CSIRO PUBLISHING

Marine and Freshwater Research, 2016, **67**, 1–6
http://dx.doi.org/10.1071/MF16269

Food chain length in a large floodplain river: planktonic or benthic reliance as a limiting factor

M. Saigo[A,C], *L. Ruffener*[A], *P Scarabotti*[A,B] *and M. Marchese*[A,B]

[A]Instituto Nacional de Limnología (INALI), Consejo Nacional de Investigaciones Científicas y Técnicas–Universidad Nacional del Litoral (CONICET-UNL), Ciudad Universitaria, Santa Fe, 3000, Argentina.
[B]Facultad de Humanidades y Ciencias–Universidad Nacional del Litoral (FHUC-UNL), Ciudad Universitaria, Santa Fe, 3000, Argentina.
[C]Corresponding author. Email: miguelsaigo@gmail.com

Abstract. Food chain length (FCL) is a key integrative variable describing ecosystem functioning. The aim of the present study was to test the hypothesis that the relative importance of planktonic and benthic energy pathways is a major factor affecting FCL in the Middle Paraná River. Samples were obtained from in eight waterbodies, measuring chlorophyll-*a* concentrations and the abundance of benthic invertebrates and the trophic position of top predators by stable isotope analysis. There was no evidence that resource availability, disturbances or ecosystem size limited FCL. Similarly, the body size and trophic position of predators were not correlated. However, the relative abundance of planktonic and benthic resources was correlated with FCL. In addition, stable isotopes analysis showed that the benthic reliance of top predators is correlated with their trophic position. The results of the present study indicate that because the major benthic primary consumer is a large fish (*Prochilodus lineatus*), the size structure of individual food chains is an important factor determining FCL. Whereas in floodplain rivers large detritivorous fishes are targets of commercial fishing, overfishing in the Middle Paraná River could be expected to increase FCL, the opposite effect to that seen in marine environments.

Additional keywords: benthos, fish, food webs, plankton, stable isotopes.

Received 1 June 2016, accepted 1 September 2016, published online xx xxxxx xxxx

Introduction

Food chain length (FCL) is a fundamental property of ecosystems (Fretwell 1987). Briefly, FCL can be defined as the number of trophic levels in a food web. Moreover, there are at least three ways to operatively define FCL. The connectance based concept defines FCL as the average number of links between basal resources and a top predator; functional FCL is defined in terms of the number of links between a basal resource and a functionally significant top consumer; and flow-based FCL, or realised FCL, is the average number of energy transferences between basal resources and top predators (Sabo *et al.* 2009). This last definition is the most widely used nowadays, in part because the use of stable isotopes has simplified its measurement. For example, although assessment of connectance-based FCL requires a fully specified food web model, realised FCL can be measured by determining the δ^{15}N of top predators and a baseline. Stable isotope techniques have prompted many FCL studies worldwide, thus renewing classic ecological question: which environmental factors limit FCL in natural ecosystems?

An early theoretical approach suggested that although trophic transferences are somewhat inefficient, FCL would be limited by primary productivity (Pimm 1982). Another feasible limiting factor is disturbance. This hypothesis is based on the assumption that long food chains are fragile in environments that are subjected to frequent perturbations (Pimm and Lawton 1978; McHugh *et al.* 2010; Sabo *et al.* 2010). More recently, Schoener (1989) proposed the productive-space hypothesis, which predicts that FCL should increase as the product of ecosystem size and some measure of per-unit size resource availability. This hypothesis has been supported by some surveys (Schoener 1989; Vander Zanden and Rasmussen 1996; Thompson and Townsend 2005; Doi *et al.* 2009) and rejected by others (Spencer and Warren 1996; Post *et al.* 2000; Post and Takimoto 2007). In addition, ecosystem size alone has been proposed as a determinant of FCL (Cohen and Newman 1991; Post *et al.* 2000).

