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Title: Differential lipid dynamics in stocked and wild juvenile lake trout

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Abstract: After 45 years of stocking, lake trout in Lake Champlain have started to exhibit strong natural recruitment, suggesting a recent change in limiting factors such as prey availability or overwinter survival. The relative abundance of juvenile wild lake trout varies among regions of Lake Champlain, which suggest the prey base, or foraging success, may vary geographically within the lake. One metric that can indicate differences in resources across regions is lake trout lipid content, which reflects the availability of food and serves as an important energy reserve for overwinter survival. We quantified total lipid content of stocked and wild age-0 to age-3 lake trout among lake regions and seasons. No spatial differences in lipid content were apparent, but wild fish had higher overall mean ± SE percent total lipid content (17.0 ± 0.7% of dry mass) than stocked fish (15.2 ± 0.7%). Lipids in fish stocked in November were high (35.1 ± 0.7% of dry mass) but dropped by spring (14.9 ± 1.3%) and continued to decline through autumn. Wild fish showed seasonal changes with winter depletion in lipids followed by summer increase, and a plateau in autumn. The lipid depletion in stocked fish poses two competing hypotheses: 1) the high lipid concentration is necessary for stocked age-0 fish to transition to foraging in the wild, or 2) the high lipid concentration is difficult to maintain on a wild diet and reduces survival in the first post-stocking year.

Authors' responses to reviewers' comments in red:

Reviewer #1: This manuscript describes the lipid status of stocked and wild lake trout captured in 3 locations and from 3 seasons in Lake Champlain. The authors note that stocked fish are placed in the lake with a very high lipid content which dramatically drops over the coming seasons to asymptote in-line with their wild counterparts. Although the authors sought to use lipid content as a proxy for lake trout condition in the three locations to help explain why they see more naturally produced fish in the central basin no differences were seen among these locations leaving the question unanswered.

General Comments

1.) The flow of logic seems to avoid talking about many other reasons why fish may be more prevalent in the center of the lake vs in other areas considered to be spawning habitats. Perhaps the lake trout are just migrating to the deeper colder parts or areas where they can be as far from shoreline areas as possible? Maybe they are avoiding predators or competitors in other locations? The alternative reasons were stated at the end of the introduction; we have expanded this section to make it clearer.

There seems to be a large focus in the introduction on the potential effects of spatial differences in diets due to spatial differences in the prey base but no actual discussion of what those differences are. Are more YOY alewife or smelt found in the central basin…are these even what YOY lake trout diets are composed of or do they mostly eat mysids and zoop? If lake trout are eating zoop, mysis, and fish which are more likely to lead to more lipid rich lake trout? Information on juvenile diets, and the lack of data on spatial differences in abundance of prey items, has been added to the second paragraph in the Introduction. YOY lake trout eat 99% Mysis, so the question of which prey has more lipid content is not relevant at this stage

Similarly, there is a lot of focus on diets of lake trout aged <4 as creating a bottleneck for the population yet no data or research is discussed that supports this claim. Where in the lake trout life cycle is the bottleneck for Lake Champlain? Are newly hatched swim-up fry abundant but nearly no age 1 (2 or 3) fish? Do we see large mortalities overwinter as is discussed repeatedly or do they experience more continual mortality? This has been added to the introduction.

Without the above two items the paper does not convince the reader of why the study was needed.

2.) I am unclear on if the analysis was done in a way that makes sense. Shouldn't the analysis be an ANCOVA with origin (stocked vs wild), Season, and Location as factors and length as the covariate? Maybe they did do this (lines 140 - 142) but the wording doesn't fully make sense of what happened and the results section does not include any results that indicate this was the case. Also with % data I have recently been seeing Beta Regression used but, if data is too sparse for the full model, perhaps some version of conditional inference trees or random forests can get at the idea of which of these factors best predicts lake trout lipid content. Yes, we used ANCOVA and have clarified this in the text. The logit transformation and the Beta Regression are both based off a Beta distribution; we have added a citation to Warton and Hui (2011) to support our use of the logit transformation for percent data.

Line Comments:
34-37 A more complete description of the lake trout population bottle neck is desired. It would then help describe why diets of juveniles are of interest in this study. What other factors may lead to differences in where lake trout are seen and why do we think it is not those that cause the differences? Added to introduction, see above.

40-41 "…risks…is" grammar does not seem correct Changed

44-51 size and age information seems necessary here since lipid content is so size dependent. Are the stocked fish that are found of similar size to the wild ones? Size information has been added to the next paragraph

53-54 This topic sentence does not seem necessary Omitted

59-61 where are these studies? An overwinter study in florida is very different from one in Nova Scotia. Location of the studies in not relevant, lipids are still needed to cope with stress
62 Convention is to generally avoid the term "health" since it cant readily be defined versus condition, relative weight, or lipid content. The word ‘health’ has been removed on line 55 but we have left the word in line 62 because it is clarified later in the sentence; Adams (1999) uses ‘health’ in the title of his paper.

74 What is the prey base and how does it differ spatially? What do these fish eat at the size you are looking at? Added to introduction, see above

75-78 sentences seem to contradict each other a bit, maybe the sentences are just missing a better description of the timeline they are talking about. We disagree, we feel the sentences work well together, but have changed the word ‘recently’ (for stocked fish) to ‘newly’ to avoid timeline confusion.

87 what size trout are stocked into these areas? This information has been added to the third paragraph

109-110 Here is some vague size information, I would repeat this in the intro and discussion as lipid content is dependent on length This information has been moved to the introduction

114 what categories were used for these size classes? We have added a citation to a paper that gives these size classes

135-137 This doesn't seem fully described, I think its missing that they subtracted from 1 (or 100) at some point in the calculation. This has been corrected

139-141 This makes it sound like I should be seeing results of an interaction test in the results and results related to covariates of origin and length but neither appear in the results. The results read as if two separate anovas were done 1.)Lipids = Location + Origin +(Length covariate) and 2.)Lipids = Season + Origin +(Length covariate)   Although neither paragraph mentions the length covariate nor that origin and the other factor could have interactions. We have added a citation that supports use of the logit transformation, and have noted that we tested for interactions and added a statement to the Results that no interactions were found

152-165   This section talks about the results of testing for location differences as well as stocked vs not stocked. However it is difficult to visualize the results dicussed due to Fig2 depicting the interaction of these two items. Consider including bar charts of both main effects and the interaction in the figure or reporting the numbers herein or in a table. Figures should show significant differences in some manner as well. Was there an interaction effect that was significant, if so it isn't discussed here. We have extensively revised this section to add clarity

208 - 213   If I am reading correctly this paragraph is only about stocked fish and this should be stated somewhere otherwise it seems contradicted by the next paragraph. This has been corrected by removing most of the cited paragraph and combining the remaining text with the following paragraph

