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Simplified food webs lead to energetic bottlenecks in polluted lakes

Graham D. Sherwood, Jennifer Kovecses, Alice Hontela, and Joseph B. Rasmussen

Abstract: Very little is known about the consequence of human activities on the flow of energy through natural ecosystems. Here, we present a trophic-based approach to describing energy relationships in pollutant-disturbed lakes, emphasizing the importance of prey diversity in maintaining energy transfer to growing fish. Both diet and community analysis indicated that the food web leading to yellow perch (*Perca flavescens*) in metal-polluted lakes was extremely simplified compared with reference lakes. Through the application of an in situ marker for fish activity costs (muscle lactate dehydrogenase activity) and through bioenergetic modelling, we show how this has severe consequences on the efficiency of energy transfer to perch from their prey; premature energetic bottlenecks (zero conversion efficiency) occur when successively larger prey types are not available to growing perch. These observations provide a much needed ecological and physiological framework for assessing how energy transfer can be affected in polluted systems. Our approach need not be limited therein but should be applicable to any aquatic system where food web structure is variable and (or) disrupted.

Résumé : Très peu est connu au sujet de la conséquence des activités humaines sur le transfert énergétique dans les écosystèmes. Cette étude présente une nouvelle approche pour décrire des relations énergétiques dans les lacs pollués, soulignant l'importance d'une diversité de proies pour la croissance des poissons. Les analyses de contenus stomacaux et de la communauté ont indiqué que la base de nourriture disponible aux perchaudes (*Perca flavescens*) provenant de lacs pollués par les métaux lourds a été extrêmement simplifiée comparativement aux lacs références. Par l'application d'un indicateur enzymatique pour les coûts d'activité (natation) chez les poissons, et par l'application d'un modèle bioénergétique, cette étude démontre que ceci a des conséquences graves sur l'efficacité du transfert énergétique de proies aux perchaudes; des entonnoirs énergétiques (aucune croissance) se produisent quand des proies de différentes tailles ne sont pas disponibles successivement aux perchaudes en croissance. Ces observations fournissent un cadre écologique et physiologique important pour évaluer comment le transfert énergétique peut être affecté dans les systèmes pollués. Cette approche n'a pas besoin d'être limitée spécifiquement aux systèmes pollués, mais pourrait être aussi appliquée à tous systèmes aquatiques où la chaîne alimentaire est variable et (ou) dérangée.

Worldwide, species are being lost at an alarming rate. In North America alone, 123 freshwater animal species have been recorded as extinct in the past 100 years and many more are presently in peril (Ricciardi and Rasmussen 1999). Aside from the species-conservation issues that this raises, there are some concerns regarding the inevitable restructuring of natural food webs and the effects that this may have on ecosystem function and stability (McCann 2000). Indeed, a growing body of evidence suggests that food-web structure is vulnerable to a wide array of human activities, including species introductions or invasions (Vander Zanden et al. 1999), habitat alteration (Wootton et al. 1996), and environmental warming (Petchey et al. 1999). Our ability to predict how ecosystem function will respond to these pressures will require a solid understanding of how energy flows within natural food webs. To this end, and in terms of tracing pathways of energy flow, stable isotopes have proven invaluable (e.g., Vander Zanden et al. 1999). However, a more com-

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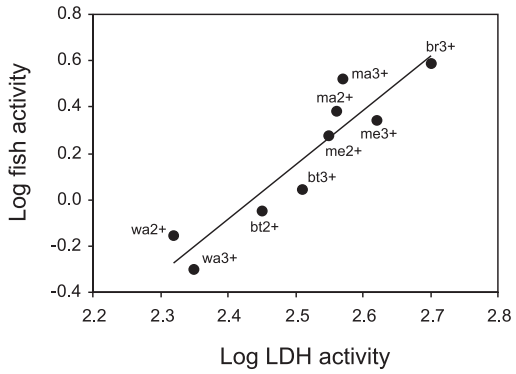
plete understanding will clearly necessitate a capacity to evaluate the efficiency of energy transfer among trophic compartments and how this may vary within and among natural and disturbed food webs.