The variable results of field studies indicate that each of these environmental factors may drive FCL in some environments but not in others. Moreover, these hypotheses may (or may not) interactively explain FCL variation. In this context, it seem that our knowledge about the underlying mechanisms by which environmental factors limit FCL is still insufficient (Young *et al.* 2013). For example, in a recent meta-analysis, Takimoto and Post (2013) concluded that the interaction between ecosystem size and FCL may be so complex that even a negative relationship could be expected. In addition, some traits of individual predators may determine their trophic position, implying additional complexity. Body size is an important trait related to the trophic level concept because predators are generally bigger than their prey.

Nowadays it seems that understanding the variation in FCL in natural ecosystems is more complex that originally presumed. This could be due, in part, to the complexity of food webs that, far from being simple chains, are often (virtually always) a set of chains of different length. Therefore, the relative importance of each chain within a food web may be an important driver of FCL.

The Middle Paraná River has a complex food web containing different chains, the lengths of which range between two and five trophic levels. A particularity of South American floodplain rivers is the importance of the bottom feeding fish *Prochilodus lineatus*, which connects top predators and benthic resources in a two-trophic level food chain. A longer food chain connects seston and top predators in at least three trophic links, which generally involves filter feeding mussels (Saigo *et al.* 2015).

The present study tested the hypothesis that, in the Middle Paraná River, the importance of the benthic pathway mediated by *P. lineatus* (hereafter referred as benthic reliance) has an important effect on the realised FCL.

Materials and methods

Sampling

AQ1 Seven waterbodies, including rivers and lakes, in the Middle Paraná River system during the low water season (November 2013; Fig. 1) and one floodplain lake were selected for sampling during December 2010. Piscivorous fish (*Salminus brasiliensis* and *Hoplias malabaricus*) were sampled using nets. Each individual was weighed and measured in the field. In addition, bottom feeding fish (*P. lineatus*) were sampled as the benthic baseline. Mussels of the species *Limnoperna fortunei* were collected from macrophyte roots and stems and considered as the planktonic baseline. Where, in some sites, *L. fortunei* was not present, seston was sampled by filtering approximately 20 L water through a 20-μm mesh. Trophic level correction of seston was made using a trophic enrichment factor of 2.55‰ for δ^{15}N and 1‰ for δ^{13}C (Post 2002; Vanderklift and Ponsard 2003). Because the isotopic signatures of seston and mussels did not differ significantly ($P > 0.05$), we considered seston and mussels as planktonic baselines.

The dorsal muscle of fish was extracted, dried at 45 ± 1°C until constant weight and then ground into a fine powder using a mortar and pestle; an approximate 1-mg sample was then placed in a tin capsule.

Mussels were kept in water for 12 h to ensure that the gut was empty and were then frozen at −18°C for ~24 h, and then unfrozen. The valves were extracted and the soft tissues were dried at 45°C until constant weight and were then ground. Samples from five to 10 individuals were pooled and placed in tin capsules.

Seston samples were filtered onto precombusted (450°C) glass fibre filters (Whatman GF/F). The filters were then dried at 45°C and frozen until isotope analysis.

Isotopic determinations were performed in a mass spectrometer (IRMS Finnigan MAT Delta S) coupled to an elemental analyser in the Instituto de Geocronología y Geología Isotópica–UBA–Consejo Nacional de Investigaciones Científicas y Técnicas (INGEIS-UBA-CONICET, Buenos Aires). Because AQ2 high lipid levels (indicated by a high C : N ratio) may drive δ^{13}C

