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Ecosystem size determines food-chain length in lakes

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Food-chain length is an important characteristic of ecological communities¹; it influences community structure², ecosystem functions¹⁻⁴ and contaminant concentrations in top predators^{5,6}. Since Elton⁷ first noted that food-chain length was variable among natural systems, ecologists have considered many explanatory hypotheses^{1,4,8,9}, but few are supported by empirical evidence^{4,10,11}. Here we test three hypotheses that predict food-chain length to be determined by productivity alone (productivity hypothesis)^{4,10,12,13}, ecosystem size alone (ecosystem-size hypothesis)^{14,15} or a combination of productivity and ecosystem size (productive-space hypothesis)^{7,16-18}. The productivity and productive-space hypotheses propose that food-chain length should increase with increasing resource availability; however, the productivity hypothesis does not include ecosystem size as a determinant of resource availability. The ecosystem-size hypothesis is based on the relationship between ecosystem size and species diversity, habitat availability and habitat heterogeneity^{14,15}. We find that food-chain length increases with ecosystem size, but that the length of the food chain is not related to productivity. Our results support the hypothesis that ecosystem size, and not resource availability, determines food-chain length in these natural ecosystems.

A major impediment to critically testing the productivity (Fig. 1a), productive-space (Fig. 1c) and ecosystem-size (Fig. 1b) hypotheses in natural systems has been the inability to measure accurately both food-chain length and ecosystem size^{12,18}. We overcame these obstacles by using stable isotope techniques to determine food-chain length and by taking advantage of the relative isolation of lakes to estimate ecosystem size. We estimated food-chain length in 25 northern temperate lakes ranging in volume from 3.8×10^5 to 1.7×10^{12} m³, and ranging in total phosphorus from 2.6 to 230 $\mu\text{g l}^{-1}$. Nearly all northern temperate lakes are phosphorus-limited and total phosphorus is a strong predictor of primary productivity in phosphorus-limited lakes¹⁹. Vander Zanden *et al.* described a positive relationship between food-chain length and both lake area and water clarity (their measure of productivity)²⁰. They could not separate, however, the influence of productivity and ecosystem size because their gradients of lake area and water clarity were correlated. Total phosphorus and lake volume are not correlated in our 25 lakes ($r = -0.21$, $P = 0.31$).

Our work differs from previous empirical tests of food-chain theory in four important ways. First, we collected data from lakes along independent gradients of productivity and ecosystem size. Second, our observations were made at the ecologically relevant scale of whole food webs. Third, our observations were made in temperate lakes that are ecologically similar, contain similar communities and are all located within a restricted geographic region.

Finally, we used stable isotope techniques to estimate maximum trophic position (MTP), a variable that is conceptually similar to mean food-chain length¹⁰. Because MTP is a continuous variable, we can detect subtle changes in food-chain length within the naturally occurring range of ecosystem size and productivity.

Stable isotope ratios of nitrogen and carbon are powerful tools for evaluating trophic structure and energy flow in ecological communities²¹. The $\delta^{15}\text{N}$ of an organism is typically enriched by 3.4‰ ($\pm 1\%$) relative to its diet²², and can be used to determine the trophic position of an organism. In contrast, $\delta^{13}\text{C}$ changes little as carbon moves through the food web and can be used to evaluate the ultimate sources of energy for an organism^{21,23}. In lakes, $\delta^{13}\text{C}$ is particularly useful for differentiating between the two major sources of available energy: littoral (near shore) production from attached algae and detritus, and pelagic (open water) production from phytoplankton²⁴. In our study lakes, the difference between littoral and pelagic $\delta^{13}\text{C}$ was between 2 and 10‰ (mean, 6.5%), with littoral $\delta^{13}\text{C}$ enriched in ¹³C relative to pelagic $\delta^{13}\text{C}$.

