

## Examining shifts in zooplankton community variability following biological invasion

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### Abstract

We examined 20 yr of zooplankton samples from Harp Lake for shifts in zooplankton variability following invasion by zooplankton predator *Bythotrephes longimanus*, using organism body size—as measured at high resolution by Laser Optical Plankton Counter (LOPC)—as the primary metric of investigation. A period of transitory high variability in the 2 yr post-invasion was observed for both body size compositional variability and aggregate variability metrics, with both measures of variability shifting from low or intermediate to high variability immediately following invasion, before shifting again to intermediate variability ~ 2 yr post-invasion. Aggregate and compositional variability dynamics were also considered in combination over the study period, revealing that the period of transitory high variability coincided with a shift from a community-wide stasis variability pattern to one of asynchrony, before a shift back to stasis 2 yr post-invasion. These dynamics were related to changes in the significant zooplankton species within the Harp Lake community over the pre- and post-invasion periods, and are likely to be indicative of changes in the stability in the zooplankton community following invasion by *Bythotrephes*. The dual consideration of aggregate and compositional variability as measured by LOPC was found to provide a valuable means to assess the ecological effects of biological invasion on zooplankton communities as a whole, extending our knowledge of the effects of invasion beyond that already revealed through more traditional taxonomic investigation.

Invasion by alien species is considered to be one of the most significant and least controlled human-mediated threats to ecosystems worldwide and is suggested to be exceeded only by habitat destruction as the primary threat to biodiversity (Almqvist et al. 2010). Although reported examples of biological invasion within freshwater ecosystems number in their thousands (Ruiz and Carlton 2003), most studies of the effects of invasion are short-term snapshots, which lack a suitable temporal context to effectively assess resulting shifts in ecosystem dynamics (Strayer et al. 2006). This knowledge gap complicates the establishment of effective invasion management strategies, because long-term data series are necessary to separate invasion-induced changes from normal system variability (Yan and Pawson 1997) and from those induced by other stressors, and to set benchmarks of recovery (Larson et al. 2011).

The invasive zooplankton predator, the spiny water flea *Bythotrephes longimanus* (Crustacea, Onychopoda, Cercopagidae) has now invaded all five Laurentian Great Lakes, Lake Winnipeg, and > 150 smaller inland lakes within North America, including the relatively small, single-basin headwater Harp Lake (Muskoka, Ontario, Canada) in 1993. As one of the Ontario Ministry of the Environment's Dorset Environmental Science Centre long-term study lakes, Harp Lake has been monitored for water quality and plankton assemblages since 1978, and thus provides researchers with a continuous data set by which to study the long-term effects of *Bythotrephes* invasion on an endemic zooplankton community. The post-invasion zooplankton

community of Harp Lake has demonstrated greater and different types of changes than those observed in nearby, uninvaded Dorset lakes, despite the chemistry of Harp Lake changing the least (Yan et al. 2008) and the algal composition changing no more than in reference lakes (Paterson et al. 2008) during this time. Although drivers such as water chemistry have possibly played a role in the observed changes within the zooplankton community of Harp Lake, their contributions are likely small compared with the *Bythotrephes* effect.

The Harp Lake data set is unique both in the long duration and fortnightly sampling frequency of its bathymetrically weighted, composite zooplankton samples (Yan et al. 2008), and its relatively small size negates the common obstacle of requiring a vast spatial scale of assessment to effectively investigate the ecological effects of a biological invader (Yan and Pawson 1997). Subsamples of the long-term species-based data set have been analyzed in a number of studies (Yan and Pawson 1997; Yan et al. 2001, 2002) to identify changes in the central macrozooplankton and microzooplankton species that may be attributed to the arrival of *Bythotrephes*. To date, there has been no study in which the preserved zooplankton samples of Harp Lake are examined in their entirety, or that uses zooplankton body size as the primary metric of investigation. Applying a more automated data collection technique—such as the use of optical plankton-counting technology—to analyze the entire samples, as opposed to the conventional microscopic analysis of subsamples, allows for both of these knowledge gaps to be addressed. In the case of Harp Lake, this results in ~ 100–200 times more zooplankton being analyzed within each sample. There are, however, also arguments for

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the use of zooplankton body size as an ecosystem metric that extend beyond the ability to more readily automate its measurement.

It has been suggested that ecosystem health may be more effectively measured by examining changes in overall community structure that are not apparent in individual species metrics (Barbiero 2003). Zooplankton body size is a central feature reflecting the physiology, ecology, and evolutionary history of an individual or community (Schmidt et al. 2006), and the size of planktonic organisms can affect how they respond to abiotic and biotic drivers of spatial and temporal heterogeneity. Organism size is one of the main scaling factors for ecological patterns and processes (Peters 1983), so body size and community size spectra metrics have been utilized to provide holistic ecosystem descriptors that facilitate ecosystem comparisons in time and space (Gaedke 1992), which addresses the growing concern that management of aquatic systems must consider ecosystems rather than individual populations (Benoit and Rochet 2004). Studies relating community size spectra metrics with ecosystem characteristics (Sprules and Munawar 1986; Gaedke 1992; San Martin et al. 2006) have revealed findings such as maintenance in biomass stability for a whole community despite variations in component species, which, in turn, provide further persuasive reasons for the development of indices of ecosystem dynamics that aggregate over species (Schindler 1990).