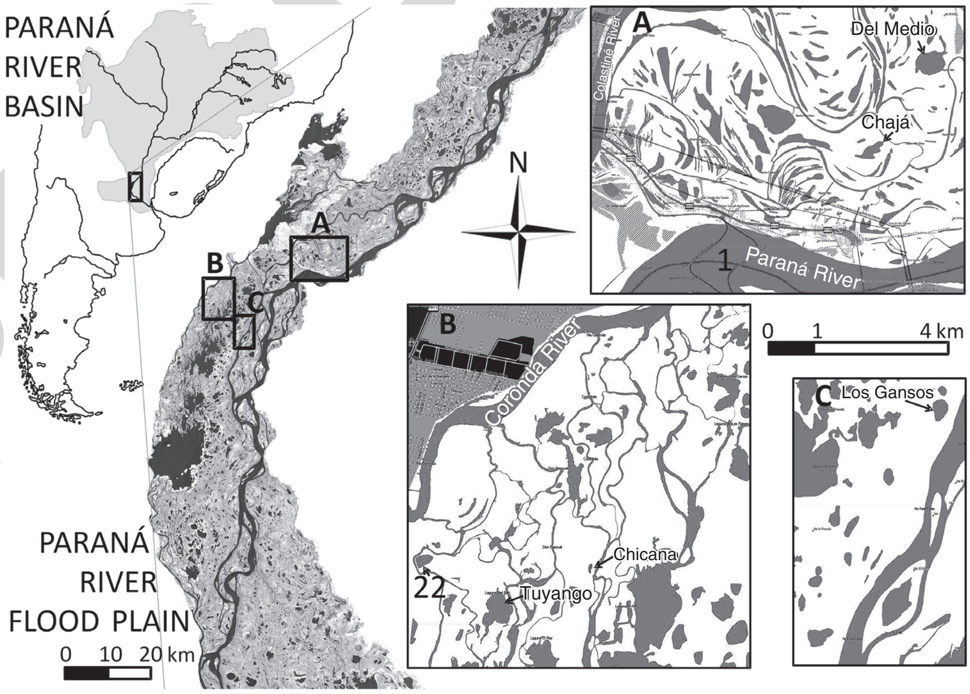

Fig. 1. Location of the sites studied.

values in a negative direction (McConnaughey and McRoy 1979; Matthews and Mazumder 2005), the δ^{13}C values of fish and mussels were normalised in the present study using the following equation when C : N ratios were higher than 3.5 according to Post *et al.* (2007):

$$\delta^{13}\text{C} = -3.32 + 0.99\,(\text{C : N})$$

Measuring environmental variables

Chlorophyll-*a* (Chl-*a*) was measured at each site as a surrogate marker of planktonic resource availability.

A variable volume of water (1000–1500 mL) was filtered through Whatman GF/F glass fibre filters. Chl-*a* was extracted from the filters with acetone (90%). The extracts were stored at 4°C for 24 h in the dark, filtered and Chl-*a* concentrations were measured using a spectrophotometer at wavelengths of 750 and 664 nm, as well as at 665 and 750 nm after acidification of samples with 0.1 M HCl (Lorenzen 1967).

In order to measure resource availability in benthos, we collected samples of benthic invertebrates in triplicate in each environment using Ekman (225 cm^2) or Tamura (325 cm^2) grabs depending on the granulometry. The number of individuals per metre squared (hereafter benthic density) was used as an estimate of benthic resource availability.

Measuring FCL

FCL was calculated using the two end member equation proposed by Post (2002) applying the δ^{15}N trophic enrichment factor proposed by Vanderklift and Ponsard (2003):

$$\text{FCL} = \lambda + \left\{\delta^{15}\text{N}_{\text{tp}} - \left[\delta^{15}\text{N}_{\text{base1}} \times \alpha + \delta^{15}\text{N}_{\text{base2}} \times (1-\alpha)\right]\right\} \div 2.55$$

where subscripts are tp, top predator; 'base 1', *P. lineatus*; 'base 2', mussels or (corrected) seston. λ is the trophic level of the organisms used to estimate $\delta^{15}\text{N}_{\text{base1}}$ and $\delta^{15}\text{N}_{\text{base2}}$ and α is defined as follows:

$$\alpha = \left(\delta^{13}\text{C}_{\text{tp}} - \delta^{13}\text{C}_{\text{base1}}\right) \div \left(\delta^{13}\text{C}_{\text{base1}} - \delta^{13}\text{C}_{\text{base2}}\right)$$

In cases where α was less than 0 or more than 1, it was treated as sampling error and set to 0 or 1 respectively (Williams and Trexler 2006).