Stable isotopes provide a continuous measure of trophic position (as opposed to discrete trophic levels) which integrates the assimilation of mass from all the trophic pathways leading to a top predator^{8,25}. Estimates of trophic position using stable isotope techniques therefore reflect the magnitude of energy or mass flow through different food web pathways and account for complex interactions such as trophic omnivory⁵. Trophic position is calculated as $\lambda + (\delta^{15}\text{N}_{\text{organism}} - \delta^{15}\text{N}_{\text{base of food web}})/3.4$, where λ is the trophic position of the organism used to estimate $\delta^{15}\text{N}_{\text{base of food web}}$ (for example, $\lambda = 2$ for primary consumers), $\delta^{15}\text{N}_{\text{organism}}$ is measured directly and 3.4 is the average enrichment in $\delta^{15}\text{N}$ per trophic level. For long-lived and mobile predators, $\delta^{15}\text{N}_{\text{base of food web}}$ (hereafter called $\delta^{15}\text{N}_{\text{base}}$) must capture the potentially high temporal variation in $\delta^{15}\text{N}$ of primary producers and detrital energy sources, and account for the spatial heterogeneity in $\delta^{15}\text{N}$ both within and among lakes. Our observations show that, within a lake, there can be seasonal differences of $>4\%$ in the $\delta^{15}\text{N}$ of phytoplankton and 3–4‰ differences in $\delta^{15}\text{N}_{\text{base}}$ between the littoral and pelagic food webs. Furthermore, we observed differences of $>13\%$ in $\delta^{15}\text{N}_{\text{base}}$ among lakes for both pelagic and littoral food webs. To account for this variability, we used filter-feeding mussels and surface-grazing

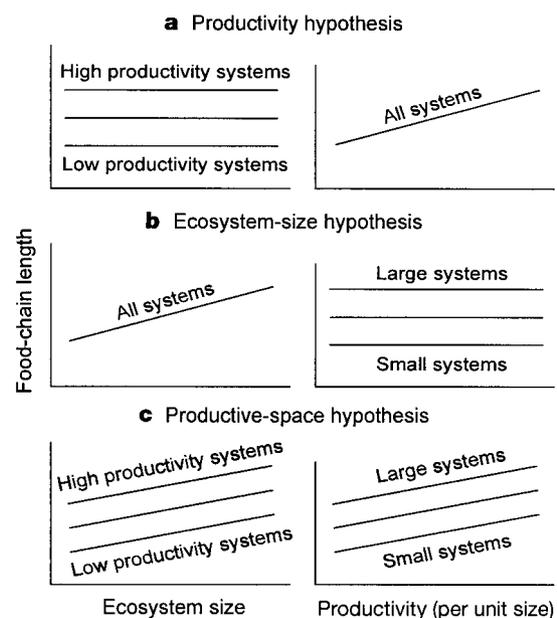


Figure 1 Hypothesized relationships between food-chain length and ecosystem size, and between food-chain length and productivity. **a**, For the productivity hypothesis; **b**, for the ecosystem-size hypothesis; **c**, for the productive-space hypothesis.

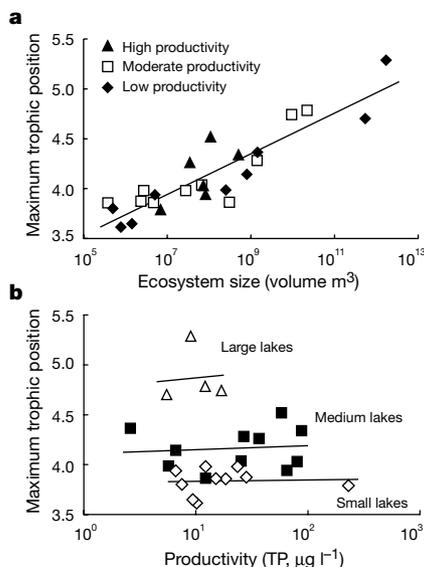


Figure 2 Relationships between maximum trophic position and ecosystem size or productivity. **a**, Ecosystem size for low (2–11 $\mu\text{g l}^{-1}$ total phosphorus (TP)), moderate (11–30 $\mu\text{g l}^{-1}$ TP) and high productivity lakes (30–250 $\mu\text{g l}^{-1}$ TP). **b**, Productivity for small (3×10^5 to $3 \times 10^7 \text{ m}^3$), medium (3×10^7 to $3 \times 10^9 \text{ m}^3$) and large lakes (3×10^9 to $2 \times 10^{12} \text{ m}^3$). Maximum trophic position is the trophic position of the species with the highest average trophic position in each of the lake food webs. The data are from 25 lakes in northeastern North America.