The exploration of compensatory dynamics within communities has been addressed in the growing field of ecological variability investigation (Micheli et al. 1999; Cottingham et al. 2001), whereby system dynamics induced by environmental fluctuations or stressors may be explored more deeply than by examining changes to species of interest (Gonzalez and Descamps-Julien 2004; Downing et al. 2008; Brown and Lawson 2010). As discussed by Micheli et al. (1999), variance of a community is not only observable in aggregate variables, such as total abundance, but also in compositional variability, here defined as changes in community size spectra (which may include variability both within and between changes in species metrics). Assuming that these two properties need not covary, aggregate and compositional variability thus provide dual probes into community variability that can provide insight into system dynamics accompanying shifts, such as those caused by invasive species.

Four variability patterns have been described by the four possible permutations of low and high aggregate variability with low and high compositional variability (see fig. 1 in Micheli et al. 1999). Thus, the two measures of variability can identify whether the temporal patterns of system dynamics are synchronous, asynchronous, compensatory, or static (Micheli et al. 1999), which provides managers with system-scale information (e.g., whether compensatory dynamics, whereby system function may be maintained despite relative population shifts [Vasseur and Gaedke 2007], are present within the system leading up to and/or following invasion-induced changes). There is also evidence that environmental fluctuations, despite their short-term destabilizing effects, may have long-term stabilizing effects on communities (Gonzalez and Descamps-Julien 2004;

Downing et al. 2008), which is of interest in the context of biological invasion.

Here we take advantage of the unique Harp Lake zooplankton data set over the period 1980–2000, processing the entire samples for the first time to examine *Bythotrephes*-induced shifts in both aggregate and compositional metrics from a body-size perspective as opposed to the previously examined species-centric perspective. We predict that this new approach will complement previous work by revealing invasion-induced changes in the variability of the zooplankton community of Harp Lake that significantly differ from normal system variability, and will in turn provide clues regarding the long-term stability of the invaded system. Given the quality of previous Harp Lake studies, this work also provides an opportunity to assess the suitability of applying body size metrics within the study of biological invasions and resulting compensatory dynamics.

## Methods

*Study sites*—Harp Lake is a 0.71 km<sup>2</sup>, single-basin, headwater lake located in the District of Muskoka, Ontario, Canada (latitude 45°23', longitude 79°07'). It has mean and maximum depths of 13.32 m and 37.5 m respectively. It exhibits physical and chemical properties typical of lakes of the same size within the surrounding region, most notably soft and nutrient-poor waters (Yan et al. 2001).

The zooplankton species richness and community structure has been tracked both prior to and following the first observation of *Bythotrephes* in Harp Lake during summer sampling in 1993. This monitoring revealed that the yearly averaged species richness of the Crustacea plankton in the lake fell by 17%, from a mean count of 9.98 to 8.25 species after the invasion (Yan et al. 2002), and the mean individual body length of Cladocera doubled in the 6 yr following invasion (Yan et al. 2001), although species richness and zooplankton composition stabilized at their new levels within 3 yr after the invasion (Helmus et al. 2010).

The nearby, uninvaded Blue Chalk and Red Chalk lakes were also used as reference lakes for part of the analysis presented here, and by Yan et al. (2002). Both are similar in size to Harp Lake, and exhibit similar water chemistry (Yan et al. 2008) and algal composition dynamics (Paterson et al. 2008) over the study period.

*Zooplankton sampling and processing*—The same zooplankton sample collection, preservation and processing methods were employed over the entire collection period (Yan and Pawson 1997; Yan et al. 2008). Zooplankton samples were collected from Harp Lake on a fortnightly basis during the ice-free seasons of 1980–2000, although not all samples were available for the purposes of this analysis, so the resulting data set contained 167 samples, with an average of 7.9 samples per year (SD = 4.4). Samples were collected from Blue Chalk and Red Chalk lakes on a monthly basis over the same period.

All samples were bathymetrically weighted composites of a series of vertical net hauls from different depths using a metered 80  $\mu\text{m}$  conical tow mesh net—exhibiting average filtration efficiency of 84% (Yan and Pawson 1997)—at a single mid-lake station, with all sample contents fixed in buffered 6% sucrose-formalin. Sample volumes were similar across the sample dates. Following collection, each sample was analyzed conventionally (Yan et al. 2008) by either Bill or Dee Geiling (Limnoservices Inc.), who identified, counted, and measured the length of a minimum of 250 crustacean zooplankters. This conventional analysis resulted in taxonomic data sets containing abundance and related density values (animals  $\text{m}^{-3}$ ) of each identified species for each sample date, for Harp Lake and for the Blue Chalk and Red Chalk reference lakes.

In December 2008, we processed the entire contents of all 167 Harp Lake samples through a Laser Optical Plankton Counter (LOPC) equipped with a Sample Circulator (Rolls-Royce Canada Ltd.—Naval Marine). As each sample passed through the LOPC, the zooplankton were counted and measured, with the resulting output data set consisting of a series of plankton counts in 15  $\mu\text{m}$  size bins, over the range 105–35,000  $\mu\text{m}$  equivalent spherical diameter (ESD). This process resulted in sample sizes that were generally 100–200 times greater than those measured using conventional means.

*Compositional variability analyses*—The first step in investigating compositional variability, as expressed by changes in the relative abundance of different-sized zooplankton over the 20 yr study period, was to perform multivariate regression tree (MRT) analysis on the Harp Lake LOPC data set. We used MRT as a form of time-constrained multivariate clustering method (Legendre and Legendre 2012) on the entire LOPC data set, following Hellinger transformation of the 15  $\mu$  size-bin abundance data (Legendre and Gallagher 2001), using the ‘mvpart’ library (De’ath 2002) of the R statistical language (R Development Core Team 2007). By using the chronological sample number (the first sample, 28 July 1980, was No. 1, and the last sample, 10 October 2000, was No. 167) as the constraint in the analysis, cross-validation within the MRT analysis resulted in the delineation of groups of samples adjacent along the time series that were fairly homogenous in community size structure. Essentially, the technique split the LOPC data into statistically similar groups along the time series.