Statistical analysis

Multiple linear regressions were performed to assess the potential controllers of FCL. First, the FCL of each site was used with total resources availability (TRA) and relative resources availability (RRA). TRA was calculated as the product of Chl-*a* and benthic density, whereas RRA was calculated as the ratio of Chl-*a* to benthic density. Then, the trophic position of individual predators was used with length, weight and planktonic or benthic reliance (α in the FCL equation above) as independent variables.

Testing the ecosystem size hypothesis requires a reliable estimate of the volume of waterbodies. When considering isolated lakes or ponds, this is quite straightforward; however, measuring (in a comparable scale) the size of rivers and connected lakes is troublesome. Moreover, the latter could even be considered as a whole large ecosystem. Besides, in floodplain rivers, ecosystem size and disturbance effects are difficult to distinguish because both depend upon hydrological connectivity. Disconnected floodplain lakes present a smaller effective size. In addition, they are subject to higher hydrological disturbances because, during low waters, these ecosystems experience greater physicochemical changes (water temperature, dissolved oxygen, turbidity, depth, vegetation coverage etc.) and can even dry out. Rivers and permanently connected floodplain lakes can be considered as larger ecosystems than isolated lakes (fish are not confined to any one area). In addition, rivers and permanently connected floodplain lakes show comparatively slighter environmental changes between the low and high water season. Therefore, we compared the trophic position of top predators of isolated lakes with those of connected lakes and rivers using a Kruskall–Wallis test. If ecosystem size or disturbance effectively limited FCL in the Middle Paraná River, then trophic positions of top predators in connected lakes and rivers (larger and more stable ecosystems) would be higher than in isolated lakes (smaller and less stable).

Results

Realised FCL varied between 1.53 and 2.81 trophic levels among sites, Chl-*a* ranged between 3 and 17 $\mu g\ L^{-1}$ and benthic density varied between 925 and 9477 individuals m^{-2} (Table 1).

The multiple linear regression model performed for sites was significant ($P = 0.044$). This model showed that RRA had a significant effect on FCL ($P = 0.017$) but not TRA ($P = 0.219$; Fig. 2). Similarly, the multiple regression model performed for individual predators was significant ($P = 0.0008$). In this model planktonic or benthic reliance had a significant effect on trophic position ($P < 0.0001$), but not length ($P = 0.597$) or weight ($P = 0.237$; Fig. 3). There were no significant differences in the trophic position of predators between connected and isolated sites (Fig. 4).

Discussion

FCL variation in the studied site could not be explained by traditional hypotheses. There was no evidence that FCL was controlled by ecosystem size or disturbance (considered here as connected *v.* isolated environments) or resource availability. However, a broader study may have been detect the effects of these variables, which, in the present study, were unperceivable. In a broad survey in Australian rivers (Warfe *et al.* 2013), traditional hypotheses could not explain FCL variation. In that study, the authors suggested that because the study was performed in seasonally connected rivers, fish could move across environments and create a regional food web overriding local constraints for FCL. Fish in the Middle Paraná River are known to perform longitudinal and lateral migrations connecting main channels and floodplain lakes (Rossi *et al.* 2007). However, even though the sampling in the present study was performed after 2 months of low waters and the isotopic turnover rate in muscle is ~2–8 weeks (Boecklen *et al.* 2011), we consider that the data reflects local conditions at least in isolated lakes.