snails collected in each of our study lakes as temporal integrators of $\delta^{15}\text{N}_{\text{base}}$ for the pelagic and littoral food webs, respectively. Snails reflect the isotopic signature of the detritus and periphyton that form the base of littoral food webs and mussels reflect the isotopic signature of seston, which forms the base of pelagic food webs. We calculated the trophic position of fish at the top of each lake food web using the equation: trophic position = $2 + (\delta^{15}\text{N}_{\text{fish}} - [\delta^{15}\text{N}_{\text{mussel}} \times \alpha + \delta^{15}\text{N}_{\text{snail}} \times (1 - \alpha)]) / 3.4$, where α is the proportion of carbon in a target organism ultimately derived from the base of the pelagic food web: $\alpha = (\delta^{13}\text{C}_{\text{fish}} - \delta^{13}\text{C}_{\text{snail}}) / (\delta^{13}\text{C}_{\text{mussel}} - \delta^{13}\text{C}_{\text{snail}})$. Trophic position is an attribute of individual fish or of a single fish species, and MTP, like mean food-chain length, is an attribute of an entire food web. MTP is the trophic position of the species with the highest average trophic position in the lake food web.

Maximum trophic position increased with lake volume but not with total phosphorus (Fig. 2), and lake volume was the only significant predictor of MTP (log volume, $t = 9.28$, $P < 0.001$; log total phosphorus, $t = 0.59$, $P = 0.56$). Lake size alone explained 80% of the variation in maximum trophic position (MTP = $2.51 + 0.2 \times \log \text{volume}$; $P < 0.001$, $r^2 = 0.80$). The increase in MTP from about 3.5 in the smallest lakes to around 5 in the Laurentian Great lakes represents an increase in food-chain length of almost 1.5 trophic levels. Four species occupied MTP in our data set: largemouth bass and northern pike in the smaller lakes, and walleye and lake trout in the largest lakes (Fig. 3a).

The increase in MTP with increasing lake size was caused by both the addition of new top predator species to larger lakes and a general increase in the trophic position of each top predator with increasing lake size (Fig. 3). For example, lake trout were not found in our smallest lakes, and in the largest lakes their trophic position increased with increasing lake size (Fig. 3). The addition of a new top predator increases MTP by adding new trophic steps to the top of the food web. In contrast, changes in the trophic position of a single top predator species must be caused by lengthening of food chains between the top and bottom of the food web. This lengthening is probably caused by some combination of functional

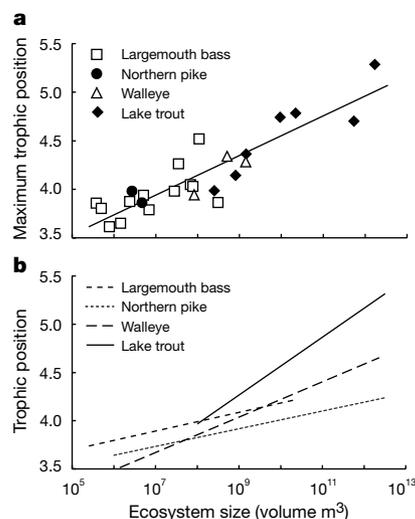


Figure 3 The increase in maximum trophic position is caused by both changes in top predator species and increases in the trophic position of each top predator. **a**, Maximum trophic position data from Fig. 2a labelled to identify the top predator species in each lake. **b**, The relationship (linear regression) between trophic position of top predator species and ecosystem size for largemouth bass (14 lakes, $n = 44$, $r^2 = 0.22$, $P < 0.01$), walleye (13 lakes, $n = 39$, $r^2 = 0.55$, $P < 0.01$), northern pike (11 lakes, $n = 25$, $r^2 = 0.20$, $P < 0.01$) and lake trout (7 lakes, $n = 22$, $r^2 = 0.89$, $P < 0.01$) plotted over the range of ecosystem size in which each species was found (data not shown).

diversification of the middle of the food web (for example, the addition of a new intermediate predator such as mysid shrimp, *Mysis relicta*, in our larger lakes) and reduced trophic omnivory at any or all trophic levels. Trophic omnivory probably declines as lake size increases because habitat heterogeneity and prey refugia increase with lake size. These changes may allow larger populations of preferred or optimal prey, which promote increased dietary specialization and reduced trophic omnivory. These changes to the top and middle of food webs are facilitated by increases in functional rather than species diversity.