Following the splitting of the data into statistically similar chronological groups, compositional variability within a given time segment may be visually assessed by the number of branches of the resulting MRT that lie within that time segment, with time segments consisting of multiple branches exhibiting higher variability than those with fewer branches. Compositional variability was also quantified by calculating the total variance of the Hellinger-transformed LOPC data—a measure of beta diversity (Legendre et al. 2005; Anderson et al. 2011)—for each of the resulting time segments.

The MRT process was repeated on the conventionally measured Red Chalk and Blue Chalk lake zooplankton

density data sets in order to compare the timing of the statistically significant splits in the data and the compositional variability during the resultant time segments between Harp Lake and the two reference lakes. Although the results of constrained clustering analysis methods such as MRT analysis do not rely on regularity of sampling, we nonetheless also confirmed the timing of the most significant split in the Harp Lake LOPC data set by repeating the MRT analysis on a subsampled LOPC data set that contained only 3 samples per year, corresponding to the smallest number of samples for any given year over the entire time series.

The cross-validation step inherent within MRT analysis provides internal validation of the results (Legendre and Legendre 2012). We also performed an external validation by relating the most statistically significant MRT split in the community size structure to the previously collected taxonomic data over the study period. Firstly, a binary variable was created to represent the most significant split in the data identified by the MRT results. This was then used as the explanatory variable within redundancy analysis (RDA) performed on the Hellinger-transformed (Legendre and Gallagher 2001) species abundance data, using the ‘rda’ function within the ‘vegan’ library of the R statistical language. The binary variable was also used within indicator-species analysis (De Caceres and Legendre 2009), in which the significant species for each of the two time periods, resulting from the MRT analysis, were identified by using the ‘indval’ function within the ‘labdsv’ package of the R statistical language on the untransformed species-abundance data.

*Aggregate variability analyses*—Before assessing aggregate variability within Harp Lake, it was first necessary to calculate aggregate metrics of density, mean body size, and biomass. For each LOPC-counted and -measured sample, density is simply a sum of the abundance of all zooplankton particles detected per unit volume of water sampled, while mean body size is the mean of all measured ESD values. Biomass values were calculated using a method developed by Sprules et al. (1998), in which the biomass present in each 15  $\mu$  size bin was calculated using the equation

$$V = (\pi/6)(\text{ESD}^3 f^{-2}) \quad (1)$$

where  $V$  is the volume of zooplankton present in the size bin, ESD is the ESD value at the midpoint of the size bin, and  $f$  is the aggregate ratio of length to width for all zooplankton present in the entire sample. The volume of each size bin was converted to wet biomass assuming a specific gravity of 1, then to a dry biomass using a multiplier of 0.2, before all size-bin biomass values were summed to give a total value for each sample. An appropriate value for  $f$  was determined by iteratively changing  $f$  using the method of Yurista et al. (2009) until a value was reached that resulted in the lowest residual variation between the resulting biomass and that calculated by extrapolating from the manually measured subsamples using published regression equations (Girard and Reid

1990). The resulting ratio of length to width for the entire zooplankton data set was  $f = 2.01$ , which was then used to calculate biomass for each sample. Similar to density values, biomass for each sample was also divided by the total volume of lake water sampled.

To assess the aggregate variability dynamics, the coefficient of variation (CV) was calculated for each aggregate metric—density, mean body size, and biomass—over the study period (Cottingham et al. 2001). CV values for pre-invasion and post-invasion intervals were first compared, before the study period was further broken down into four intervals—two prior to invasion and two following invasion—to assess the changes in aggregate variability at higher resolution. These four intervals were determined from the compositional variability results, in which the data were split into four distinct time segments based on statistical differences in community composition.

*Ecosystem dynamics analyses*—Ecosystem dynamics over the study period were then explored by fitting the aggregate and compositional variability to the framework proposed by Micheli et al. (1999). In order to fit the system variability within each interval to one of the four described patterns—synchrony, asynchrony, compensation or stasis (Micheli et al. 1999)—it was necessary to fit both types of variability onto a scale of ‘low’ to ‘high.’ For the compositional variability, this was achieved by comparing the total variance of the Hellinger-transformed LOPC data within each time interval to the total variance of these data over the entire study period. If the total variance within an interval was less than the total variance over the entire study period, it was classified as exhibiting low compositional variability. High compositional variability within a time interval was declared when the total variance in that interval was greater than double the total variance over the entire study period. Values that fell between the upper bound of low and the lower bound of high were described as being of intermediate variability. To fit the compositional variability into the Micheli et al. (1999) framework, the bound between synchrony and asynchrony, and between stasis and compensation, was set at the midpoint of the intermediate compositional variability range.