Similarly, we did not find a relationship between body size and the trophic position of predators. This result contradicts that

Table 1. Type of system, chlorophyll-*a* and average benthic density of sites studied
Sample sizes are given in parentheses. Chl-*a*, chlorophyll-*a*

	Coronda	Colastiné	Del medio	El Tuyango	El chajá	Los gansos	La chicana
Type of system	River	River	Connected lake	Connected lake	Isolated lake	Isolated lake	Isolated lake
Chl-*a* (μg L^{-1})	4.80	3.00	4.90	9.90	8.70	5.10	17.30
Benthic density (individuals m^{-2})	925.00	2933.33	1192.00	6508.80	9477.78	8047.80	3200.00
Benthic reliance (α)	0.16	0.71	0.07	0.75	0.26	1.00	0.18
FCL	2.54	2.06	2.34	1.96	2.39	1.53	2.81
$\delta^{15}N$ of top predators (‰)	12.30 (2)	11.90 (2)	12.20 (3)	13.30 (2)	11.30 (2)	8.90 (2)	13.60 (3)
$\delta^{13}C$ of top predators (‰)	−24.77 (2)	28.12 (2)	−25.85 (3)	−22.82 (2)	−27.50 (2)	−27.00 (2)	−23.88 (3)
$\delta^{15}N$ of benthic source (‰)	12.20 (1)	9.74 (2)	9.41 (2)	11.50 (2)	9.70 (1)	7.50 (1)	9.90 (3)
$\delta^{13}C$ of benthic source (‰)	−20.42 (1)	−29.40 (2)	−28.57 (2)	−21.92 (2)	−29.27 (1)	−24.45 (1)	−26.43 (3)
$\delta^{15}N$ of planktonic source (‰)	7.60 (4)	7.80 (2)	8.70 (2)	8.80 (2)	7.10 (4)	7.50 (2)	8.80 (2)
$\delta^{13}C$ of planktonic source (‰)	−25.60 (4)	−25.06 (2)	−25.68 (2)	−26.32 (2)	−26.88 (4)	−21.80 (2)	−23.30 (2)

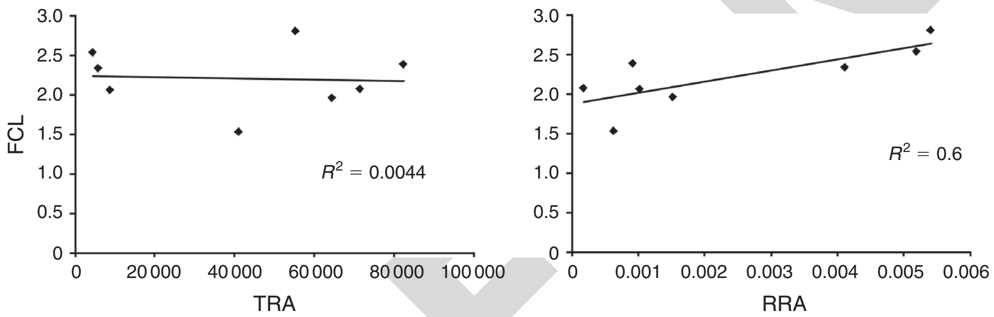

Fig. 2. Correlations between food chain length (FCL) and total resources availability (TRA) and relative resources availability (RRA).

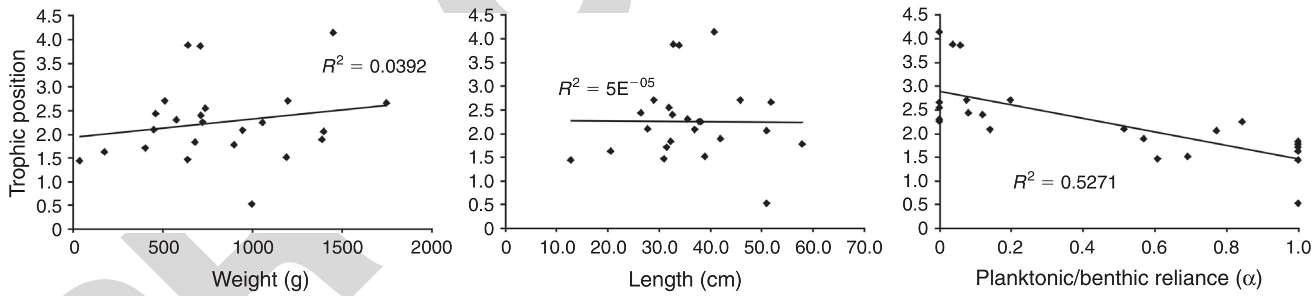

Fig. 3. Correlation between the trophic position of predators and their weight, length, and planktonic or benthic reliance.