If functional diversity is a determinant of maximum trophic position, then the ultimate cause of variation in food-chain length will be factors that influence the functional diversity of species in food webs. Human-mediated modifications of natural systems that affect the functional diversity of food webs, such as cultural eutrophication, species invasions and habitat fragmentation, will also affect food-chain length. Changes in food-chain length may, in turn, cause further changes in community dynamics, ecosystem function and contaminant concentrations in apical predators.

The importance of primary productivity in explaining variation in food-chain length has been debated for 40 years^{4,10,12,16}. Unlike the few studies that indicate some relationship between productivity and food-chain length^{12,20,26,27}, we found that ecosystem size was the only determinant of food-chain length in our study lakes. In contrast to many previous studies^{12,20,26,27}, we adopted a continuous measure of food-chain length and used independent gradients of productivity and ecosystem size in a natural setting. Although productivity must be an ultimate constraint of food-chain length^{16,17}, and may be important in small systems^{12,26} or where productivity is very low^{16,18}, our results and those of others^{4,8,10} show that productivity is a poor predictor of, and has a limited direct role in controlling food-chain length. □

Methods

To test the use of mussels and snails as indicators of the isotopic signature of the base of the littoral and pelagic food webs, we collected time series of periphyton (attached algae) and detritus samples from the littoral zone, and zooplankton and seston (a mixture of phytoplankton and other particulate organic matter) from integrated epilimnetic water

samples from the pelagic zones of three lakes (Spencer, Oneida and Cayuga). Periphyton and detritus were brushed from rocks, macrophytes and logs and pre-filtered through a 75-µm mesh to remove large invertebrates. Seston was pre-filtered through a 75 or 30 µm mesh. All periphyton and seston samples were then filtered onto pre-combusted glass fibre filters. Zooplankton were filtered from the water using a 150-µm mesh and visually inspected to remove particulate contaminants and predatory zooplankton. Each lake was sampled every two weeks from early June to late August (5–6 dates) in 1997 and 1998. Zooplankton were collected to provide the $\delta^{13}\text{C}_{\text{base}}$ of the pelagic food web because zooplankton are a better indicator of $\delta^{13}\text{C}_{\text{base}}$ for the pelagic food web than seston samples²⁸. Snails and mussels were sampled in late August. In Spencer Lake we used unionid mussels (Unionacea) and in Cayuga and Oneida lakes we used zebra mussels (*Dreissena polymorpha*). In three other New York lakes (Champlain, Conesus and Keuka lakes), where unionid and zebra mussels occurred together, we found no difference in their $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values (nested ANOVA with species nested in lake, d.f. = 3, $F = 2.85$, $P = 0.06$ for $\delta^{13}\text{C}$; d.f. = 3, $F = 1.79$, $P = 0.19$ for $\delta^{15}\text{N}$). Mussels and snails effectively captured the spatial variation and integrated the temporal variation in the $\delta^{15}\text{N}_{\text{base}}$ and $\delta^{13}\text{C}_{\text{base}}$ of pelagic and littoral food webs. Using lake-by-habitat combinations as replicates ($n = 6$ for both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$), we found no significant differences between the median $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of each time series and the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of snails and mussels (paired t -test for means: $t = 2.29$, $P = 0.07$ for $\delta^{13}\text{C}$; $t = 2.19$, $P = 0.08$ for $\delta^{15}\text{N}$, where we subtracted 3.4‰ from the $\delta^{15}\text{N}$ of snails and mussels to remove the expected one trophic level of enrichment).