Here, to illustrate this point, we show how heavy-metal loading into lakes (from copper-smelting operations) alters food-web structure and eventually leads to a blockage of energy transfer to growing fish. Our approach combines physiological measurements, diet and community analysis, and bioenergetic modelling. The first step of our investigation involved measuring the activity of the glycolytic enzyme, lactate dehydrogenase (LDH), in the muscle of yellow perch (*Perca flavescens*) from five non-metal-impacted populations with varying levels of predetermined fish activity (*A*, swimming) costs (Boisclair and Leggett 1989). We observed a strong, positive relationship between muscle LDH activity and fish activity (Fig. 1; $r^2 = 0.86$, $p < 0.001$, $n = 9$; $\log A = 2.35 \log \mathrm{LDH} - 5.70$). This result was not surprising as LDH is involved in supplying quick, burst energy to spontaneous movements (Somero and Childress 1980), such as would be necessary for attacking prey when feeding, and its activity in the muscle has been suggested to be related to patterns of locomotory activity in at least one other study (Somero and Childress 1980; see also Sherwood et al. 2002). Measurements of LDH activity may provide a snapshot of recent (days–weeks) energetic requirements; its activity in fish has been shown to respond to altered energetic demands within at least, but not earlier than, 7 days (Schulte et al. 2000).

We then applied this enzymatic marker to inferring patterns in fish activity in perch from four lakes along a gradient of metal contamination. LDH activity scaled positively to body size in all cases (Fig. 2*a*), as has previously been noted (Somero and Childress 1980). In the case of the two reference lakes, the mass-dependent rise in LDH activity appeared to break suddenly between 15 and 20 g and again at about 200 g, creating a distinct "saw-toothed" pattern. The timing of the first break corresponded to a well-defined shift in feeding habit (zooplanktivory to benthivory, Figs. 2*b*, 2*c*) and would have involved a drop in fish activity of about twofold, an enormous savings in terms of a fish's energy budget. The second break occurred around the point where perch became almost entirely piscivorous (Fig. 2*b*) and would have meant an even larger savings. We interpret these sudden drops in LDH activity as a function of foraging costs; attack rate, in terms of number of feeding bursts, will be high for fish foraging on disproportionately small prey, and it would appear that perch shift to progressively larger prey when the cost of fish activity gets prohibitively high. Alternatively, shifts to larger prey could result from overcoming gape limitation. No distinct diet shifts or associated saw-toothed patterns in LDH activity were evident for either of the two polluted populations; although both stable isotope ($\delta^{13}C$) and stomach content analyses suggested an important contribution of chironomids to the diet of lake Osisko perch (Figs. 2*b*, 2*c*), it was unclear where exactly this shift from zooplanktivory occurred (chironomids are a prey type that perch normally avoid in favour of attacking larger, more accessible taxa such as ephemeropterans; G. Sherwood, unpublished data). Finally, LDH activity diverged quite early so that it was always higher in the two polluted populations than in the two reference populations (Fig. 2*a*). It is possible

Fig. 1. Log muscle LDH activity (absorbance units per minute per milligram white muscle protein) versus log fish activity (unitless) for yellow perch from five non-metal-impacted Eastern Townships populations; br, Lake brome; bt, Lake Bromont; ma, Lake Magog; me, Lake Memphremagog; wa, Lake Waterloo; suffix "2+" or "3+" refers to age-class. Fish activity values are from Boisclair and Leggett (1989).



that this was related to a higher dependence on smaller, faster-moving zooplankton (i.e., copepods) in metal-polluted lakes; the numerical ratio of copepods to larger-bodied cladocerans in the diet of zooplanktivorous perch was 2:1 in metal-polluted lakes compared with 1:3 in reference lakes. This may reflect higher predatory pressures on "easy-to-catch" zooplankton (cladocerans) in polluted lakes where diet options following zooplanktivory are limited.

The final step of our analysis involved fitting lines to the observed LDH activities versus body size and inserting the appropriate slopes, intercepts, and breaks for related fish activity into a bioenergetic model (Kitchell et al. 1977), which can then be used to estimate conversion efficiency based on body size (Fig. 3). It should be noted that fitting lines in a manner consistent with observed diet shifts, as opposed to simple linear regression techniques, led to a considerable improvement in tightness of fit between LDH activity and body size (Fig. 2*a*). The overall result of our modelling exercise was to show that perch conversion efficiency was always lower in polluted lakes than in reference lakes. Perch from reference lakes continued to grow more efficiently because they had the option of resetting fish activity by periodically switching to larger prey types. Perch from the two metal-polluted lakes could not do this. They had either no prey choice (Lake Dufault) or very limited choice (Lake Osisko) following zooplanktivory (Figs. 2*b*, 2*c*, 3*b*(inset)); a more in-depth analysis (J. Kovecses, unpublished data) suggests that this was a result of metal toxicity whereby most benthic invertebrates with close association to contaminated sediments were removed from the community. As a result, both Lake Dufault and Lake Osisko perch could not benefit from the energetic advantages of switching prey, and their growth bottlenecked at comparatively small sizes (Fig. 3). This may explain why perch from both metal-polluted lakes, unlike their reference counterparts, never reached piscivory. For Lake Dufault, this did not seem to be related to low prey-fish availability. In fact, our seining efforts have revealed considerable densities of common prey fish such as spottail shiners (*Notropis hudsonius*) in this lake. Rather, it seems that Lake Dufault perch were deprived of the benthi-