reported by Romanuk *et al.* (2011), who used a global dataset and concluded that the body size of fish is positively correlated with their trophic position. However, Layman *et al.* (2005) proposed that, in complex food webs that are characterised by a broad range of primary consumer body size, there is no correlation between body size and trophic position. In the Middle Paraná River, primary consumers show a wide range of body size. For example, the body mass of *P. lineatus* can easily reach 2 kg, whereas filter feeding consumers such as *L. fortunei* rarely exceed 1 g. The fact that primary consumers of benthic and planktonic resources have such different body sizes explains the different lengths of these pathways. The significant relationship found between planktonic or benthic reliance and the trophic position of top predators supports this idea. This result is in contrast with findings in The Everglades, where the relative contribution of sources did not affect FCL (Williams and Trexler 2006). In that study, scuds and snails were used as baselines, whereas in the present study *P. lineatus* and *L. fortunei* we used as baselines. In the Middle Paraná River, the benthic food chain is short because its most important primary consumer is a large detritivorous fish that is consumed directly by top predators. If, in The Everglades, the main detritivores are invertebrates (scuds), then the detrital food chain should not be expected to be especially short.

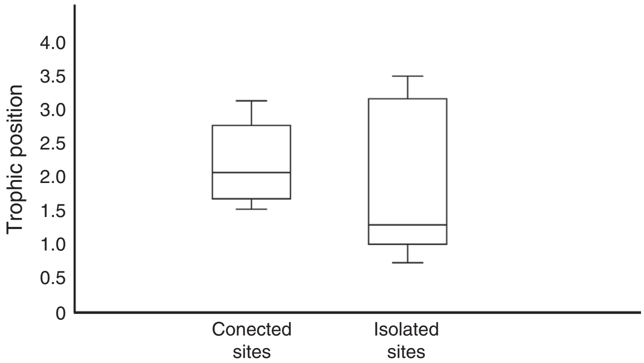

Fig. 4. Box plot comparing the food chain length (FCL) of isolated lakes with that of rivers and connected lakes. The boxes show the interquartile range, with the median value indicated by the horizontal line; whiskers show the range.

Moreover, the results of the present study also indicate that RRA is an important factor determining FCL in the Middle Paraná River. Similar results were reported by Hoeinghaus *et al.* (2008), who concluded that in the Upper Paraná River food chains were longer in reservoirs than in high-gradient rivers. The authors attributed this result to the presumed relatively higher importance of planktonic source in reservoirs and the body size of detritivorous fish. In the Middle Paraná River, there is no impoundment and all lotic systems are low-gradient rivers. Thus, the spatial patterns proposed by Hoeinghaus *et al.* (2008) for reservoirs *v.* high-gradient rivers could not be applied to this reach of the river. However, the significant correlation between FCL and RRA suggests that in non-regulated rivers, the natural variation in the availability of benthic or planktonic resources is an important driver of FCL.

We consider that the planktonic or benthic reliance hypothesis is a useful frame for the assessment of FCL variation in complex ecosystems such as large rivers. Moreover, the findings of the present study provide important insights for fisheries management. In marine systems, fisheries decrease FCL because large fish (those targeted by fisheries) are generally top-level predators (Rice and Gislason 1996; Pauly *et al.* 1998). Conversely, in large floodplain rivers, detritivorous fish are targets of commercial fisheries, in part because of their relatively large size. As in other food webs in which prochilodontids determine short chains to top predators (Layman *et al.* 2005), the overfishing of this species is expected to produce an increase in the FCL in the Middle Paraná River.

Acknowledgements

The authors are grateful to CONICET and the Universidad Nacional del Litoral (UNL) for financial support. The authors thank Dr M. Devercelli and Dr G. Mayora for assistance in the field and laboratory.