Prior to fitting the aggregate variability values into the Micheli et al. (1999) framework, it was first necessary to standardize each of the three CV values for each interval by dividing it by the mean CV plus two standard deviations (the maximum value of high CV values) of the corresponding aggregate metric. These standardized values, which were similar across all three metrics at each of the intervals, were then averaged to obtain an average standardized CV value to represent the aggregate variability at each time interval. In a similar fashion to the method used for compositional variability, aggregate variability values were classified into low, intermediate, and high ranges within each time interval, using classification criteria presented by Romano et al. (2005): low =  $CV \leq y - s$ ; intermediate =  $y - s < CV \leq y + s$ ; high =  $y + s < CV \leq y + 2s$ ; where  $y$  = mean value for CV and  $s$  = standard deviation for CV. The bounds between these low, intermediate, and high variability classifications were also averaged across the

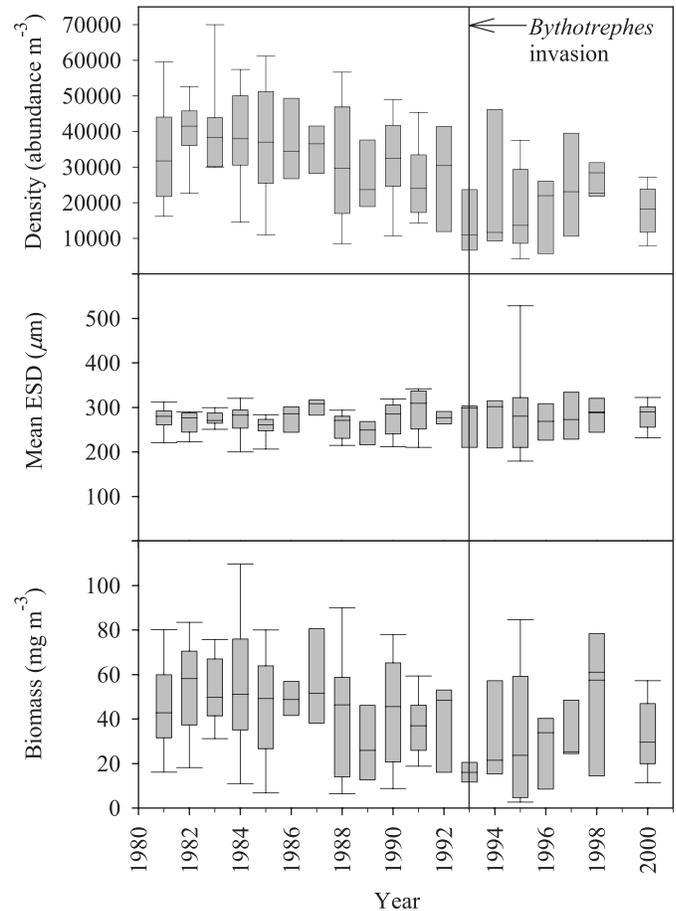


Fig. 1. Annual box plots of density (abundance per cubic meter sampled), mean body size as expressed in equivalent spherical diameter (ESD), and total biomass per cubic meter sampled for each zooplankton sample processed with the LOPC.

three metrics, and the bound between synchrony and stasis, and between asynchrony and compensation, was set at the midpoint of the intermediate aggregate variability range to fit the aggregate variability values into the Micheli et al. (1999) framework.

## Results

The aggregate metrics of density, mean body size (represented as mean ESD), and biomass over the study period revealed that the introduction of *Bythotrephes* in 1993 was accompanied by reduced mean density and increased variability in mean body size. General trends of annual variability and marked within-year variability in the aggregate metrics are shown in Fig. 1.

The optimal division of the chronologically ordered LOPC data by the least-squares criterion within MRT analysis occurred between samples 118 and 119. This split divided the data into two time segments: 1980–1992 (pre-*Bythotrephes*) and 1993–2000 (post-*Bythotrephes*). This, most significant, split in the data between 1992 and 1993 was also repeated in the results of the MRT analysis of the subsampled LOPC data set, confirming that the division

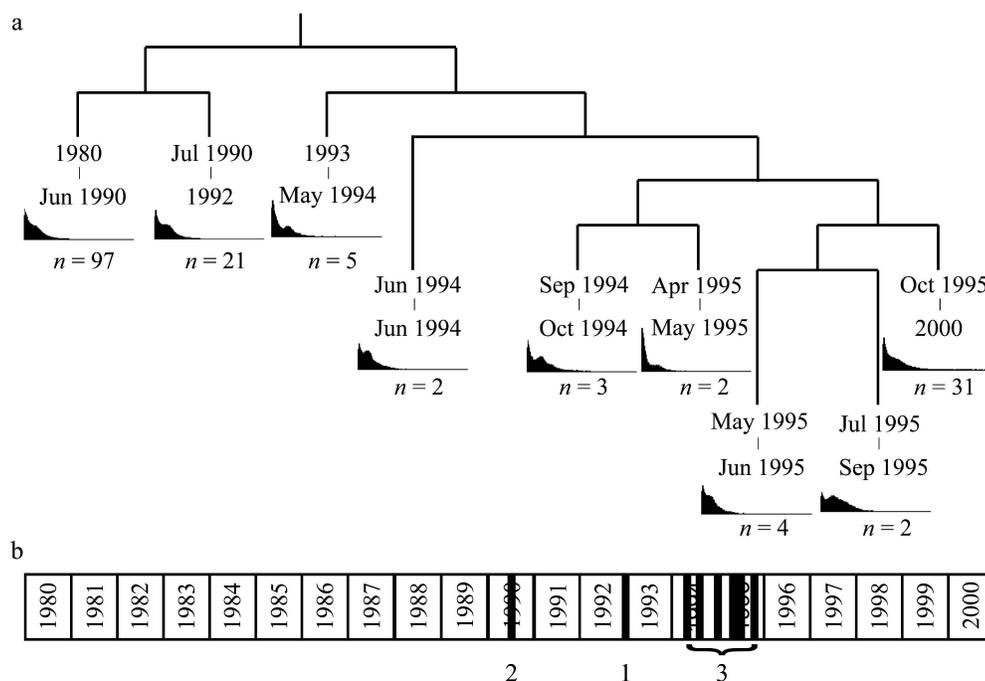


Fig. 2. Multivariate regression-tree analysis results for chronologically ordered LOPC data. (a) The numbers at the end of each leaf of the tree indicate the time period and number of samples ( $n$ ), while the bar plots show the average abundances for each size bin at each terminal node. (b) The splits of the tree are further demonstrated here by the bold black lines in the timeline, while the bold numbers indicate the level of the split (1 being the most significant). Note the abscissa time scale in (a) is unrelated to that in (b).

