habitat coupling induced by predators, with an unprecedented precision in the field. Nevertheless, I am aware that a large amount of data is still needed for a better understanding of the ecosystem functioning. For instance, while benthic invertebrates are known to constitute a large part of the brook charr feeding regime (Magnan et al. 1988, Bourke et al. 1997), I was not able to assess their abundance, size structure and spatial distribution. In order to complete this work, but also to deepen our knowledge of predator-prey relationships, habitat coupling and ecosystem functioning, additional ecosystem features should be evaluated and integrated. While evaluating this is a non-trivial task, that requires extensive sampling as well as considerable funding, advances in technologies and artificial intelligence represent a potential avenue of these limitations. In Lake Ledoux for example, I set up a network of underwater cameras with the aim to capture the zooplankton community structure at a high temporal resolution. These prototypes need to be improved to stay immersed but could be used to cheaply monitor zooplankton, zoobenthos or other small organisms. Furthermore, recent advances in deep learning and image recognition algorithms offer the possibility of efficiently analyzing millions of pictures and even films (Lopez-Vazquez et al. 2020, Salman et al. 2020, Zhang et al. 2022). In order to better understand the habitat coupling related to predator-prey relationships, we could also imagine to equip a predator with a camera, in addition to the tracking device, to continuously monitor its environment or prey responses (Ryan et al. 2022, Sales-Baptista et al. 2022). Ideally, these questions should be investigated from both the predator and the prey perspectives, given the action of the former can have variable impacts on the latter.

Monitoring predator movements is the first step to appreciate the habitat coupling resulting from predator-prey relationships. Yet, knowing a predator's movement is not enough to be certain of its role in the connection between trophic networks. Although the detection of changes in the prey community can support the hypothesis for such a connection, actual information on the consumption of prey by predators

is needed to have robust conclusions. Stomach contents are often used to determine what an animal feed (Magnan 1988, Lacasse & Magnan 1992), but this is limited when for example one want to adopt a long-term approach (i.e., without sacrificing individuals), and combining such data with movement data can be challenging. A potential solution would be the automated detection of signature movements with “path segmentation” which would indicate a predator is searching for prey, hence confirming the connection between the predator and this specific trophic network (Edelhoff et al. 2016). However, such methods must be fine-tuned for each species given the variation in searching techniques (e.g., handbuch vs. active searching predators). It is more difficult to estimate the nature of the consumed prey without stomach content or video data. For this reason, the best alternative, to date (the one I applied in this thesis), is to indirectly get this information by studying the response of the different prey species, even if other processes are possibly involved, like competition. A survey based on isotopes is also a possibility, but this implies the capture and the sacrifice of the predator at the end of the study period, which is not a simple task. In addition, while this technique allows data collection on different feeding habits at different time scales (Rainville et al. 2021b), matching the temporal resolution to the movement monitoring would be challenging.

Furthermore, predator movement analyses to describe predator-prey relationships are conducted within a relatively small portion of the population and is necessary to increase sample size to ensure that the data are representative of the population studied. In the case of Lake Ledoux, I excluded juvenile brook charr from the project since they were too small to bear an acoustic transmitter. However, they significantly predate on zooplankton and their thermal tolerances are not the same than those of the adults, affecting differently the trophic network and the habitat coupling (Marchand et al. 2002). This should therefore be considered in further studies. Moreover, the concern about the representativeness of the monitored fraction of the predator population can be extended to the community of predators. Indeed, several predator species can have cumulative or interactive consequences on the prey community (Magnan 1988, Lacasse & Magnan 1992). In the Canadian Shield lakes for example, white suckers (*Catostomus commersonii*) and creek chubs (*Semotilus atromaculatus*) were introduced by bait fishers and are other predators which influence the brook charr habitat use due to interspecific competition (Magnan 1988, Lacasse & Magnan 1992, Rainville et al. 2021a). Investigating the role of the three species in the habitat coupling (i.e., movement analyses), could therefore improve our understanding of the habitat coupling in presence of introduced or invasive species (Magnan 1988, Lacasse & Magnan 1992, Rainville et al. 2021a).

Prey community should be surveyed at the same time than predators to disentangle the predator actions from the actual effect they have on the prey community. In this thesis, I confirmed that a minimal taxonomic resolution in addition to size measurements are required to encompass the community response to predation in aquatic systems. Furthermore, the spatial resolution of the sampling method should match the order of magnitude at which the predator moves and makes decisions. Achieving such a match between prey and predator sampling is key to accurately studying the predator-prey relationships in an ecosystem and the resulting habitat coupling. In order to increase the integration of trophic compartments, future studies should optimize their sampling with new technologies as I did in the thesis and go further into the integration of the whole trophic network. For example, next-generation of echo sounders are able to autonomously obtain the spatial distribution of organisms, encompassing zooplankton, juvenile fish or fish in a very non-invasive way (ASL Acoustic Zooplankton Fish Profiler™). Associating high-frequency sampling of different trophic compartments is a promising path to unravel the functioning of ecosystems. Therefore, I recommend the combined use of instruments such as the UVP or echo sounder for zooplankton, and eventually zoobenthos and juvenile fish, the fluoroprobe or automated flow cytometer for phytoplankton, acoustic telemetry for predators and Laser In-situ Scattering and Transmissiometry for bacteria, phytoplankton and particulate matter (Serra et al. 2001, Leroux et al. 2018). This will allow investigation on the cascading effect of the variation of the habitat coupling induced by top predators on trophic networks compartments.

The comprehensive approach I recommend, integrating prey and predator relationships at the level of ecosystems, could also inform us about the impact of habitat coupling disruptions at the individual level. The data I collected and the resulting conclusions about the impact of the thermal constraint on the ability of fish to feed during summer raise questions about how a predator could be impacted when its main feeding habitat becomes inaccessible. An interesting way to predict this phenomenon at the individual level would be to implement bioenergetic models based on the prey distribution, temperature and the metabolic rates of predators (Deslauriers et al. 2017). This could determine if the predator has enough food to ensure the maintenance of its metabolism, growth and reproduction when the habitat coupling is disrupted. Identifying such phenomena are necessary to assess potential impacts of anthropogenic disturbances of the habitat coupling on predators, which could result in cascading effects, strengthening the disruption of this same habitat coupling.

Conclusion

In this thesis, I portrayed the trophic dynamic in a typical boreal system. I showed the strong influence a thermal barrier preventing the habitat coupling had on the predator-prey relationships and, hence, on the structure and behavior of the populations in the system. This project provides precious information to appreciate the specific role of predators in the habitat coupling and ecosystem functioning, especially in relation to thermal constraints. We showed at a small scale that the consequences of such constraint could be diverse and numerous, from a change of the size structure, composition and behavior of the prey population to the modification of the predator behavior, with impacts on their ability to feed. In a context of rapid global changes, these changes in habitat connectivity will occur more often and for longer periods in the future, disturbing existing trophic networks. This thesis showed how important the impacts of such changes could have on ecosystems and trophic networks. Future studies should extend the approach presented in this work to fully understand this field of ecology.

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