244-246   Is the effect size shown a biologically important one? It seems to be a lipid content difference of 2 or 3%, is this enough to cause differences in mortalities? We have restated the sentence to address the relationship between lipid content and survival rather than construe that the 2-3% difference will change survivorship

257   what metabolic burdon is being mentioned? This is not explained.
Text has been clarified with additional information in this paragraph to explain our two competing hypotheses

Figures 2 and 3 only depict the interaction effects (season by origin or location by origin) yet the results discuss the main effects only making it difficult to follow. Main effects could be shown alongside the current bar graphs. We have substantially changed the figures to clarify the interactions among factors and covariate

Reviewer #2. I like the ideas presented in this paper and believe that the results merit publishing. Incomplete presentation of the methods and results, especially concerning the sample designs and the stats, needs to be corrected. I think the authors should revisit their statistical tests and provide a more complete description of what they did and how the tests were structured. The authors had 4 independent variables that require careful attention to interaction testing and the balance of the sampling design. These issues have been changed, see comments above. The sample values presented on the figures seem too small in relation to the collections and give the appearance of an unbalanced sample structure that will often complicate comparisons by supporting independent variable interactions.   Lipid storage is age and size dependent in fish and careful explanation of their treatment of these 2 similar but separate effects should be included. Even if the authors find no significant differences, I think for their results to be fully understandable as they relate to their hypotheses they need to present them for wild and hatchery fish separately. I think they should only present grand means for all samples if they believe that the measured values are homogeneous across the population. I also believe that their data will be valuable to future studies and would like to see some useable presentation of it within or attached to this paper. Presentation of the data has been substantially changed, with new figures that address these concerns.

Abstract

Line 13: What is "relative abundance" relative to. Suggest changing "The distribution of" to "The abundance of" and delete "in relative abundance". We made this change, except we retained the term ‘relative’, as absolute abundance was not measured and relative abundance is standard fisheries metric.

Lines 19-20: Rather than defining spatial and temporal just delete those terms and the parentheses around "lake regions" and "seasonal". Changed

Line 22: It would be helpful to give the month that pre-winter refers to. Done

Lines 22-23: This sentence would be simple if it were written in chronological order with winter depletions followed by summer increase. What is a cyclical summer increase - suggest deleting cyclical as the authors are already referring to times of year. These changes have been incorporated into the sentence

Line 24: The high lipid content of hatchery fish? The authors previously state that wild fish were higher. Since "hatchery" isn't introduced as an origin grouping until later in the paper it's difficult to understand what the authors are presenting here especially since it seems to conflict with their previous statement about wild be higher in lipids than stocked. Sentence has been changed to add the necessary clarity

Line 25: Not sure what feeding alteration is being suggested here? In other evaluations time of year when stocking took place was very important with fall and winter stockings being associated with greater post-stocking mortality. Again a lack of the context here (that appears later in the paper) affects the clarity. We have removed this sentence from the abstract.

Introduction

Line 31 Change was to were. Species (lake trout) is singular, ‘was’ is correct

Line 35: Suggest changing "did not begin" to "was not observed". Changed

Line 40: Over winter thermal stress is likely not a problem for lake trout; and for some prey species that move deeper in the winter their vulnerability to lake trout predation may increase. Changed sentence to state winter can be a period of high mortality – this is the conclusion reached by Hjort and generally understood in recruitment dynamics

Lines 44: Need more information concerning what juvenile surveys? More text has been added

Lines 53-54: Lipid storage would be a fish physiological response/activity, the metric would be lipid concentration. Done

Line 65: Suggest deleting "how fish respond to winter depletion to energy reserves". How does total lipid concentration tell you how fish respond, it's a measure of the presumed response. Done

Line 74: The prey base also has to be attainable (not thermally separated) to lake trout. Summertime is the most productive season, it should be self-evident that we are referring to lake trout prey, i.e., coldwater species

Line 77-78: "However, post-release stress could contribute" seems like discussion and I'd suggest moving the idea there (or earlier in the introduction) unless it bares directly on the statement of objectives. We disagree, this is part of the rationale/justification for the study

Methods
Line 85: Lake Champlain is along the borders of New York, Vermont and Quebec with waters in each jurisdiction rather than between them. This is incorrect, the border runs along the center of the lake

Lines 113-115: Ageing by finclip and size class overlap was presented here and later in the discussion the authors indicate fish compared were of the same age class (line 191) but they don't indicate how size class overlap was measured, how ageing differed between wild and stocked fish and whether age entered the analyses. The authors do use TL as a scaling factor, but do not indicate how they did that and don't elaborate in the results or discussion on whether it mattered. These issues have been addressed; see response to comments from reviewer 1

Line 113: Suggest inserting "for" before total length and removing the parentheses. Done

Line 132: How did the authors determine if samples were dry after the 3-day drying period? Was the amount of water left inconsequential in comparison to the amount of lipid removed? These are standard methods; all samples were treated the same way and were all less than or equal to 1 g. We did preliminary tests to ensure drying time was adequate

Lines 135-136: How did the authors come up with lipid percentage by dividing total sample weight by the weight of the extracted sample - that would give a value greater than 100%. Percent lipids would be calculated by weight of the lipids extracted divided by initial sample weight. So in order for the authors to get there they would need to subtract sample weight post lipid removal from sample weight prior to lipid removal and divide the difference by the pre-removal sample weight. Not sure what the authors are referring to in their description and they should clarify what they mean by converting their quotient to a percent. This was a mistake and has been corrected

Line 142: Is something missing from the parentheses? This is standard format for an R function.

Line 140: The methods are a bit confusing and seem incomplete. What were the interactive tests? Were those tests for interactions among the independent variables - if so what interactions were tested? The author's description here indicates they may have run ANCOVAs with length as the covariate - true? Why did they use a logit transformation? It is hard for me to follow the authors test structure here and whether is was appropriate. There is enough information published that indicate lipids are size and age related so they should present how specifically TL was used as a scaling factor. Also the authors talk about aging the fish in the methods and comparing same aged fish in the discussion so how was age used in the analyses? I think I would have approached this analysis by running ANCOVAs first with origin, season and site as the independent factors and TL as the covariate and tested for all interactions using type III SS. If interactions were present, I would have looked at the sample design to see if unbalanced samples were driving them or the factors themselves and then applied the appropriate tests for simple effects. Also where possible I'd examine whether the slope of the TL vs MPTLC relation remained constant across factors. We have substantially changed the presentation of the results; the new text and figures address these issues (see replies to reviewer 1).

Results

What was the variance for extracted fat values among samples tested from each fish? Was it generally low (e.g., <5%)? It would be very helpful to include this info somewhere so the reader can judge how much measurement variation contributed to ecological variation. We have added coeeficient of variation in the methods section to address this issue.

Line 147: A sample design table incorporating the distribution of samples across all factor levels (site, season, origin, TL (size range)) tested would help the reader understand how balanced the sample population was. We have added a table with this information.