was not an artifact of a change in sampling availability around this period. The tree for the entire LOPC data set, pruned to nine leaves (end groups) by cross-validation, is shown in Fig. 2. Following the most significant division in the data between 1992 and 1993, a further division occurred during 1990, producing the two leftmost leaves. The data then split into six statistically separate intervals, as shown by the six leaves, between 1993 and September 1995. As shown in Fig. 2, this series of divisions broke the study period into four distinct intervals—the two (leftmost) intervals in which the data were contained within a single branch, one in which the data split into six leaves over the 1993–1995 interval, and then one (rightmost) long interval in which the data were again contained within a single branch. This chronological division of the data suggests that the three single-branch intervals exhibited low compositional variability, while the interval in which the data split into six separate branches exhibited high compositional variability. This is further shown in Fig. 3, in which the total variance of the Hellinger-transformed LOPC data—a measure of beta diversity, used here to quantify compositional variability—was plotted over the study period. The total variance in the post-*Bythotrephes* period was greater than in the pre-*Bythotrephes* period, but Fig. 3b revealed this increase to be primarily due to a spike in total variance during the 1993 to September 1995 interval.

MRT analysis results for Red Chalk and Blue Chalk lakes varied from those of Harp Lake. Compared with the confirmed timing of the most significant split in the Harp

Lake data between 1992 and 1993, the most significant splits in the Red Chalk and Blue Chalk lake data sets occurred between 1994 and 1995, and between April and July 1989, respectively. Subsequent splits in the Red Chalk Lake data were observed in July 1980 and September 1989, with subsequent splits in the Blue Chalk Lake data set occurring in June 1988 and May 1996. After dividing the data for each reference lake into four time intervals based on these splits, the total variance within each interval was then calculated and is shown in Fig. 3c. Both Red Chalk and Blue Chalk lakes exhibited unique patterns in total variance over the study period, and both were different from the temporal pattern of the Harp Lake total variance. Both reference lakes also exhibited increases in total variance in their latest time interval, in contrast to the reduction in total variance evident in the Harp Lake total variance pattern as shown in Fig. 3b.

Aggregate variability of Harp Lake (as expressed by CV values for zooplankton body size, density, and biomass) exhibited similar patterns to the total variance over the study period, as shown in Fig. 3. There was a clear increase in variability for all three metrics between the pre- and post-*Bythotrephes* intervals as shown in Fig. 3a, although this increase was primarily due to the spike in the 1993 to September 1995 interval as shown in Fig. 3b.

External validation of the Harp Lake MRT results was carried out by relating (by RDA) the species abundance data to the most statistically significant MRT split in the community size structure. The canonical relationship was highly significant ( $p = 0.0001$ ). It also identified 10