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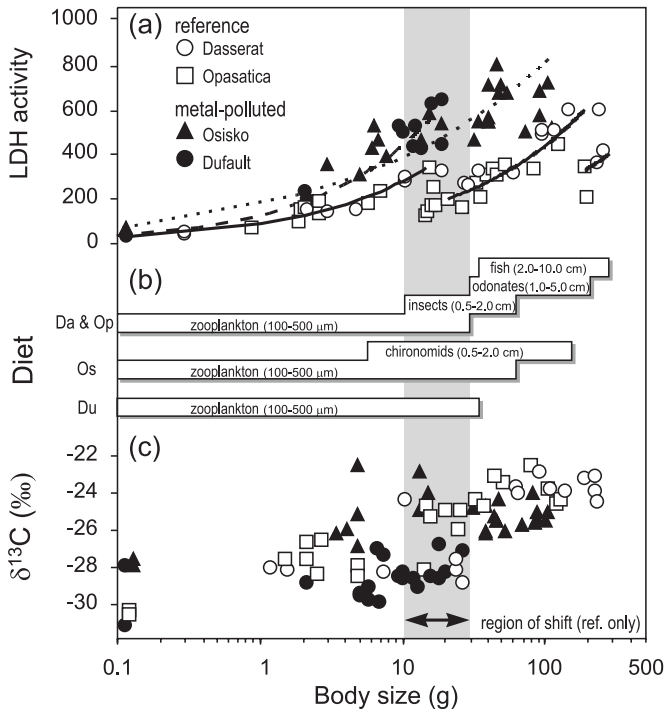
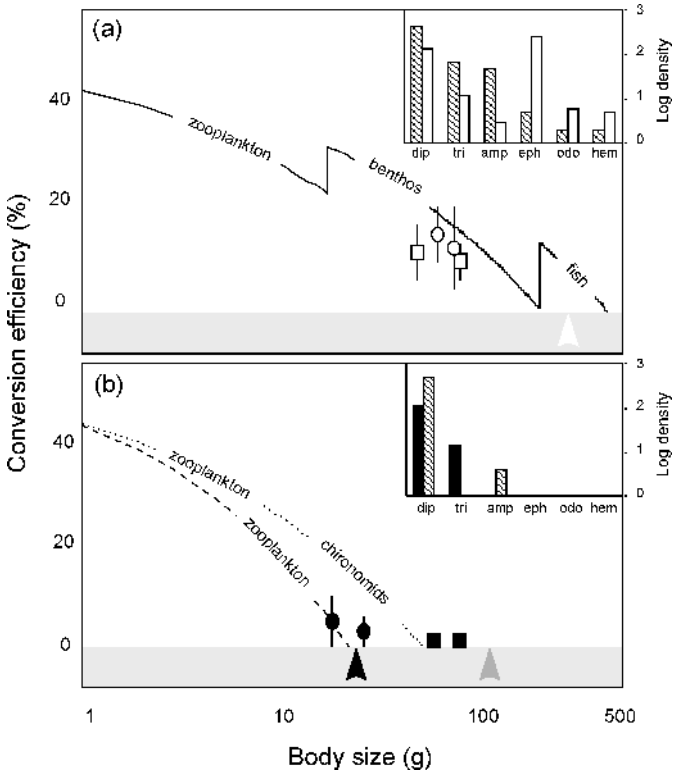
Fig. 2. (*a*) Muscle LDH activity vs. body size in Abitibi perch from four lakes along a gradient of metal contamination. For reference perch, log LDH = 0.49(0.04)·log*W* + 1.97(0.03) – 0.30(0.06) – 0.56(0.10) (r^2 = 0.89, p < 0.0001, n = 45; all model terms were significant to p < 0.0001; area of diet overlap (10–30 g, shaded area spanning all panels) was not included in regression; for model without diet shifts, r^2 = 0.75; values in parentheses are standard error). For Lake Osisko perch, log LDH = 0.30(0.02)·log*W* + 2.30(0.04) (r^2 = 0.85, p < 0.0001, n = 25; no diet shifts were considered). For Lake Dufault perch, log LDH = 0.53(0.04)·log*W* + 2.11(0.04) (r^2 = 0.96, p < 0.0001, n = 12; no diet shifts were considered). (*b*) Perch diet expressed as range of perch body size for various diet stages. Approximate prey size ranges for prey types are in parentheses: "zooplankton" includes copepods and cladocerans; "insects" includes all aquatic insects, plus amphipods, other than "odonates" (which have their own category because of their large size); and "fish" is mostly young-of-the-year yellow perch and spottail shiner (*Notropis hudsonius*). (*c*) δ^{13}C vs. body size for Abitibi perch populations. More negative values (~–30‰) are indicative of pelagic feeding (i.e., zooplankton), and more positive values (~–24‰) are indicative of littoral feeding (i.e., benthic invertebrates and littoral fish) (Vander Zanden et al. 1999).