Line 148: What time periods, sites and fish sizes were the means representative of? Was this just total sample means per fish origin? Does the interaction results and size relationships indicate that these values are appropriate? Since size is an important factor in lipid accumulation, the size corrected lipid values would make more sense in these comparisons. What were the size correction stats and how important was size to lipid values? Also since the stocked fish have a unnaturally high level of lipids leaving the hatchery the authors should state here what samples (seasons in particular) were included to produce the means. Why were the N values so low in the figures if 30 fish were used per collection? These questions are all addressed in the revised results section; the N of 30 fish was a target, not the final number sampled.

Lines 152-163: The authors indicate in the methods that they did "interactive tests", were there any significant interactions among the independent variable effects? Did the authors account for those in their comparisons (i.e., main vs. simple effects)? Were comparisons corrected for fish size and/or age and how? Were there significant size effects on lipid concentrations and were the slopes of those size effects consistent among comparisons? Was age used as an independent variable? These questions are all addressed in the revised results section

Line 152: Were origins tested for separately? Without knowing if interactions were significant, whether the authors were using a main effects model incorporating all variable effects including length or some other model was used looking at simple effects, what test was used (the methods seem to point both at ANOVA and ANCOVA), and what sums of squares was looked at, it is difficult to follow the results. Initially we tested origins separately, but based on the suggestion from reviewer 1, we have now included origin as a factor in a three-way ANCOVA

Lines 154-155: Where the authors state the wild fish showed more lipid content than stocked fish at the central and southern sites, were they referring to tests constrained within origins and seasons, or were those tests for values constrained within origins and grouped across seasons? Figure 2 doesn't seem to agree with the results text, it looks like only the north (and maybe the central) had a significant difference between stocked and wild? Also the caption on figure 2 does not give a complete description of what is being presented - are samples grouped across factor levels? The sample n's seem too low for grouping 30 fish each across 2 or 3 collection dates. We have changed the analysis and the figure, and included sample sizes for each group in the new table to clarify group size.

Line 158: Did the authors group wild with stocked fish for this result or did they group stocked and hatchery fish? This paragraph doesn't provide useful information that bares directly on the hypotheses, did wild and stocked have different values within seasons and different trends across seasons? Were comparisons size corrected? Did independent variable effects interact? What was the structure of the pairwise comparisons being referred to? Stocked vs. Wild grouped across site and TL? As above, the new analysis addresses these issues; size is now a factor in the analyses, interactions have been tested and reported, etc. These comments have been very useful for helping us to improve the analyses and description of the methods, and we trust our revisions are clear.

General: Why don't the authors look or present a closer look at the results for the wild and stocked fish separately? I suggest first looking within sites, seasons and origins to see if there is a significant length effect. If so run ANCOVAs separately for stocked and wild origins incorporating length as a covariate and both class variables (site and season) and all interactions including with TL in the interaction term. Examine seasonal and site trends, grouping where interaction tests indicate appropriate, still within origins. If you can't get to a point where you can test between origins over levels of site and season due to interactions try to constrain origin tests within site and season. Also examine the seasonal trend within origins especially if it is possible to group across sites. Based on comments from reviewer 1 we have elected to run a single 3-way ANCOVA and report the results of the main effects and interactions.

If the fish are different ages the TL/lipid relation may be complicated as the stocked fish within their first year of life will lose lipids from their artificially high hatchery levels as they figure out how to find their own food (like the authors indicate). After that first summer though the survivors should start making up lipid content and the overall seasonal cycle should be more natural and correspond more to environmental conditions. Did the authors parse out those stocked fish in their first year at large, or group those with stocked fish that survived into their second year? This point has been clarified earlier in the manuscript by explaining the size-at-age difference between hatchery and wild fish

Discussion
Line 166: Suggest changing "was" to "were" for smoother readability. Did not change. Suggestion would result in incorrect grammar.
There are many instances where the authors point out differences in MPTLC between wild and stocked fish so to indicate that there were no differences among sites is confusing, this makes me think that this main effect discussion is inappropriate. For example, In the results on lines 154-

155 the authors state there were differences at two sites (although the data presented in figure 2 seems to conflict with that). We have changed our text as no site differences were found. Additionally, the authors statement on lines 169-171 that wild lake trout had higher MPTLC refutes that there were no differences among sites, there had to be a difference somewhere if in general they were different. We did have a source effect, but that does not mean we need to have a site effect. This comment is confusing to us. However, our Results section has changed quite a bit so the text should be much more clear to the reader.

Lines 171-173: Figure 3 suggests that MPTLC was rather stable from summer-fall for stocked fish? In the original figure 3 (now replaced), lipids showed a steady decline from fall to the following fall. Fig 3 caption needs more explanation of the comparison structure for this graph, were samples grouped across sites? We have created new figures

Lines 175-181: Figure 2 suggests that wild fish have significantly higher MPTLC at the north site? But there was no statistically significant difference, likely due to small sample size and high variability in the data.

Line 188: Authors indicate hatchery fish lipids were higher than wild fish of the same size, but were those fish the same age? Age is not the relevant metric, as size varies substantially by age; this has been clarified in the additional text in the introduction.

Line 190: The authors begin this discussion talking about size and finish talking about age, confusing. Were the fish measured at the hatchery the same age as the fish they were compared to from wild samples? This has been clarified by the addition of text that indicates the ages of each of the fish being compared. This is the only comparison where age (not size) is particularly important.

Line 191: The authors indicated in the methods that fish were aged from fin clips and non-overlapping size classes (they should clarify the size class determinations more), but did they explain somewhere that fish were of the same age class in comparisons prior to this instance? In the methods they said TL was a scaling factor in analyses (but didn't explain how) which seems different to the statement on line 191. How did they know this for wild unclipped fish when length varied from <150mm up to 300mm? Please elaborate on whether same aged (in addition to size) fish were used in the analyses across each collection (e.g., within site and season)? This has been taken care of with the new analysis.

Lines 194-196: Please clarify this statement on foraging efficiency to indicate that you are speaking about fish within the size and age ranges tested in your study. We have edited the paper as suggested.

Line 200: I think that density dependent growth and condition should be removed from this statement because both current hatchery procedures and the authors own results suggest that this was not a factor for the fish in this study. Agreed, text has been removed

Line 208: Again, this seems to conflict with the first sentence of the discussion. We hypothesized that total lipid content of wild juvenile lake trout would be highest in the summer, whereas in fact it was lower than other seasons; line 208 confirms that our hypothesis was not supported (first line of discussion)

Lines 208-209: Clarify who you are talking about, hatchery, stocked or wild fish, or some combination of them. Discussion of results from grouped samples of wild and stocked fish is confusing. Done

Lines 224-225: It takes reading this sentence a couple of times to get the cohort delineation clear here. It can seem from reading this fast that the authors sample the hatchery fish the following spring in the lake, but they actually got their fish after they took their in-lake samples, dates would help readability here. Sentence has been rewritten

Line 233: Suggest changing "necessary" to "advantageous". Done

Lines 237-241: This reads like introduction, suggest connecting strongly to your results (e.g., "our results indicated that…..") or deleting theses 2 sentences. Text has been changed as suggested, and we deleted the first two sentence.