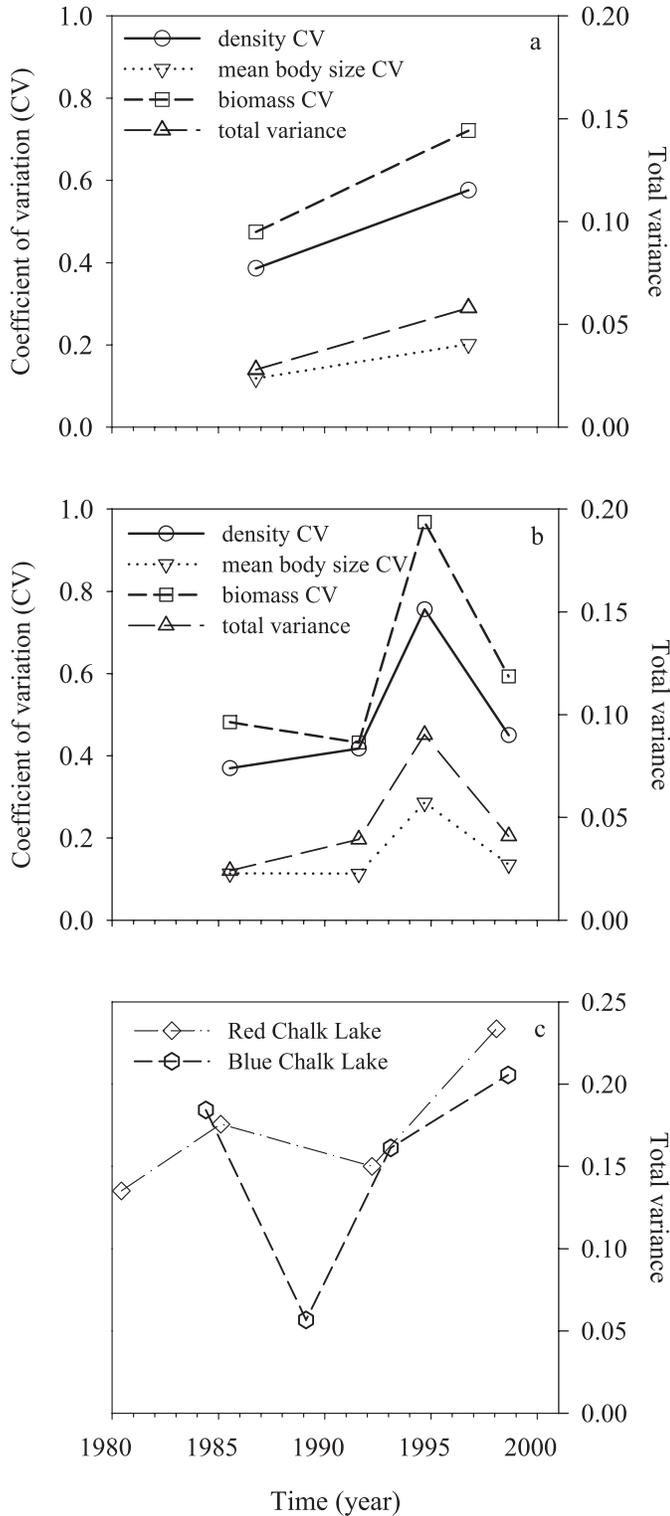


Fig. 3. Aggregate variability as expressed by coefficient of variation (CV, left ordinate scale) of the mean body size, density, and biomass; and compositional variability as expressed by total variance (right ordinate scale) of the Hellinger-transformed LOPC abundance data for (a) pre-*Bythotrephes* (1980–1992) and post-*Bythotrephes* (1993–2000) periods of Harp Lake; and (b) for four time intervals over the entire study period (1980–June 1990, July 1990–1992, 1993–September 1995, October 1995–2000) of Harp Lake.

zooplankton species or groups (hereafter simply referred to as species) for which > 5% of their variance was explained by the MRT split: Sp110 (*Bosmina longirostris*), Sp118 (*Chydorus sphaericus*), Sp122 (*Daphnia galeata mendotae*), Sp127 (*Daphnia retrocurva*), Sp133 (*Eubosmina tubicen*), Sp152 (*Diaphanosoma birgei*), Sp301 (Cyclopoid copepodid), Sp208 (*Leptodiatomus sicilis*), Sp215 (Calanoid nauplius), and Sp312 (*Tropocyclops prasinus mexicanus*). Of these, > 20% variance of the abundance of Sp118 (*C. sphaericus*), Sp122 (*D. galeata mendotae*), Sp133 (*E. tubicen*), Sp152 (*D. birgei*), Sp208 (*L. sicilis*), and Sp312 (*T. prasinus mexicanus*), and > 30% of the variance of Sp110 (*B. longirostris*) abundance was explained by the MRT split. Indicator-species analysis extended the RDA results further, identifying two groups of indicator species—seven that were significant ( $p < 0.05$ ) prior to the most significant split in the MRT results (1980–1992), and three that were significant ( $p < 0.05$ ) following the most significant split in the MRT results (1993–2000). The significant species during 1980–1992 were Sp110 (*B. longirostris*), Sp118 (*C. sphaericus*), Sp127 (*D. retrocurva*), Sp133 (*E. tubicen*), Sp152 (*D. birgei*), Sp301 (Cyclopoid copepodid), and Sp312 (*T. prasinus mexicanus*; left-hand group in Fig. 4); while the significant species during 1993–2000 were Sp122 (*D. galeata mendotae*), Sp208 (*L. sicilis*), and Sp215 (Calanoid nauplius; right-hand group in Fig. 4).

Prior to examining Harp Lake zooplankton community variability, compositional and aggregate variability values were classified as low, intermediate, or high. It was found that compositional variability, as expressed by total variance, was low during 1980 to June 1990, intermediate–low during July 1990 to 1992, high during the 1993 to September 1995 interval immediately following invasion, and finally returned to intermediate–low from October 1995 onward. Classification of aggregate variability, as expressed by the mean of the standardized CV values for the three aggregate metrics, revealed that aggregate variability was low during the two intervals of 1980 to 1992, high during the 1993 to September 1995 interval immediately following invasion, and intermediate–low from October 1995 onward.

Examining community variability, through the combined consideration of aggregate and compositional variability, revealed that only immediately following invasion by *Bythotrephes* were both aggregate and compositional variability high. As shown in Fig. 5, in which both aggregate and compositional variability of Harp Lake were fit onto the framework based on that of Micheli et al. (1999), this corresponded to an asynchronous pattern of community variability during the time interval of 1993 to

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Compositional variability as expressed by total variance of the Hellinger-transformed species-density data for the Red Chalk and Blue Chalk reference lakes are also shown (c), for the Red Chalk Lake time intervals of May–July 1980, July 1980–August 1989, October 1989–1994, and 1995–2000; and the Blue Chalk Lake time intervals of 1980–May 1988, June 1988–October 1989, November 1989–May 1996, and June 1996–2000. All values are plotted at the mid-point of their respective time interval.