vory stage that provides the crucial "stepping-stone" to piscivory (i.e., they bottlenecked at sizes too small to become piscivorous). Lake Osisko perch, on the other hand, may have never become piscivorous because no other fish species are present in this lake and availability of small young perch is low.

Our approach is unique in that it combines both physiological and ecological principles and is simple in that it involves only a minor, yet important, modification to existing bioenergetic models (i.e., variable fish activity costs). In fact, fish activity has already been found to be highly variable among species (Rowan and Rasmussen 1996), popula-

Fig. 3. Bioenergetic modelling results for conversion efficiency of (*a*) reference (Lake Dasserat and Lake Opasatica combined; solid line) and (*b*) metal-impacted (broken line for Lake Dufault and dotted line for Lake Osisko) perch in relation to body size. Arrowheads represent maximum observed body size for reference (open), Lake Dufault (solid), and Lake Osisko (shaded) perch. Individual data points are conversion efficiency values for age-4+ perch obtained from independent, in situ, growth and consumption estimates (Sherwood et al. 2000; open circles are for Lake Dasserat, open squares for Lake Opasatica, solid circles for Lake Dufault, and solid squares for Lake Osisko; vertical bars represent 95% confidence interval). Inset graphs are average density (individuals/m^2) of dipterans (dip), trichopterans (tri), amphipods (amp), ephemeropterans (eph), odonates (odo), and hemipterans (hem) in the littoral zone of Lake Dasserat (hatched bars, panel *a*), Lake Opasatica (open bars, panel *a*), Lake Dufault (solid bars, panel *b*), and Lake Osisko (hatched bars, panel *b*).



tions (Boisclair and Leggett 1989), and stages of maturity (Rowan and Rasmussen 1996). Field measurements of LDH activity can provide a fast and effective way of evaluating this important and often neglected energetic variable in wild-living fish.

The results of this study underscore the importance of understanding possible indirect (i.e., food web) effects of toxicity on fish bioenergetics (e.g., fish activity rates) when collecting and interpreting field data in ecotoxicological studies. In a more general sense, our findings should have application to any aquatic system where natural feeding relationships are disrupted. For example, stable isotopes showed that bass invasion into Canadian-shield lakes forced indigenous lake trout to feed much more heavily than usual on zooplankton (Vander Zanden et al. 1999). Final trout size in zooplanktivorous versus piscivorous populations from the

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same system of lakes is almost an order of magnitude smaller (Ivano Pazzia, McGill University, Montreal, Quebec, unpublished data), suggesting that invasion-impacted lake trout are caught in a bass-induced energy bottleneck. It is these types of observations, coupled with what we observe here, that provide a compelling argument for the conservation of aquatic food webs in as much their natural state as possible. The trophic linkages that have evolved over time (and continue to develop through diet ontogeny) may represent the most energetically efficient pathways of energy flow through natural food webs.

Methods

Fish capture and diet analysis

Yellow perch were sampled in June of 1998 for five Eastern Townships populations (southern Quebec, 45°N, 72°W; these populations are all nonpolluted) and in June of 1999 for four Abitibi populations (northwest Quebec, 48°N, 79°W; these populations are at the extremes of a metal-contamination gradient (Cd, Cu, and Zn), Sherwood et al. 2000). Fish were captured by seine, gill net, and hook-and-line. Fish were sacrificed within 1 h of capture, and a cross-sectional sample of white muscle tissue was dissected from the tail section and immediately stored in liquid nitrogen for enzyme analysis.

Diet and community analysis

Stomachs were removed from frozen perch carcasses and contents were identified to order. Additional data for Abitibi lakes was available from previous years. The total number of full stomachs examined for Abitibi populations was 256 and never less than 40 per lake. Independent invertebrate sampling of the littoral zone of Abitibi lakes was carried out in June of 1999 by kicknet (8 samples per lake; 0.5 m^2 per sample). Individuals from each sample were counted and identified to order. Only those taxa that formed a major part of the benthic diet of perch were considered.