Lines 250-251: There are examples in the literature where season of stocking was compared to recruitment. See Elrod's Lake Ontario work on lake trout and the Cornell University's work on walleye for some local cites. We are not examining factors that affect recruitment of stocked fish – as stated in the paper, stocked fish survive well after stocking. We only discuss the issue of lipid content at stocking – possibly fish could be stocked with less lipid content and maintain survival.

Lines 251-254: Sentence structure is clunky. Maybe split this into 2 sentences at the semicolon. Think about doing that also for the previous sentence. You already include a subject in the phrase after the semicolon. Done, though we have kept the semicolon in the previous sentence.

Line 253: Need a pronoun or a noun after "but" (e.g., "it" or "abundance"). Sentence has been changed

Lines 256-259: The authors could also call these null and alternative hypotheses. We prefer to use ‘competing hypotheses’ as it is unclear which would be considered the null.

Differential lipid dynamics in stocked and wild juvenile lake trout

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Abstract:

After 45 years of stocking, lake trout in Lake Champlain have started to exhibit strong natural recruitment, suggesting a recent change in limiting factors such as prey availability or overwinter survival. The relative abundance of juvenile wild lake trout varies among regions of Lake Champlain, which suggest the prey base, or foraging success, may vary geographically within the lake. One metric that can indicate differences in resources across regions is lake trout lipid content, which reflects the availability of food and serves as an important energy reserve for overwinter survival. We quantified total lipid content of stocked and wild age-0 to age-3 lake trout among lake regions and seasons. No spatial differences in lipid content were apparent, but wild fish had higher overall mean ± SE percent total lipid content (17.0 ± 0.7% of dry mass) than stocked fish (15.2 ± 0.7%). Lipids in fish stocked in November were high (35.1 ± 0.7% of dry mass) but dropped by spring (14.9 ± 1.3%) and continued to decline through autumn. Wild fish showed seasonal changes with winter depletion in lipids followed by summer increase, and a plateau in autumn. The lipid depletion in stocked fish poses two competing hypotheses: 1) the high lipid concentration is necessary for stocked age-0 fish to transition to foraging in the wild, or 2) the high lipid concentration is difficult to maintain on a wild diet and reduces survival in the first post-stocking year.

Keywords: *Salvelinus namaycush*, recruitment, lipids, Lake Champlain, hatchery

Introduction

Lake trout *(Salvelinus namaycush)* was extirpated from Lake Champlain by 1900 (Plosila and Anderson, 1985). Restoration efforts began in 1972 with an intensive stocking program to reestablish a self-sustaining population and a recreational fishery (Marsden et al., 2010; Marsden and Langdon, 2012). Successful spawning and hatching were documented at several sites starting in 2000 but sustained natural recruitment was not observed until 2012, four decades after the stocking program commenced (Marsden et al., 2018). Thus, a survival bottleneck appears to have been present between newly hatched age-0 and age-1 wild lake trout; age-0 lake trout stocked in fall at the size of age-1 wild fish survive to maturity and have established a population in Lake Champlain. Recent natural recruitment may be due to a change in limiting factors such as food quality or quantity. For example, the Lake Champlain prey base was diversified in 2003 by the invasion of alewife (*Alosa pseudoharengus*), a known diet item of juvenile lake trout (Marsden et al. unpublished data; Madenjian et al., 2006). Additionally, winter can be a period of high mortality for juvenile fishes when the risks of starvation, thermal stress, and predation are high (Hjort, 1914; Hurst, 2007). Increased prey availability, milder winter conditions, or other factors could help juvenile lake trout survive through the winter.

Bottom trawl surveys were initiated in 2015 to target age-0 to age-3 juvenile lake trout. Trawl catches indicated that relative abundance of these year classes of stocked and wild lake trout varied among regions of Lake Champlain (Marsden et al., 2018; Wilkins and Marsden in revision). Annual stocking occurs at two spawning sites, Whallon Bay in the southern Main Lake and Gordon Landing in the northern Main Lake, and both sites produce high densities of newly hatched lake trout (Ellrott and Marsden, 2004). Few spawning sites have been identified in the central lake, so we expected to find higher abundance of wild lake trout near Whallon Bay and Gordon Landing than in the central lake. However, the highest proportion and relative abundance (catch-per-unit-effort, CPUE) of age-0 to age-3 wild fish has been consistently found in the central lake (Marsden et al., 2018; Wilkins and Marsden in revision). This difference in expected versus observed distributions suggests that (1) unknown spawning sites that successfully produce age-0 lake trout are present in the central Main Lake, (2) wild lake trout move from the northern and southern spawning sites to the central Main Lake, or (3) mortality of age-0 wild lake trout in the north and south is higher than in to the central Main Lake. Either of the latter two possibilities may be due to an asymmetrical distribution of prey resources across the lake. Age-0 lake trout primarily consume *Mysis diluviana*. By age-1, juvenile lake trout begin to consume small alewife (*Alosa pseudoharengus*), smelt (*Osmerus mordax*), and slimy sculpin (*Cottus cognatus*) (unpublished data). However, estimates of the relative abundance of these prey species in different areas of the Main Lake are not available.

Lake trout are stocked in Lake Champlain in fall as ‘fingerlings’, i.e., age-0, but at a range of sizes (149-211 mm) similar to fall age-1 wild lake trout (145-232 mm, Wilkins and Marsden in review, Marsden et al. 2018). Consequently, size is a more relevant metric than age when evaluating diet, growth, and condition. Lipid concentration in juvenile lake trout could provide insight into the recent surge in natural recruitment as an indirect measure of foraging success – lipids serve as energy resources and help fish to cope with environmental stressors (Adams, 1999; Tocher, 2003). In particular, lipids are used for basic maintenance and other metabolic needs during winter, when prey availability is presumably low and typically reduced by the end of the open-water season (Adams, 1999; MacKinnon, 1972; Rikardsen and Elliott, 2000). For example, juvenile rainbow trout (*Oncorhynchus mykiss*) and juvenile Atlantic salmon (*Salmo salar*) exhibited depleted lipid reserves (by 60-90% and 34-57%, respectively) over winter (Biro et al., 2004; Naesie et al., 2006). Additionally, the health of fish can often be predicted by lipid content; fish with low growth and condition factor have correspondingly low lipid content (Amara et al., 2007). Accordingly, total lipid content provides an assessment of the energy status of a fish (Naesie et al., 2006; Trudel et al., 2005), and may indicate how well fish are prepared to survive the winter. Differences in lipid content may help explain why lake trout in Lake Champlain are exhibiting natural recruitment and how different areas of the lake might support the growth of juvenile wild fish. Variation in lipid content between stocked and wild juvenile fish could also reveal differences in the abilities of wild and stocked fish to survive stressors such as the winter season.