References

Boecklen, W. J., Yarnes, C. T., Cook, B. A., and James, A. C. (2011). On the use of stable isotopes in trophic ecology. *Annual Review of Ecology Evolution and Systematics* **42**, 411–440. doi:10.1146/ANNUREV-ECOLSYS-102209-144726

Cohen, J., and Newman, C. (1991). Community area and food-chain length: theoretical predictions. *American Naturalist* **138**, 1542–1554. doi:10.1086/285299

Doi, H., Chang, K. H., Ando, T., Ninomiya, I., Imai, H., and Nakano, S. I. (2009). Resource availability and ecosystem size predict food chain length in pond ecosystems. *Oikos* **118**, 138–144. doi:10.1111/J.1600-0706.2008.17171.X

Fretwell, S. D. (1987). Food chain dynamics: the central theory of ecology? *Oikos* **50**, 291–301. doi:10.2307/3565489

Hoeinghaus, D. J., Winemiller, K. O., and Agostinho, A. A. (2008). Hydrogeomorphology and river impoundment affect food-chain length of diverse Neotropical food webs. *Oikos* **117**, 984–995. doi:10.1111/J.0030-1299.2008.16459.X

Layman, C. A., Winemiller, K. O., Arrington, D. A., and Jepsen, D. B. (2005). Body size and trophic position in a diverse tropical food web. *Ecology* **86**, 2530–2535. doi:10.1890/04-1098

Lorenzen, C. (1967). Vertical distribution of chlorophyll and phaeopigments: Baja California. *Deep-Sea Research* **14**, 735–745.

Matthews, B., and Mazumder, A. (2005). Temporal variation in body composition (C : N) helps explain seasonal patterns of zooplankton δ^{13}C. *Freshwater Biology* **50**, 502–515. doi:10.1111/J.1365-2427.2005.01336.X

McConnaughey, T., and McRoy, C. P. (1979). Food-web structure and the fractionation of carbon isotopes in the Bering Sea. *Marine Biology* **53**, 257–262. doi:10.1007/BF00952434

McHugh, P. A., McIntosh, A. R., and Jellyman, P. G. (2010). Dual influences of ecosystem size and disturbance on food chain length in streams. *Ecology Letters* **13**, 881–890. doi:10.1111/J.1461-0248.2010.01484.X

Pauly, D., Christensen, V., Dalsgaard, J., Froese, R., and Torres, F. (1998). Fishing down marine food webs. *Science* **279**, 860–863. doi:10.1126/SCIENCE.279.5352.860

Pimm, S. L. (1982). 'Food Webs.' (Chapman & Hall: London, UK.)

Pimm, S. L., and Lawton, J. (1978). The number of trophic levels in ecological communities. *Nature* **275**, 542–544. doi:10.1038/275542A0

Post, D. M. (2002). Using stable isotopes to estimate trophic position: models, methods, and assumptions. *Ecology* **83**, 703–718. doi:10.1890/0012-9658(2002)083[0703:USITET]2.0.CO;2

Post, D. M., and Takimoto, G. (2007). Proximate structural mechanisms for variation in food-chain length. *Oikos* **116**, 775–782. doi:10.1111/J.0030-1299.2007.15552.X

Post, D. M., Pace, M. L., and Hairston, N. G. (2000). Ecosystem size determines food-chain length in lakes. *Nature* **405**, 1047–1049. doi:10.1038/35016565

Post, D. M., Layman, C. A., Arrington, D. A., Takimoto, G., Quattrochi, J., and Montaña, C. (2007). Getting to the fat of the matter: models, methods and assumptions for dealing with lipids in stable isotope analyses. *Oecologia* **152**, 179–189. doi:10.1007/S00442-006-0630-X

Rice, J., and Gislason, H. (1996). Patterns of change in the size spectra of numbers and diversity of the North Sea fish assemblage, as reflected in surveys and models. *ICES Journal of Marine Science* **53**, 1214–1225. doi:10.1006/JMSC.1996.0146

Romanuk, T. N., Hayward, A., and Hutchings, J. A. (2011). Trophic level scales positively with body size in fishes. *Global Ecology and Biogeography* **20**, 231–240. doi:10.1111/J.1466-8238.2010.00579.X

Rossi, L., Cordiviola, E., and Parma, M. J. (2007). Fishes. In 'The Middle Paraná River: Limnology of a Subtropical Wetland'. (Eds M. H. Iriondo, J. C. Paggi and M. J. Parma.) pp. 303–326. (Springer: Berlin.)