Enzyme analysis

Lactate dehydrogenase assays were performed on frozen white muscle tissue according to kit specifications (LDH-Optimized, Sigma Diagnostics®, St. Louis, Mo.) following a sample preparation protocol described in Somero and Childress (1980) with some modifications. Before homogenisation (using a rough glass and Teflon apparatus) in buffer solution (50 volumes; 0.07 M pH 7.0 KPO_4 buffer + 1 mM EDTA + 0.2% v/v Triton X-100), muscle samples were crushed to a fine powder in liquid nitrogen. This allowed for a more complete homogenisation. Fifteen microlitres of sample preparation, versus the 100 μL prescribed by Sigma Diagnostics®, were sufficient to initiate and sustain the LDH reaction for a duration of 1 min for the entire range of LDH activities encountered. LDH activities, measured at 20°C and expressed as arbitrary absorbance units (at 340 nm), were normalized to protein concentration to account for variations in homogenized tissue content from one sample preparation to another as well as variations among individuals. Protein analysis was carried out on sample preparations according to BioRad® (Hercules, Calif.) specifications. The average coefficient of variation for all LDH assays run in duplicate was less than 5%.

Stable carbon isotope analysis

Carbon isotope (^{13}C) analysis was performed on dried perch muscle tissue (dorsal, directly posterior to the head) packed in 4 mm × 6 mm tin capsules. ^{13}C, which is a naturally occurring heavy isotope of carbon, is useful for inferring feeding habits of fish because prey from different habitats of lakes (e.g., benthic vs. pelagic) typically have distinct ^{13}C signatures ($^{13}C/^{12}C$) and consumers acquire ^{13}C signatures similar to that of their prey (Vander Zanden et al. 1999). Stable carbon signatures were expressed in delta (δ) notation, defined as the parts per thousand (‰) deviation from a standard material (PeeDee belemnite limestone): $\delta^{13}C = ([R_{sample}/R_{standard}] - 1) \times 1000$; $R = {}^{13}C/{}^{12}C$). The average standard error for a subset of one-tenth of the samples run in duplicate was 0.09‰.

Bioenergetic analysis

Conversion efficiency ($K1$) was given as

$$K1 = (G/C) \times 100 \quad (1)$$

where G is specific growth rate ($g \cdot g^{-1} \cdot day^{-1}$) and C is specific consumption rate ($g \cdot g^{-1} \cdot day^{-1}$). G can be estimated from the following bioenergetic model (Kitchell et al. 1977):

$$G = C - \mathrm{SMR} \times A - \mathrm{SDA} - F - U \quad (2)$$

where SMR is the standard metabolic rate, A is fish activity, SDA is specific dynamic action, F is egestion, and U is excretion. In traditional formulations of this model, both C and A are unknowns (although changes in activity are usually assumed to be negligible) and SMR, SDA, F, and U are generic parameters derived from laboratory experiments; SMR is a function of body size and water temperature, and SDA, F, and U are fixed proportions of C (totalling about 40%) (Kitchell et al. 1977). For our purposes, C was assumed (4% body weight per day for all lakes and body sizes); no systematic difference exists in consumption rates of perch among lakes of varying metal contamination (Sherwood et al. 2000), and C is isometric in yellow perch (Sherwood et al. 2002). Fish activity was included as a function of LDH activity (Fig. 1), which scales positively to body size and is dependent on diet on a lake-specific basis (Fig. 2).

Statistics

For perch from the Eastern Townships, published fish activity values (Boisclair and Leggett 1989) were regressed against measured LDH activity values (log transformed). Fish activity values were for age-2+ and age-3+ perch from five different lakes (Brome, Bromont, Magog, Memphremagog, and Waterloo; total $n = 9$; data for Brome age-2+ perch was lacking). LDH values were from regression of LDH versus body size in these five lakes. For Abitibi perch, size- and diet-related changes in LDH activity were described by the following regression model:

$$\log \mathrm{LDH} = a \cdot \log W + b - c - d \quad (3)$$

where W is body size, a is slope, b is intercept, and c and d are saw-toothed breaks that apply when fish shift diets, sequentially, to benthivory and piscivory (see Sherwood et al.

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(2002) for a comparison of diet-shift model with more traditional line-fitting techniques). The inclusion and positioning of saw-toothed breaks (i.e., parameters *c* and *d*) was aided from both diet and $\delta^{13}C$ observations. Saw-toothed breaks were not included when diet shifts were not well defined as in the case of the two metal-polluted lakes where simple linear regression was employed. Area of diet overlap (region of shift, Fig. 2) was not included in regression analysis (eq. 3) for reference perch.

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