We hypothesized that total lipid content of wild juvenile lake trout would be greatest in the central Main Lake where wild recruits are most abundant (Marsden et al., 2018; Wilkins and Marsden in revision), and would be highest in the summer when the prey base is most abundant. We also hypothesized that newly stocked lake trout would have a higher lipid content than wild juveniles because hatchery fish are typically fed a highly nutritious diet under ideal conditions prior to their release. However, post-release stress and adaptation to a wild-caught diet could result in a substantial reduction in lipid content. To test our hypotheses, we measured total lipid content of stocked and wild juvenile lake trout (ages 0-3) in Lake Champlain from three areas of the Main Lake basin during three seasons, and lipid content of age-0 hatchery lake trout prior to stocking.

Methods

Study System

Lake Champlain is situated among New York and Vermont, USA, and Quebec, Canada (Figure 1). The lake is 193 km long, with a maximum width of 20 km. The Main Lake is meso-oligotrophic, with a maximum depth of 122 m. Since 1995, lake trout have been primarily stocked at Whallon Bay, Gordon Landing, and Burlington Bay (Figure 1; Marsden et al., 2018).

Sample Collection

Fish were sampled in 2018 at three areas in the Main Lake, near Burlington Bay, Whallon Bay, and Grand Isle (hereafter referred to as the central, south, and north sites) (Figure 1). Sampling efforts for juvenile lake trout have been concentrated at these locations over the previous four years, and provided information on variation in relative abundance of stocked and wild lake trout throughout the Main Lake (Marsden et al., 2018).

Sampling was conducted between 8 June and 28 September 2018 to assess potential seasonal changes in lake trout condition. The central site was sampled every 2-3 weeks, and north and south sites were each sampled twice (June and August). We used a three-in-one bottom trawl with an 8-m headrope, 9.3-m footrope with chains, and 1.25-mm stretch cod end liner (Marsden et al., 2018). Trawl tows were taken along-contour at depths from 28 m to 64 m, with the majority of tows concentrated around 40 m, for 10 or 20 min at ~5.5 km/h. Approximately 30 lake trout were selected from the trawls on each sampling date to represent the range of sizes captured up to 300 mm, and included both stocked and wild fish from each site (i.e., 15 stocked and 15 wild fish were targeted). Stocked fish were identified based on presence of a fin clip

(Marsden et al. 2018). Fish were immediately frozen on dry ice and stored at -80°C until lipid extraction. A sample of hatchery-reared lake trout (15 fish) was collected from the Ed Weed Fish Culture Station, Grand Isle, VT, on 15 November 2018 to assess lipid content of the lake trout a week prior to release into Lake Champlain.

Sample Preparation

In the laboratory, lake trout were thawed, measured for total length, weighed, and re-assessed for fin clips. Age of stocked fish was known based on fin clips and estimated for wild fish based on non-overlapping size classes (Marsden et al., 2018). Fish were dissected and stomach contents removed to avoid any influence of recently consumed prey on the estimate of total lipid concentration. Each lake trout >150 mm in total length was homogenized in a Ninja BL500 Professional Blender, and a 30-g subsample was removed. Lake trout <150 mm in total length were dried whole. Subsamples and whole small fish were dried to a constant mass at 65°C for 72 hours. Once dry, samples were ground into a fine powder by mortar and pestle.

Lipid Extractions

Three 1-g (for lake trout >150mm) or 0.5-g (for lake trout <150mm) samples were measured from the dried mass of each fish, and placed into pre-weighed 50-ml conical centrifuge tubes. Samples were analyzed for total lipid content according to a modified version of the Folch et al. (1957) method. Briefly, 10 or 20 ml (depending on sample weight) of a 2:1 chloroform:methanol solution was added to each centrifuge tube. Samples were agitated for 30 seconds using a vortex, and centrifuged for 10 minutes at 3,000 rpm. The lipid-containing supernatant was pipetted off to avoid disturbing the pellet, and the process was repeated a second time. The resulting pellets were then dried for 24 hr at 65°C to evaporate any remaining chloroform:methanol solution. Samples were weighed again in the centrifuge tubes to estimate the final lipid-free dry mass measurement.

Data Analysis

Mean percent total lipid concentration (MPTLC) of the dry fish weight was determined by dividing the post-extraction weight of each sample by the pre-extraction weight and converting to a percent. The three subsamples for each fish were used to calculate average MPTLC; the average coefficient of variation among all fish was 0.09

MPTLC was transformed using the logit function (Warton and Hui, 2011) and compared across sites (north, south, and central) and seasons (spring, summer, autumn). We used a three-way Analysis of Covariance (ANCOVA) with length as a covariate to test our hypothesis that total lipid content of juvenile lake trout varied between sources, and among seasons and locations. The data did not conform to the parametric assumption of homoscedasticity and sample sizes were low for some locations and seasons. Therefore, we used a non-parametric bootstrapping approach to conduct our analyses. We generated a bootstrap sample by randomly assigning, with replacement, a lipid content, length, source, season, and location to a bootstrapped fish, for each of 182 fish (i.e., the number of observations in the original sample). The F statistic of the covariate (length), independent variables (source, season, and location), and all 11 possible interactions of these variables, were extracted from the bootstrap sample ANCOVA. The resampling procedure was repeated 10,000 times to create a distribution of bootstrapped F statistics for each variable and interaction. We calculated 95th percentile F statistics from each bootstrap distribution. The observed F statistic from the ANCOVA of the original data was deemed statistically significant if it fell above the 95th percentile of the bootstrap distribution of F statistics. All analyses were conducted using the R statistical environment v3.6.1. (R Core Team, 2019).

Results

One-hundred and ninety-seven juvenile lake trout (86 wild and 111 stocked, including 15 hatchery-sampled fish in the stocked group) were analyzed for MPTLC (Table 1). The overall average (± SD) MPTLC was 15.2 ± 7.1% of dry mass for stocked fish in the lake and 17.0 ± 6.8% for wild fish. MPTLC of lake trout from the hatchery, just prior to stocking into Lake Champlain, was 35.1 ± 2.9% of dry mass.

All significant main effects were confounded with interaction effects and do not allow for interpretation. We found significant interaction effects between MPTLC in length and season ($p < 0.01$), length and source ($p < 0.0001$), and season and location ($p < 0.01$; Figures 2 & 3). Seasonal variation in MPTLC increased with length in stocked and wild juvenile lake trout, with spring MPTLC higher than summer and autumn as length increases. Stocked juvenile lake trout showed a continuous drop in MPTLC from pre-winter levels at stocking in November to the following autumn. Wild juvenile lake trout displayed higher MPTLC than stocked juvenile lake trout but a slower increase in MPTLC by length (Figure 2).

Spatial variation in stocked and wild juvenile lake trout was confounded with seasonal variation.