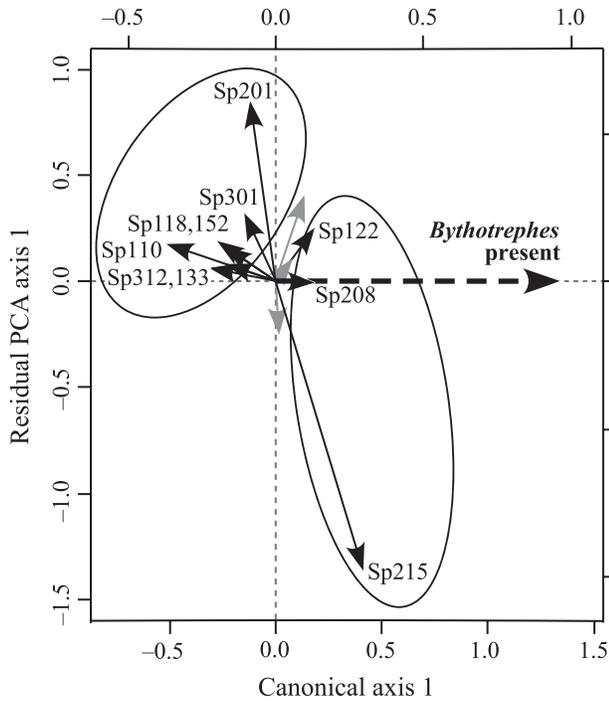


Fig. 4. RDA bi-plot relating Harp Lake zooplankton species abundances (solid arrows) to the presence of *Bythotrephes*, based on timing of sample (dashed arrow). Scaling type 2 was used to display the covariances among the species. Indicator species analysis results are also included here, indicated by ellipses and species number labels. Seven zooplankton species were significant indicators of the pre-*Bythotrephes* period, and three zooplankton species were significant indicators of the post-*Bythotrephes* period. Species labels for the least significant species, indicated by the grey arrows, are not included.

September 1995. The three other time intervals all fell within the stasis pattern of community variability, although the community variability during the final interval of October 1995 to 2000 was closer to the pattern of synchrony than during the two pre-invasion intervals. The interval immediately prior to the 1993 invasion exhibited neither unusual nor high aggregate or compositional variability, and actually had the lowest mean aggregate variability of all four time intervals, due to a decrease in biomass CV.

Discussion

Significant shifts in the aggregate and compositional zooplankton variability of Harp Lake reveal a period of transitory high variability, significantly greater than normal system variability, during the 1993–1995 period immediately post-invasion. Taxonomic studies have previously identified a rapid reduction in zooplankton species richness, and significant changes in the abundance and mean Cladoceran size, in Harp Lake following invasion (Yan and Pawson 1997; Yan et al. 2001, 2002), before these metrics stabilized from 1995 onward (Yan et al. 2001). Here we see evidence of this stabilization in the LOPC-measured data, because the aggregate and compositional variability

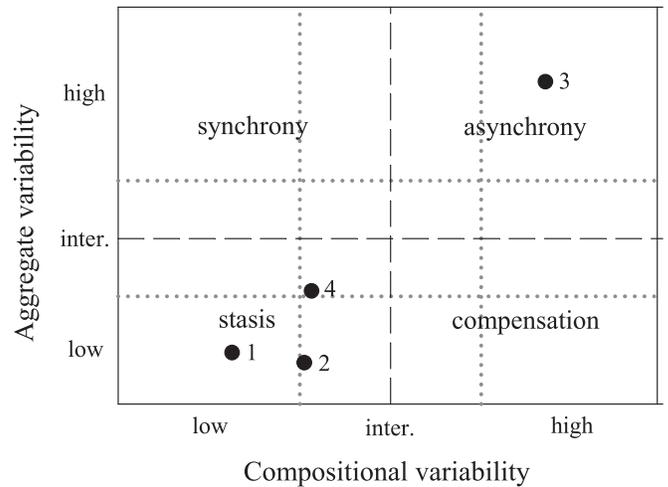


Fig. 5. Standardized community variability as represented by the combination of aggregate and compositional variability over the four intervals of the study period, each represented by a circle (1: 1980–June 1990; 2: July 1990–1992; 3: 1993–September 1995; 4: October 1995–2000). The dashed lines represent the bounds between the four types of variability patterns (synchrony, asynchrony, stasis, and compensation), while the dotted lines indicate the bounds between low, intermediate (inter.), and high aggregate and compositional variability.

metrics return to intermediate–low values from 1995 until the conclusion of the study period in 2000. External validation by RDA and indicator-species analyses also reveal taxonomic significance of the size-centric Harp Lake MRT results, with RDA analysis identifying 10 species that were significantly affected by the introduction of *Bythotrephes* to Harp Lake (as represented by the binary variable constructed from the timing of the most significant MRT split), while the indicator-species analysis revealed that 7 of the 10 were significant species prior to invasion, while the remaining 3 were significant post-invasion.

MRT analysis on nearby reference lakes of Red Chalk and Blue Chalk revealed that neither reference lake exhibited a statistically significant split in their species composition data around the time of the 1992–1993 split observed in the Harp Lake LOPC data set. Varying temporal patterns in total variance were also observed among the three lakes, which suggested that there was no clear regional trend in total variance over the study period. These differences, and the fact that both Red Chalk and Blue Chalk lakes exhibited similar water chemistry (Yan et al. 2008) and algal composition dynamics (Paterson et al. 2008) over the study period, strengthen the argument that the significant split in the LOPC data during 1992–1993 and the subsequent observations in aggregate and compositional variability dynamics within Harp Lake are attributable to the unique driver of invasion by *Bythotrephes* within the lake.

The dual consideration of aggregate and compositional variability provides a means to extend our knowledge by assessing the ecological effects of invasion by *Bythotrephes* on the community as a whole and as a collection of varyingly sized organisms. In the two pre-*Bythotrephes*

periods the general community variability pattern was one of stasis, which in a stable environment may be indicative of either strong consumer self-limitation or strong competitive interactions (Micheli et al. 1999). During the period of high aggregate and compositional variability immediately after invasion, the community variability shifted to a pattern of asynchrony. Asynchrony has been demonstrated to be the dominant pattern in communities in which an intermediate time lag exists between predator and prey (Micheli et al. 1999). This is interesting to note, given that in addition to showing that the zooplankton community of Harp Lake stabilized from 1995 following the period of significant change following invasion, Yan et al. (2001) also found marked changes in the phenology of *Bythotrephes* in Harp Lake post-1995. These changes may have played a role in the shift from asynchrony after October 1995 when the system returned to a pattern of stasis, albeit one that was closer to synchrony than the two pre-*Bythotrephes* patterns, given that synchrony has been shown to be a dominant pattern in communities for which no time lag exists between predator and prey (Korpimäki 1994).