In each lake, we collected all fish species that were likely to feed at the top of the food web. Because trophic position can increase with fish length, we collected adult fish of each species and held length as constant as possible across the lake size gradient. The fish species collected and the lengths of fish analysed were: largemouth bass (*Micropterus salmoides*; 250–440 mm), smallmouth bass (*Micropterus dolomieu*; 250–450 mm), northern pike (*Esox lucius*; 450–840 mm), chain pickerel (*Esox niger*; 390–530 mm), walleye (*Stizostedion vitreum*; 300–700 mm), burbot (*Lota lota*; 580–740 mm), lake trout (*Salvelinus namaycush*; 460–730 mm), brook trout (*Salvelinus fontinalis*; 410 mm), chinook salmon (*Oncorhynchus tshawytscha*; 800–1000 mm), rainbow trout (*Oncorhynchus mykiss*; 340–480 mm), Atlantic salmon (*Salmo salar*; 490–770 mm) and brown trout (*Salmo trutta*; 310–610 mm). Snails and mussels were collected from each lake between late July and early September each year. Most fish were collected in late July to October, but for a few lakes we used fish collected in June. We used total phosphorus from integrated epilimnetic water samples taken in late July or August as an index of lake productivity. Our range of total phosphorus (2.6 to 230 µg l⁻¹) corresponds to a range of primary productivity of ~30–450 g C m⁻² yr⁻¹ (ref. 29). We used previously documented volume estimates for 21 of our lakes. For the remaining four lakes, we estimated lake volume as a hyperbolic sinusoid: $0.43 \times \text{area} \times \text{maximum depth}$.

We took a small section of muscle tissue from each fish for isotopic analysis. Snails and mussels were dissected and aggregated, particulate contaminants were removed and only soft tissue was used for isotopic analysis. Samples were dried at 40 °C for >48 h and ground to a fine powder. We then extracted lipids (using methanol-chloroform extraction) from all animal samples because lipids are depleted in ¹³C compared with whole organisms^{21,30} and lipid content in our tissue samples was variable (ranging from about 5% by mass in largemouth bass to >30% in some lake trout). Stable isotope analysis was performed using a Europa Geo 20/20 continuous flow isotope ratio mass spectrometer at the Cornell Laboratory for Stable Isotope Analysis. The standard error of the replicates of all our analyses were 0.05‰ for $\delta^{13}\text{C}$ and 0.18‰ for $\delta^{15}\text{N}$. All stable-isotope values are reported in the δ notation: $\delta^{15}\text{N} = ((^{15}\text{N}_{\text{sample}}/^{14}\text{N}_{\text{sample}})/(^{15}\text{N}_{\text{standard}}/^{14}\text{N}_{\text{standard}})) - 1) \times 1,000$, where the global standard is atmospheric nitrogen, and $\delta^{13}\text{C} = ((^{13}\text{C}_{\text{sample}}/^{12}\text{C}_{\text{sample}})/(^{13}\text{C}_{\text{standard}}/^{12}\text{C}_{\text{standard}})) - 1) \times 1,000$, where the global standard is Pee Dee Belemnite²¹.

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Cryptophyte algae are robbed of their organelles by the marine ciliate *Mesodinium rubrum*

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***Mesodinium rubrum* (Lohmann 1908) Jankowski 1976 (= *Myrionecta rubra*)^{1,2} is a common photosynthetic marine planktonic ciliate which can form coastal red-tides³. It may represent a 'species complex'^{4,5} and since Darwin's voyage on the *Beagle*, it has been of great cytological, physiological and evolutionary interest⁴. It is considered to be functionally a phytoplanktoner because it was thought to have lost the capacity to feed and possesses a highly modified algal endosymbiont^{5,6}. Whether *M. rubrum* is the result of a permanent endosymbiosis or a transient association between a ciliate and an alga is controversial⁷. We conducted 'feeding' experiments to determine how exposure to a cryptophyte alga affects *M. rubrum*. Here we show that although *M. rubrum* lacks a cytostome (oral cavity)⁸, it ingests cryptophytes and steals their organelles, and may not maintain a permanent endosymbiont.**