Wild juvenile lake trout from the south and central Main Lake had lower MPTLC in spring (June) and higher MPTLC in summer (July – August; Figure 3). MPTLC of wild juvenile lake trout in the central Main Lake decreased in autumn to similar levels found in spring (September; Figure 3). Wild juvenile lake trout in the north Main Lake showed high MPTLC in summer but was based on low sample size (n = 3; Figure 3 & Table 1). Stocked juvenile lake trout from the south and central Main Lake had higher MPTLC in spring (June) and lower MPTLC in summer (July – August). MPTLC of stocked juvenile lake trout in the central Main Lake continued to decrease in autumn (September). MPTLC of stocked juvenile lake trout in summer showed a continuous decrease from the south to north Main Lake (Figure 3).

Discussion

Our results were unexpected, and each of our hypotheses was refuted. First, we did not find any differences in MPTLC in lake trout sampled from the three different areas of the Main Lake despite higher CPUE and higher proportions of wild lake trout in the Main Lake relative to the northern and southern areas (Wilkins and Marsden in revision). Second, wild lake trout had higher MPTLC than stocked lake trout, despite a two-fold higher MPTLC in stocked lake trout just prior to release into the lake. Further, the high lipid concentration when hatchery fish were stocked was rapidly lost over their first winter in the lake, and the decline in lipid concentration continued over summer and into autumn.

We hypothesize that the spatial heterogeneity in abundance of wild juvenile lake trout in Lake Champlain was due to differences in prey quantity or quality across the different regions of the Main Lake that draw juveniles from the north and south to the central lake. Alternatively, lake trout hatched in the north and south could have lower survival than in the central region if prey resources were higher in the central lake. However, the lack of variation in lipid concentration among the three regions suggests that lake trout do not experience differences in prey availability across the Main Lake. The differential mortality hypothesis remains to be tested.

Hatchery-reared fish are typically fed a high-ration diet rich in lipids that is reflected in their body composition (Reinitz, 1983). Thus, we expected stocked juvenile lake trout would possess a higher MPTLC than their wild counterparts, similar to other stocked species (e.g., Atlantic salmon *Salmo salar*; Bergstrom, 1989). Analysis of lake trout collected from the Ed Weed Fish Culture Station just prior to stocking in November showed that hatchery-reared lake trout had a MPTLC approximately two times higher than wild lake trout of the same size in Lake Champlain. However, lipid content of newly stocked lake trout dropped markedly over their first winter to the level of age-1 wild fish in spring, and continued to drop throughout summer until by autumn the stocked juvenile lake trout were lower in MPTLC than wild juvenile lake trout of the same age class, although the stocked fish were longer than wild lake trout.

The high lipid content of wild juvenile lake trout compared to stocked juvenile lake trout suggests that wild lake trout may be more efficient foragers than stocked fish at the same size range. The artificial environment in which stocked fish are raised may not select for traits such as boldness and aggressiveness that are adaptive in natural settings (Brown and Laland, 2002; Brown et al., 2003; Saikkonen et al. 2011). In general, hatchery-raised fish post-stocking tend to consume less food and fewer prey types than wild fish, and exhibit reduced ability to switch to new prey types in the wild (e.g., Saikkonen et al., 2011). Inferior anaerobic capacity and swim performance have also been documented for fish raised in hatcheries (McDonald et al., 1998). Hatchery-raised brook trout (*Salvelinus fontinalis*) also exhibited lower survival rates once released compared to wild fish because of poor foraging ability (Ersbak and Haase, 1983). The body of evidence suggests that hatchery-raised salmonids are less efficient foragers than wild fish in a natural lake environment, potentially resulting in lower lipid levels compared to wild fish, as we found in our study.

We also found seasonal differences in MPTLC of juvenile lake trout in the central Main Lake. Stocked and wild fish showed different trends in seasonal lipid levels; the pattern in lipid content in the stocked fish appeared to influence the overall trend when all fish were analyzed together. Lipid content of wild fish was consistent with other piscivorous fishes, in which lipids are greatest in the summer and lower in spring and autumn (e.g. Madenjian et al., 2000; Metcalfe et al., 2002). In summer, age-1 to 3 lake trout have access to young-of-year smelt and alewife that hatch in June and July, respectively (Simonin et al 2016), and this prey base appears to be sufficient to allow accumulation of lipid storage in addition to growth. Stocked fish, in contrast, showed significant declines in lipid content from spring to summer to autumn. Lipid levels of the hatchery fish also declined substantially after stocking in November, as lipid levels in fish sampled from the hatchery just prior to stocking were substantially higher than in stocked fish caught in the lake in spring. Although this comparison was made between two cohorts (i.e., lake trout sampled prior to stocking in November, and the previous cohort sampled in spring and summer of the same year), hatchery conditions and diet are consistent from year to year, and we can assume reasonable consistency in lake conditions in two consecutive years. The consistent seasonal decline in lipid content of stocked juvenile lake trout suggests that these fish will have less energy reserves than wild juveniles to survive through their second winter in the lake. The high-nutrient diet that stocked lake trout were fed in the hatchery does not appear to give them a lasting advantage over wild lake trout, as wild fish surpass stocked fish in lipid content by the summer following their first winter in the lake. However, the high lipid content of stocked fish may be advantageous for survival through the first post-stocking winter, as they learn to feed on active prey and cope with stresses associated with predators.

Our results do not help to explain the greater abundance of wild recruits in the central Main Lake relative to other regions of Lake Champlain because we did not find spatial variation in lipid content in juvenile lake trout. Larger sample sizes and additional years of data would be useful to confirm this result. The increase in lipid levels of wild recruits during the summer is predictable and encouraging, as the data suggest that wild juvenile lake trout are feeding well; higher lipid content is associated with high survival potential. However, we only examined juveniles from June to September. Analysis of juvenile lake trout throughout the year would provide a more complete picture of lipid acquisition and depletion over the winter. The dramatic loss of the lipid advantage of the hatchery lake trout have at stocking is interesting; hatchery fish may be at a substantial disadvantage during their first winter as they acclimate to wild conditions and therefore need the higher lipid content provided by the hatchery. However, we do not know the survival rate of stocked lake trout during the first winter after stocking. The population of lake trout in Lake Champlain has been maintained by fish stocked with high lipid content, but we do not know whether post-stocking survival is dependent on this high lipid content. That is, could the same size lake trout population be supported by stocking fish with half the lipid content at stocking? We propose two competing hypotheses: high lipid content either 1) provides the necessary energy reserves for stocked fish to acclimate to life in the wild and learn to forage, or 2) imposes an energetic penalty that cannot be sustained in the wild. To test these hypotheses, hatcheries could evaluate post-stocking performance and survival of lake trout raised with normal and reduced hatchery diets. If the second hypothesis is supported and the first refuted, hatcheries may be able to achieve the same level of survival by stocking smaller lake trout earlier in the year (e.g., May), when seasonal prey production is increasing and transition to a wild diet may be easier than in November.