The asynchronous dynamics immediately following invasion also suggest the presence of competitive interactions between species of varying sensitivity to the stressor of *Bythotrephes* invasion. The role of biotic interactions in facilitating asynchronous or compensatory dynamics when a community experiences an environmental fluctuation has been demonstrated theoretically (Doak et al. 1998; Ives et al. 1999; Ives and Cardinale 2004), though empirical investigations are rare (Descamps-Julien and Gonzalez 2005; Downing et al. 2008). In the case of Harp Lake, it appears likely that indirect responses to the invasion expressed through biotic or trophic interactions were acting in conjunction with direct organism responses to result in community asynchrony (Downing et al. 2008).

The presence of compensatory or asynchronous dynamics has also been shown to stabilize ecosystems (Tilman et al. 1998; Ives et al. 1999; Ives and Cardinale 2004), though this stability may be dependent on the manner in which the community responds to the initial stressor. Although general loss of species within a community may decrease its ability to exhibit compensatory or asynchronous dynamics—and thus buffer against further environmental changes—in the future, an ordered loss of species most sensitive to the stressor will result in a community with greater overall tolerance to its effects (Ives and Cardinale 2004). Additionally, the role of biotic interactions and resulting compensation or asynchrony in response to a stressor can also mask the sensitivity of a given species to the community stressor, and enhance the difficulty in predicting how the system may then respond to further changes (Ives and Cardinale 2004). The asynchrony identified here, in conjunction with the loss of species previously identified (Yan et al. 2001) during the period immediately following *Bythotrephes* invasion, may suggest that the more apparently stable Harp Lake community post-1995 is one with greater tolerance to *Bythotrephes*, but that it may be more sensitized to future stressors. It is also possible that widespread calcium loss (Jeziorski et al. 2008) across the softwater boreal lake system in which Harp is

located has reduced the resilience of lakes to the threat of invasion by *Bythotrephes*, which has been found to have much lower calcium requirements than its prey (Kim et al. 2012). As argued by Ives and Cardinale (2004), such potential sensitivities within an ecosystem highlight the importance of a whole-ecosystem approach to conservation of biodiversity, because stressor-induced loss of species may result in previously insignificant species playing critical roles in buffering against future stressors.

The changing roles of species within systems undergoing change is also highlighted here in the MRT external validation results. Of the seven species identified as significant during the 1980–1992 period, *B. longirostris*, *C. sphaericus*, *E. tubicen*, and *D. birgei* were the dominant cladoceran taxa within Harp Lake during the 1980s and all but *B. longirostris* disappeared following invasion (Yan and Pawson 1997). Of the three species identified here as being significant during 1993–2000, the abundances of both *D. galeata mendotae* and *L. sicilis* increased appreciably in Harp Lake post-invasion (Yan and Pawson 1997; Yan et al. 2001). Species that were significant prior to invasion have been replaced by fewer newly significant species, and it remains to be seen how this new hierarchy may respond to future stressors.

This study has implications for the use of organism body size as a metric in the study of invasion-induced community dynamics, and for the LOPC as a means to collect high-resolution zooplankton community data. The consistency between this work and previous studies into changes of the zooplankton community following invasion by *Bythotrephes*, and more importantly the increased insight into community dynamics this new approach provides, give clear value to the more automated method of collecting zooplankton body size data by processing samples through a LOPC. The LOPC-based method allows for rapid counting and measuring of all zooplankton within the 105–35,000  $\mu\text{m}$  ESD size range, as opposed to the more time-consuming method of manually identifying, counting, and measuring zooplankton from subsamples. It also does not require as high a level of biological expertise as does the traditional process of identifying, counting, and measuring. The price of this gain in time efficiency and requisite expertise is a lack of species-specific data in the resulting LOPC data set, and the continued value of taxonomic data collection is recognized. As shown here, the benefits of analyzing high-resolution LOPC-collected data relating to zooplankton samples in their entirety, and not only subsamples of species of interest, not only allows us to more deeply investigate community-wide zooplankton dynamics such as variability, but can also be easily and constructively related to traditional taxonomic data. Looking ahead, there is also scope to extend the approach presented here to LOPC data collected in situ, thus extending the data collection to capture those taxa that may be difficult to catch or retain by net, and also allowing for changes in the vertical and horizontal spatial distribution of zooplankton communities to be assessed.

This study clearly demonstrated a significant shift from normal system variability in both aggregate and compositional variability metrics within a zooplankton community, triggered

by colonization by the invasive species *B. longimanus*. Studying the combined effects of changes in aggregate and compositional variability, as primarily measured through organism body size, provided insights into community dynamics in the lead up to, during, and following the invasion that were not apparent through previous consideration of species metrics alone. The importance of increasing the knowledge base of the effects of *Bythotrephes* invasion is clear, given the current lack of an appropriate management strategy for its removal from invaded systems, the generally inevitable nature of such invasions once they have begun (Ricciardi et al. 2011), and the rapid spread of *Bythotrephes* through Ontario coupled with the prevalence of lakes in the region with suitable habitat for *Bythotrephes* (Yan et al. 2001). Such work provides managers with information about the possible ecological consequences to expect if their system should become invaded, and may be used to develop effective management and monitoring strategies for invaded systems and prevent further ecosystem degradation by future stressors on the potentially newly sensitized systems.

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