Sabo, J. L., Finlay, J. C., and Post, D. M. (2009). Food chains in freshwaters. *Annals of the New York Academy of Sciences* **1162**, 187–220. doi:10.1111/J.1749-6632.2009.04445.X

Sabo, J. L., Finlay, J. C., Kennedy, T., and Post, D. M. (2010). The role of discharge variation in scaling of drainage area and food chain length in rivers. *Science* **330**, 965–967. doi:10.1126/SCIENCE.1196005

Saigo, M., Zilli, F. L., Marchese, M. R., and Demonte, D. (2015). Trophic level, food chain length and omnivory in the Paraná River: a food web model approach in a floodplain river system. *Ecological Research* **30**, 843–852. doi:10.1007/S11284-015-1283-1

Schoener, T. W. (1989). Food webs from the small to the large. *Ecology* **70**, 1559–1589. doi:10.2307/1938088

Spencer, M., and Warren, P. H. (1996). The effects of habitat size and productivity on food web structure in small aquatic microcosms. *Oikos* **75**, 419–430. doi:10.2307/3545882

Takimoto, G., and Post, D. M. (2013). Environmental determinants of food-chain length: a meta-analysis. *Ecological Research* **28**, 675–681. doi:10.1007/S11284-012-0943-7

Thompson, R. M., and Townsend, C. R. (2005). Energy availability, spatial heterogeneity and ecosystem size predict food-web structure in streams. *Oikos* **108**, 137–148. doi:10.1111/J.0030-1299.2005.11600.X

Vander Zanden, M. J., and Rasmussen, J. B. (1996). A trophic position model of pelagic food webs: impact on contaminant bioaccumulation in lake trout. *Ecological Monographs* **66**, 451–477. doi:10.2307/2963490

Vanderklift, M. A., and Ponsard, S. (2003). Sources of variation in consumer-diet $\delta^{15}N$ enrichment: a meta-analysis. *Oecologia* **136**, 169–182. doi:10.1007/S00442-003-1270-Z

Warfe, D. M., Jardine, T. D., Pettit, N. E., Hamilton, S. K., Pusey, B. J., Bunn, S. E., Davies, P. M., and Douglas, M. M. (2013). Productivity, disturbance and ecosystem size have no influence on food chain length in seasonally connected rivers. *PLoS One* **8**(6), e66240. doi:10.1371/JOURNAL.PONE.0066240

Williams, A. J., and Trexler, J. C. (2006). A preliminary analysis of the correlation of food-web characteristics with hydrology and nutrient gradients in the southern Everglades. *Hydrobiologia* **569**, 493–504. doi:10.1007/S10750-006-0151-Y

Young, H. S., Mccauley, D. J., Dunbar, R. B., Hutson, M. S., Ter-Kuile, A. M., and Dirzo, R. (2013). The roles of productivity and ecosystem size in determining food chain length in tropical terrestrial ecosystems. *Ecology* **94**, 692–701. doi:10.1890/12-0729.1

AUTHOR QUERIES

AQ1: Is the text ‘Seven’ OK? In the summary and in the replies to the copyeditor you had said this should be eight in ‘Stable isotope analysis was used in eight waterbodies of the Middle Paraná River.’

AQ2: Please define UBA.

AQ3: Relative to what? ‘Relatively’ needs a corresponding statement to which it can be compared for it to add meaning to a sentence. Should this be ‘fairly’ or ‘reasonably’, or should ‘relatively’ be deleted?

AQ4: Again large relative to what?