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Table 1. Size range (mm total length) and sample sizes (in parentheses) of stocked and wild juvenile lake trout collected for lipid analysis from three areas of the Main Lake of Lake Champlain in three seasons, 2018. Samples were also obtained from the Ed Weed Fish Culture Station in early November, 2018.

		Pre-winter	Spring	Summer	Autumn
Stocked	North	--	--	159 – 306 (15)	--
	Central	--	181 – 285 (13)	192 – 269 (16)	192 – 292 (15)
	South	--	152 – 310 (31)	206 – 243 (6)	--
	Hatchery	149 – 211 (15)	--	--	--
Wild	North	--	--	236 – 280 (3)	--
	Central	--	81 – 245 (15)	106 – 332 (27)	121 – 275 (15)
	South	--	95 – 237 (10)	104 – 287 (15)	--

Figure headings

Figure 1: Lake Champlain, showing north, central, and south sampling areas in the Main Lake and two major known lake trout spawning sites at Gordon Landing and Burlington Bay where lake trout are also stocked annually.

Figure 2: Seasonal comparison of mean percent total lipid content of the dry mass of wild and stocked juvenile lake trout ages 0-3 in Lake Champlain captured between 8 June and 29 September 2019. The colors denote seasons in which lake trout were captured: spring (June), summer (July – August), and autumn (September); the black line is the regression for all fish combined by season. Numbers on the graph indicate age of each fish; age-0 stocked fish were collected in November 2018 from the Ed Weed Fish Culture Station just prior to stocking (pre-winter).

Figure 3: Seasonal and spatial comparison of mean percent total lipid content of the dry mass of wild and stocked juvenile lake trout ages 1-3 in Lake Champlain captured between 8 June and 29 September 2019. Error bars show standard error. North, central, and south refer to the sampling regions in the Main Lake basin. The seasons refer to the month in which lake trout were captured: June (spring), July – August (summer), and September (autumn).

Figure

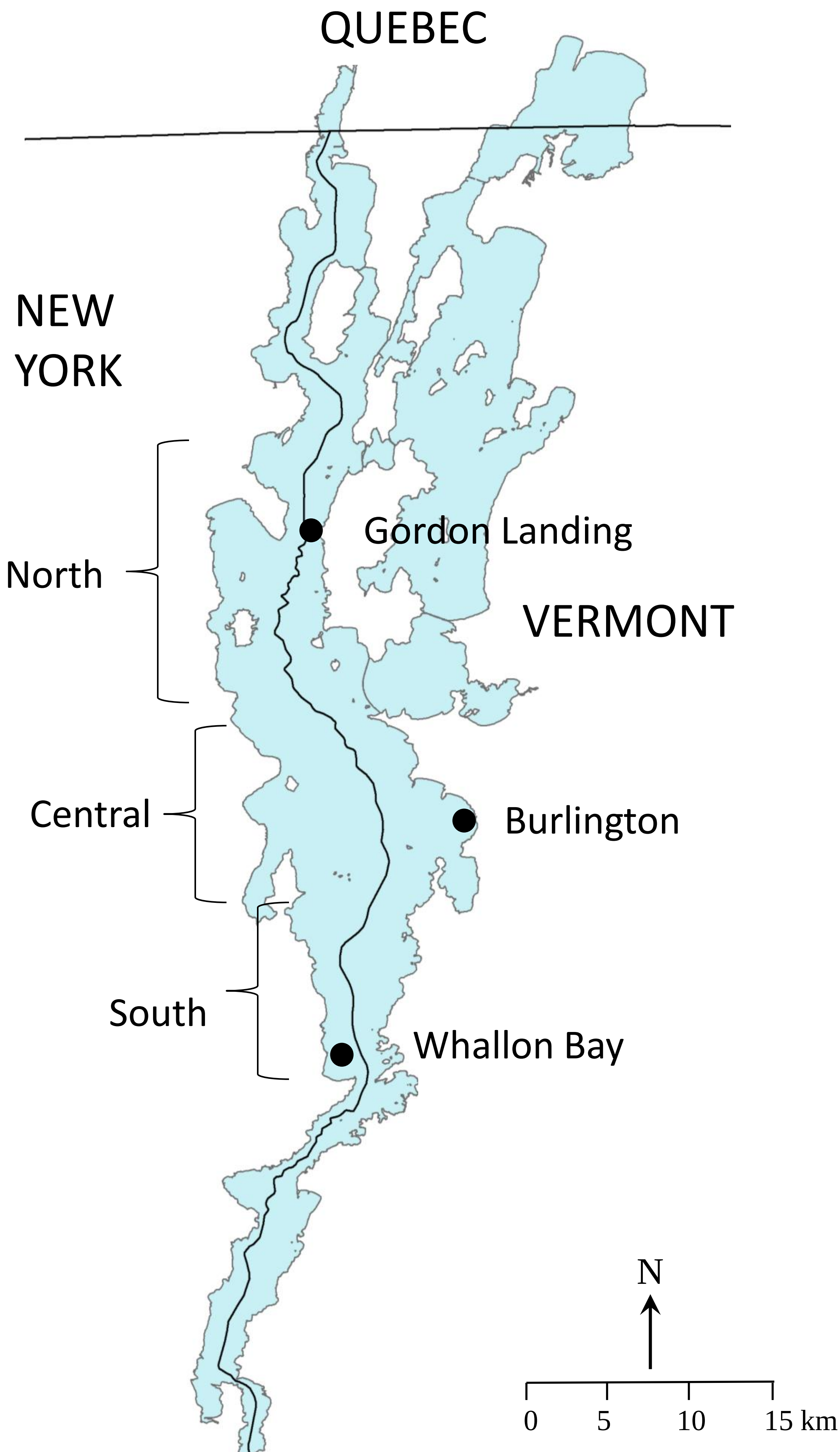
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Figure
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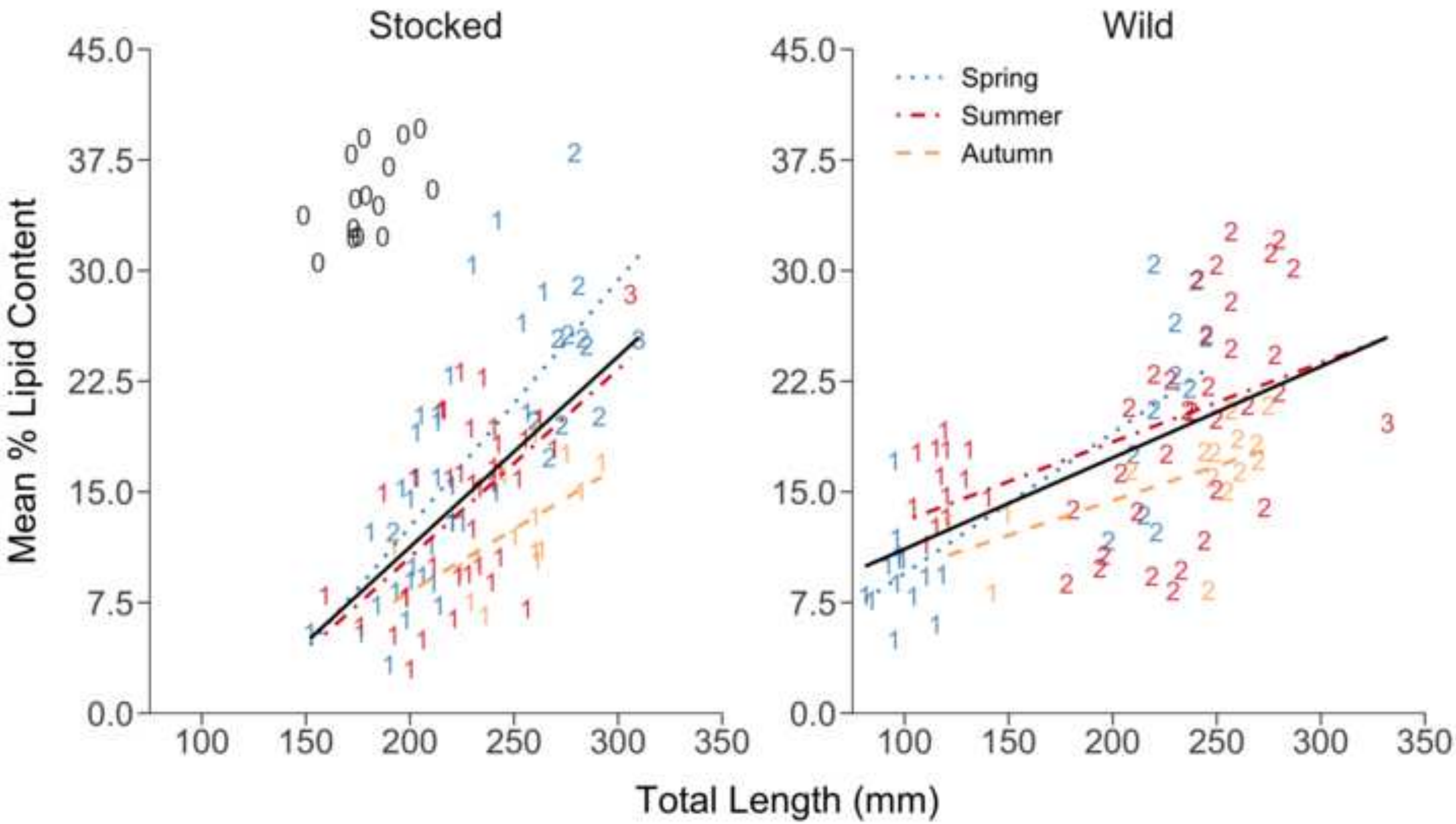

Figure 3
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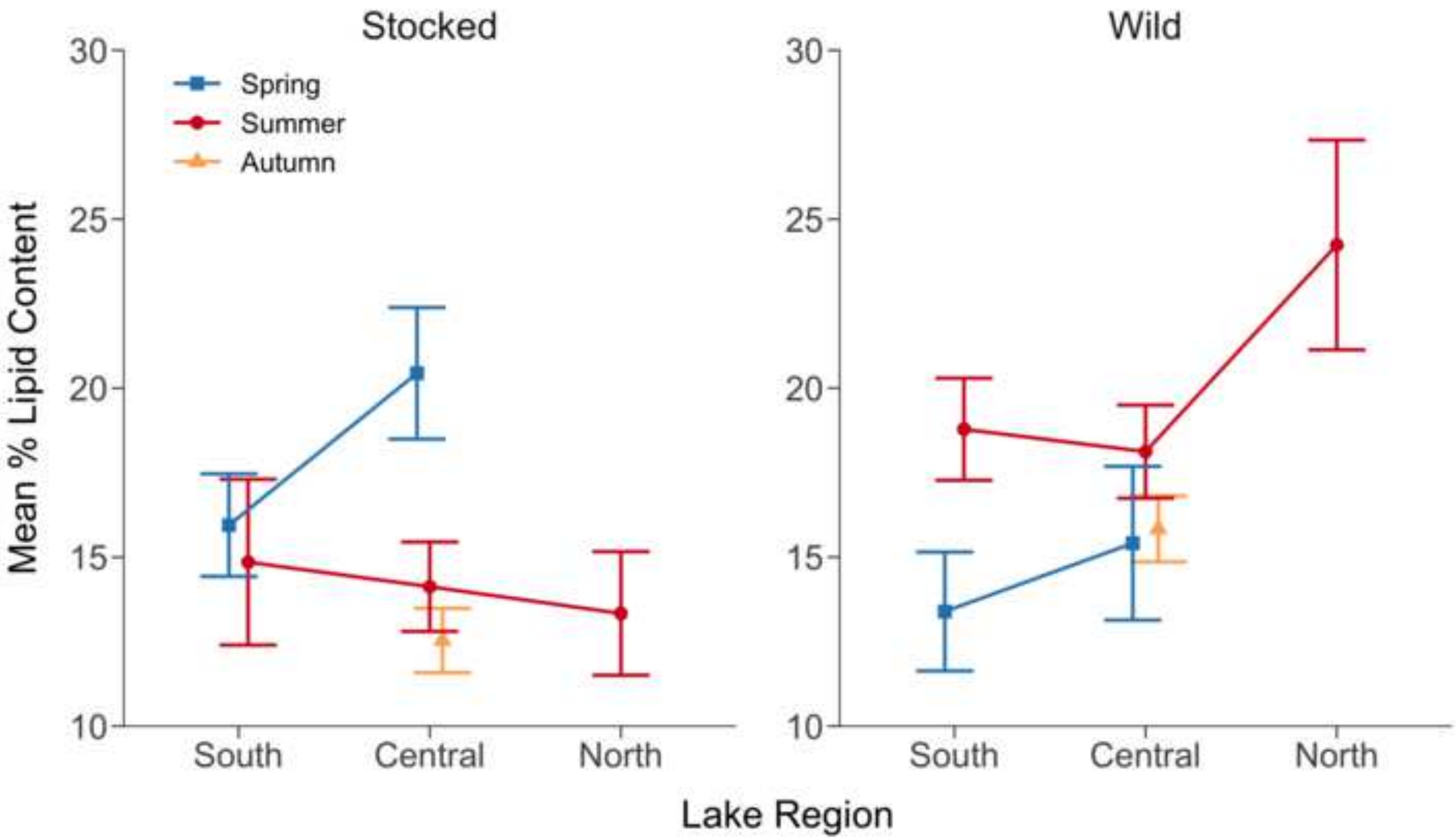

***Declaration of Interest Statement**

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: