Estimating diet in individual pumpkinseed sunfish *Lepomis gibbosus* using stomach contents, stable isotopes and parasites

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The diets of 99 pumpkinseed sunfish *Lepomis gibbosus* from a pair of small, adjacent lakes in Ontario, Canada, were estimated from their stomach contents, trophically transmitted parasites and stable isotopes of carbon and nitrogen in fish tissue. The three methods provided virtually unrelated information. There was no significant correlation in the importance of any prey item across all three methods. Fish with similar diets according to one method of estimating diet showed no tendency to be similar according to other methods. Although there was limited variation in fish size and the spatial scale of the study was small, both fish size and spatial origin showed comparatively strong associations with diet data obtained with all three methods. These results suggest that a multidisciplinary approach that accounts for fish size and spatial origins is necessary to accurately characterize diets of individual fish.

INTRODUCTION

Fish diet is important to numerous aspects of aquatic ecosystems, ranging from the behavioural ecology of individual fishes to the structure of food webs. Three methods commonly used to characterize the diet of individual fishes are the examination of stomach contents (Hyslop, 1980), the analysis of chemical isotopes in fish tissue (Michener & Kaufman, 2007) and the survey of trophically transmitted parasites (Knudsen *et al*., 1996). Each technique has distinct advantages and limitations. Stomach contents often can be identified with relatively high taxonomic resolution, but provide only a snapshot that may differ from long-term diet. In addition, slowly digested prey may be overrepresented in stomach contents, and empty stomachs provide no information. In contrast, the ratios of stable isotopes of carbon (δ^{13}C) and nitrogen (δ^{15}N) in fish tissues reflect diet on a longer time scale. The identity

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and relative importance of prey can be inferred by comparing isotopic ratios in tissues of a fish to those of samples from its potential prey. These inferences are not straightforward, however, because different combinations of prey, or prey species other than those sampled, can produce similar isotopic composition in a predator, and also because isotopic fractionation and turnover rates vary with the growth rate, size and age of fishes (Harvey *et al.*, 2002; Herzka, 2005; Caut *et al.*, 2008). Trophically transmitted parasites also present longer term but indirect information on prey species in fish diets. Uninfected hosts, however, provide no information, and prey that transmit no parasites go undetected. In addition, parasites can be difficult to identify to species, and may have life cycles that are poorly known or that include alternate transmission routes, all of which can obscure the identity of prey implied by their presence.

Fish diet has been studied by a number of authors using two of these methods (Knudsen *et al.*, 1996; Beaudoin *et al.*, 1999; Bertrand *et al.*, 2008, 2011; Valtonen *et al.*, 2010) or in rare cases, all three (Johnson *et al.*, 2004; Dick *et al.*, 2009). The degree of association among the three methods is, however, not well known. Stomach contents and parasites may provide estimates of diet that are strongly consistent (Walkey, 1967; Knudsen *et al.*, 1996) or only partially related (Valtonen *et al.*, 2010). Parasites may be more strongly correlated with isotopes of nitrogen, which track trophic level, than with carbon, which reflects the habitat of food sources (Johnson *et al.*, 2004; Dick *et al.*, 2009; Bertrand *et al.*, 2011), yet parasites can also reflect foraging habitat (Wilson *et al.*, 1996; McCairns & Fox, 2004; Bertrand *et al.*, 2008). The stomach contents and isotopes of fishes can be somewhat or strongly consistent (Vander Zanden *et al.*, 1997; Beaudoin *et al.*, 1999; Uchii *et al.*, 2007), but may also provide substantively different information (Harvey *et al.*, 2002). As isotopes and parasites both reflect longer time scales, Johnson *et al.* (2004) suggested that they should be more strongly associated than either is to the relatively ephemeral information provided by stomach contents, but did not observe this using carbon isotopes of yellow perch *Perca flavescens* (Mitchill 1814). It could also be hypothesized that individual diets inferred from isotopes might be more strongly correlated with those known from stomach contents, because the species of prey sampled for isotope mixing models are often identified from preliminary examination of stomach contents.

All three methods of estimating diet are affected by local habitat conditions, hence the spatial origins of fishes. Indeed, both parasites and isotopes (particularly $\delta^{13}C$) are used to discriminate the spatial origins of fishes (Williams *et al.*, 1992; Bertrand *et al.*, 2008, 2011). Another factor potentially influencing dietary data is the size of fishes. Within species, fish of different sizes vary in stomach contents and in rates of gastric evacuation (Keast, 1978*a*; Hyslop, 1980; Godinho *et al.*, 1997), in isotopic turnover (Herzka, 2005) and in their parasite communities (Poulin, 2000; Poulin & Valtonen, 2001). Fish size may also influence relationships among data obtained with these methods. For example, Beaudoin *et al.* (1999) recorded significantly higher $\delta^{15}N$ in nine pike *Esox lucius* L. 1758 with only fish remains in their stomachs than in 22 *E. lucius* that recently had fed only on invertebrates. Paradis *et al.* (2008), however, found that $\delta^{15}N$ indicated no difference in trophic levels among 66 *E. lucius* classified as either piscivores or invertivores according to their stomach contents, after accounting for fish length. This suggests that the relationship between stomach contents and isotopes observed by Beaudoin *et al.* (1999) may be attributable to the moderating effect of size.

Overall, it is difficult to form even a general, much less quantitative picture of the relationships among the data that emerge from existing studies using at least two of these three methods of estimating diet. This probably reflects both real variability in different systems and the wide range of goals and methods in previous studies. Most previous studies have used one or more of these tools to address specific questions about fish diet. The aim of this study, in contrast, is to determine if the different data provide similar information in individual adult pumpkinseed sunfish *Lepomis gibbosus* (L. 1758). Adult *L. gibbosus* commonly feed on benthos in the littoral zone, but in the absence of sympatric bluegill *Lepomis macrochirus* Rafinesque 1819, some *L. gibbosus* feed in open water, and both strategies are strongly evident from stomach contents and parasites (Robinson *et al*., 2000; McCairns & Fox, 2004). Here, the importance of different food items and the similarity of diets are compared using each method at the scale of the individual fish. Although sampling focused on fish with limited variation in size collected on a small spatial scale, the effects of spatial autocorrelation and of fish size are also taken into account.

MATERIALS AND METHODS

COLLECTION OF *L. GIBBOSUS* AND ITS PREY

Between 28 July and 9 August 2010, 99 *L. gibbosus* were collected from seven localities in Lake Opinicon ($n = 64$) and four localities in Rock Lake ($n = 35$), Ontario, Canada, by hook and line (Fig. 1). Fish were killed upon capture by cerebral percussion using a small mallet, weighed and measured (total length, L_T, ± 1 mm and mass, M_T, ± 1 g), kept on ice and dissected within 36 h. The gastrointestinal tract and viscera of freshly killed fish were examined under a stereomicroscope, and stomach contents and parasites were preserved in ethanol. Only parasites that are trophically transmitted, *i.e.* gastrointestinal helminths, and certain helminths from the viscera, were retained. A sample of lateral muscle tissue was frozen.

Samples of prey items were collected using dip nets or an Ekman dredge. In Lake Opinicon, these were zebra mussels, gastropods (Viviparidae, Physidae and Planorbidae), amphipods and isopods, which were the four most frequently observed items in the stomachs of fish from this lake, and Odonata, which were not observed in many fish. In Rock Lake, only gastropods and Trichoptera were collected.

ESTIMATION OF *L. GIBBOSUS* DIET USING STOMACH CONTENTS

Items in stomachs that clearly corresponded to an individual prey (*e.g.* whole prey, heads of insects, septa of bivalves and columellae of gastropods) were identified to the species, family or order level. The proportion of each prey item in the diet of each fish was estimated by dividing the number of individual prey in a given taxon by total prey abundance.

ESTIMATION OF DIET USING TROPHICALLY TRANSMITTED PARASITES

All trophically transmitted parasites were either cleared in glycerol and examined in wet mounts (nematodes) or stained in acetocarmine, cleared in clove oil and mounted on slides for identification using the keys of Arai (1989), Gibson (1996) and Hoffman (1999). Adult *Leptorhynchoides thecatus* in the gastrointestinal tract and larvae (cystacanths) in the body cavity were considered separate species in similarity-based analyses described below (Steinauer *et al*., 2007). In cases where parasite life cycles included alternate transmission pathways and implicated different possible prey, the distribution of the parasite and potential
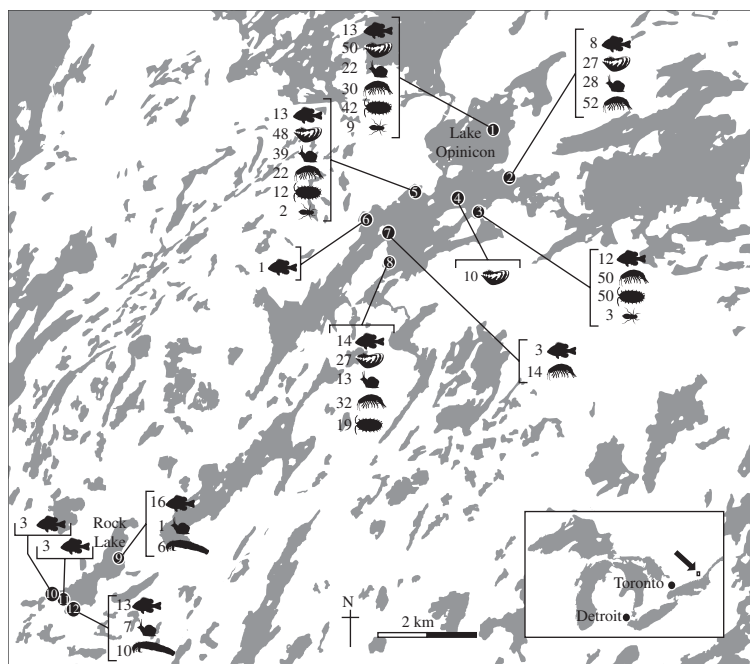
FIG. 1. Localities where *Lepomis gibbosus* and potential prey were collected from Lake Opinicon and Rock Lake, Ontario, in July and August 2010. The number of fish (🐟) and prey (🦪, *Dreissena polymorpha*; 🐚, Gastropoda; 🦐, Amphipoda; 🪲, Isopoda; ✳, Odonata; 🪱, Trichoptera) collected at each locality (●) are indicated. Study area is indicated by ⟶ in inset. Locality coordinates are 1 = 44·576° N, 76·309° W; 2 = 44·568° N, 76·304° W; 3 = 44·561° N, 76·312° W; 4 = 44·563° N, 76·318° W; 5 = 44·566° N, 76·327° W; 6 = 44·561° N, 76·342° W; 7 = 44·558° N, 76·336° W; 8 = 44·552° N, 76·337° W; 9 = 44·500° N, 76·405° W; 10 = 44·493° N, 76·422° W; 11 = 44·492° N, 76·4210° W; 12 = 44·490° N, 76·420° W.

transmitting prey species were examined in more detail. For example, *L. gibbosus* may become infected with enteric *L. thecatus* after consuming infected fish (paratenic host) or amphipods. Because fishes were never observed in stomachs of adult *L. gibbosus* in Lake Opinicon (Keast, 1978*a*) enteric *L. thecatus* were assumed to have been acquired from amphipods. For similar reasons, other acanthocephalans and *Camallanus oxycephalus* were assumed to have been acquired from invertebrates rather than paratenic fish hosts, and *Phyllodistomum* cf. *superbum* was assumed to have been acquired from insects rather than other potential hosts.

Thus, all prey implicated by parasites were invertebrates, in which infection intensities are generally low (Ward, 1940*a*; Marcogliese, 1995; Scholz, 1999). It was therefore assumed that each parasite corresponded to the consumption of a single prey item. The proportion of each prey taxon in the individual's diet was estimated by dividing its abundance, as implied by parasites, by the total abundance of all implied prey.

ESTIMATION OF DIET USING ISOTOPES OF CARBON AND NITROGEN

Freeze-dried samples of fish muscle and prey were analysed with a mass spectrophotometer at the G. G. Hatch Stable Isotope Laboratory at the University of Ottawa. Except for molluscs, in which only the foot muscle was analysed, prey were analysed whole in composite samples of two to 50 individuals from the same locality. The ratios (R) of carbon ($R = {}^{13}C \, {}^{12}C^{-1}$) and nitrogen ($R = {}^{15}N \, {}^{14}N^{-1}$) are reported with reference to a standard, *i.e.* as $\delta^{13}C$ or $\delta^{15}N$
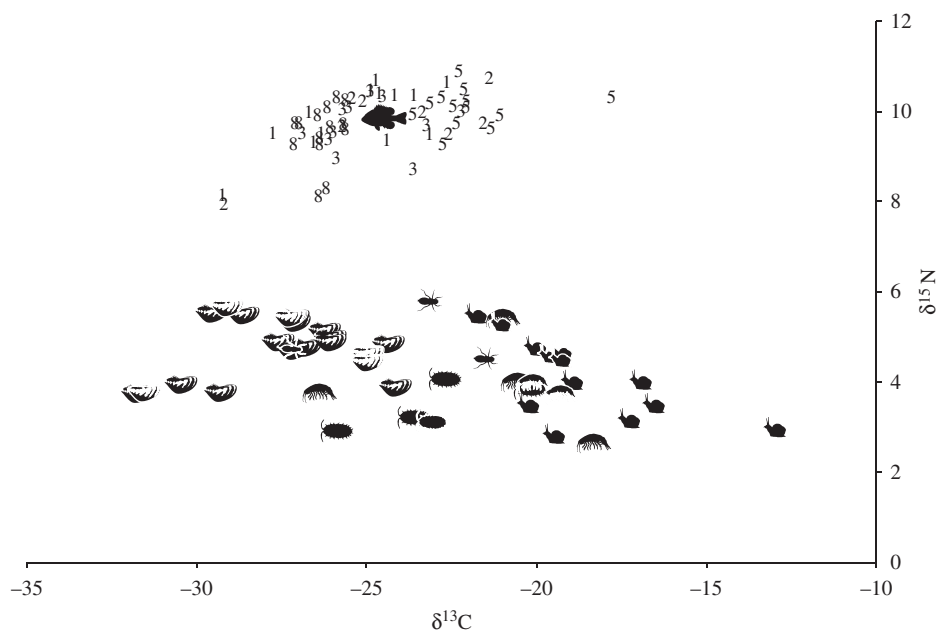
FIG. 2. Biplot of stable isotopes of carbon (δ^{13}C) and nitrogen (δ^{15}N) from the muscle of 64 *Lepomis gibbosus*, and from potential prey (zebra mussel *Dreissena polymorpha*, Odonata, Amphipoda and Isopoda; see Fig. 1), collected in July and August 2010 in Lake Opinicon, Ontario. Individual fish are indicated by numbers corresponding to collection locality in Fig. 1.

$= 1000 \ [(R_{sample} \ R_{standard}^{-1}) - 1]$. The mean absolute deviation between replicate measurements ($n = 32$ repeated measurements) was 0·23‰ for δ^{13}C and 0·16‰ for δ^{15}N.

   The composition of diet in individual *L. gibbosus* was estimated from fish and prey isotope ratios using a Bayesian mixing model implemented by the SIARSOLO command in the SIAR package in R (R Core Development Team; www.r-project.org; Parnell *et al*., 2010). Reconstruction of individual diets with isotopes was only possible in fish from Lake Opinicon because prey samples necessary for SIAR were not obtained from Rock Lake. The diet-dependent method proposed by Caut *et al*. (2009) was used to estimate the trophic enrichment factor (F_{TE}) for carbon (2·6‰). The nitrogen F_{TE} thus obtained (3·13‰), however, led to implausibly high trophic levels for *L. gibbosus* (Keast, 1978*a*; Mercer *et al*., 1999; Andraso, 2005). A nitrogen F_{TE} of 5‰ was therefore used. This value is within the range reported for fish muscle (Caut *et al*., 2009) and yielded plausible trophic levels for *L. gibbosus* from both lakes. To reflect the uncertainty of the F_{TE}, a large F_{TE} s.d. (2‰) was used in the mixing model. Initial modelling indicated that the diets of most fish were composed of ≥94% odonatans. This is highly implausible in light of what was observed in stomachs in the present and previous studies, including prior work in the same system (Keast, 1978*a*), and considering inferences from parasites. These unrealistic estimates may have arisen because the number of prey ($n = 5$) was large compared to the number of isotopes ($n = 2$), a situation which can reduce the predictive power of SIAR (Parnell *et al*., 2010), and which may have been exacerbated by the overlap in the isotopic signatures of odonatans, isopods and amphipods (Fig. 2). Because odonatans were the least common of these prey in stomach contents, they were excluded from the mixing model, which was restricted to estimation of the proportion of zebra mussels, gastropods, amphipods and isopods in the diet of individual fish.

   Mean δ^{13}C differed markedly in viviparid gastropods from Rock Lake (−25·3‰) and Lake Opinicon (−18·85‰), indicating that baseline values also differ in the lakes (Post, 2002). To accommodate this, the differences in the mean isotope ratios (δ^{13}C and δ^{15}N) between

gastropods (Viviparidae) from Rock Lake and Lake Opinicon were added to the respective values of fish from Rock Lake. The trophic position of each fish was calculated using this baseline-corrected $\delta^{15}N$ and the formula of Vander Zanden & Rasmussen (2001).

STATISTICAL ANALYSIS

Two approaches were used to compare the three different methods of estimating diet. Correlations were used to assess whether the proportion of a given prey item in the diet of an individual fish, as estimated by one method, was related to its proportion as estimated by each of the other two methods. Correlation tests of prey proportions were limited to fish from Lake Opinicon, because the SIAR mixing model could only be constructed in fish from this lake. Fish without parasites or stomach contents were not included in these analyses.

To analyse fish from both lakes, raw data were used instead of inferred prey proportions. Pair-wise resemblances between individual fish based on each type of data (isotopes, parasites and stomach contents) were computed in PRIMER 6 (PRIMER-E Ltd; www.primer-e.com) and exported into and further analysed with zt (Bonnet & Van de Peer, 2002). The latter software performs partial Mantel tests (using 10 000 permutations) of associations between two types of resemblance while controlling for a third. As described below, zt was used to test whether individual fish with similar isotopic signatures have similar parasites or stomach contents, and whether those with similar stomach contents have similar parasites, while controlling for similarity in fish size or their spatial proximity.

The similarity of stomach contents and parasite communities in individual fish among and within both lakes was calculated using the Bray–Curtis index. Reported results are based on square root-transformed abundance of stomach contents and parasites, which was carried out to reduce the influence of numerically dominant prey and parasites, while still preserving the quantitative signal in Bray–Curtis. Other data treatments (*e.g.* no or $\log_{10}$-transformed abundance) produced similar results. This includes the addition of a dummy food item and parasite to all fish, which allows inclusion of fish without parasites or stomach contents (Clarke *et al.*, 2006).

The isotopic similarity of fish was based on a single Euclidean distance derived from $\delta^{13}C$ and $\delta^{15}N$. Distances were calculated from the baseline-corrected $\delta^{13}C$ and the trophic position of the fish, which was based on the baseline-corrected $\delta^{15}N$ value. Analyses run with each isotope considered separately produced results similar to those reported below.

The effect of similarity in size and spatial proximity on the dietary resemblance of fish was assessed with Euclidean distances in L_T and M_T, and latitude and longitude, respectively, both within lakes and among all sites in both lakes. Euclidean distances in which paired data were in different units (*i.e.* in L_T and M_T, and baseline-corrected $\delta^{13}C$ and $\delta^{15}N$ trophic level values) were first normalized (*i.e.* transformed to range from −1 to 1) so that they would be on the same scale. Analysis of similarities (ANOSIM, with 9999 permutations) was used to test for sex-related differences in isotopes, parasites and stomach contents.

RESULTS

The mean ± s.d. in L_T of fish collected in Rock Lake was 153 ± 20 mm, while in those taken from Lake Opinicon it was 174 ± 24 mm. Eleven categories of prey items were found in stomachs (Table I). Thirteen species of trophically transmitted parasites were observed, implicating up to 11 categories of prey (Table II). The overall mean $\delta^{13}C$ in fish from both lakes was −24·9 (range −29·3 to −17·7) and mean $\delta^{15}N$ was 9·3 (7·4 to 10·9). In Lake Opinicon, isotopes indicated that most fish fed mainly on zebra mussels (Fig. 2), which also emerged from the mixing model analysis (Table III).

TABLE I. Mean abundance (*n* observed) of stomach contents in 99 *Lepomis gibbosus* collected in July and August 2010

	Lake Opinicon (*n* = 64)	Rock Lake (*n* = 35)
Arthropoda		
Crustacea		
Decapoda	0·17	0·09
Isopoda	0·65	1·15
Amphipoda	1·43	1·85
Insecta		
Ephemeroptera	0·30	0·62
Odonata	0·45	1·88
Trichoptera	0·36	1·74
Other insects	1·48	4·71
Mollusca		
Dreissena polymorpha	20·85	0
Other bivalves	0·28	2·65
Gastropoda	7·32	14·82
Other	0·23	0·06

n, Number of fish examined.

COMPARISON OF DIET COMPONENTS (LAKE OPINICON)

In the 64 fish from Lake Opinicon in which explicit estimates of prey proportions were available for all three methods, there were large discrepancies in prey proportions among the different methods (Fig. 3). The isotope mixing model and stomach contents indicated that zebra mussels were the most important food source, but parasites did not indicate any consumption of zebra mussels. Gastropods made up the second largest proportion of stomach contents but ranked last in the isotope mixing model, and parasites provided no evidence of gastropod consumption. The food item indicated by parasites to be of greatest importance, copepods, was never observed in stomachs. Similarly, the consumption of ostracods and oligochaetes was indicated by parasites, but these were not observed in stomachs, and important food items according to stomach contents (zebra mussels and decapods) were not implicated by parasites. The proportion of isopods in individual stomach contents was weakly correlated with the proportion in the isotope mixing model ($r = 0.369$, $P < 0.05$). Among the remaining 12 prey items in Fig. 3, however, stomach contents, parasites and the isotope mixing model yielded proportions of prey items that were unrelated to each other in individual fish ($P > 0.05$). For example, the lack of correspondence among estimates of the proportion of amphipods in individual diets is shown in Fig. 4. In fish in which parasites indicated that the diet consisted mostly or even exclusively of amphipods, stomach contents and the isotope mixing model indicated little or no amphipod consumption. The isotope mixing model indicated uniformly low levels of amphipod consumption, even in fish in which amphipods were numerous in stomachs. The lack of correspondence in the proportion of amphipods in individual diets is particularly interesting because, at the scale of the population, all three methods indicated that amphipods were of intermediate importance (Fig. 2).

TABLE II. Trophically transmitted parasites observed in 99 *Lepomis gibbosus* collected in July and August 2010 and prey items implicated

| | Mean abundance | | Transmission | |
	Lake Opinicon ($n = 64$)	Rock Lake ($n = 35$)	Implicated prey	Reference
Acanthocephala				
Leptorhynchoides thecatus (adult)	0·047	2·229	Amphipoda/fishes	1
L. thecatus cystacanth†	0	1·343	Amphipoda	1
Neoechinorhynchus cylindratus	0·016	0	Ostracoda/fishes	2
Pomphorhynchus bulbocolli	0·172	0·743	Amphipoda/fishes	2
Pomphorhynchus rocci	0·003	0·086	Amphipoda/fishes	2
Nematoda				
Camallanus oxycephalus	1·094	0·200	Copepoda/fishes	3
Eustrongylides sp.	0·078	0·086	Oligochaeta	3
Philometra sp.†	0	0·029	Copepoda	3
Spinitectus gracilis	0	0·029	Ephemeroptera, Odonata, Plecoptera	3
Platyhelminthes				
Cestoidea				
Bothriocephalus cuspidatus	0	0·029	Copepoda	4
Proteocephalus sp.†	0·250	0·486	Copepoda	5
Digenea				
Azygia angusticauda	0·234	0	None (free-living stage consumed directly)	4
Phyllodistomum cf. *superbum*	1·437‡	0·143	Sphaeriidae or Trichoptera	4, 6, 7

Sources: 1, Steinauer *et al.* (2007); 2, Ward (1940*a*, *b*); 3, Anderson (2000); 4, Hoffman (1999); 5, Scholz (1999); 6, Bellfuss (1954); 7, Schell (1985).

n, Number of fish examined.

†Extra-enteric parasites.

‡The mean abundance of *P*. cf. *superbum* in Lake Opinicon strongly reflects one fish infected with 60 of the 92 parasites observed in this lake. The mean abundance of this parasite in 63 other fish in this lake was 0·510.

TABLE III. Mean proportion (range) (%) of four prey items in the diets of 64 *Lepomis gibbosus* collected from Lake Opinicon in July and August, 2010, according to stable isotope mixing model analysis

Arthropoda	
Crustacea	
Isopoda	15 (6−24)
Amphipoda	14 (5−26)
Mollusca	
Dreissena polymorpha	61 (22−84)
Gastropoda	10 (4−28)

In both lakes, a combination of intestinal and extra-enteric parasites indicated that 57 fish had consumed copepods (Table II). Extra-enteric parasites are often longer lived than intestinal parasites and therefore may reflect diet on a longer temporal scale, *i.e.* copepod consumption in the more distant past. The intestinal nematode *C. oxycephalus* was the only copepod-transmitted parasite in 34 fish, larval
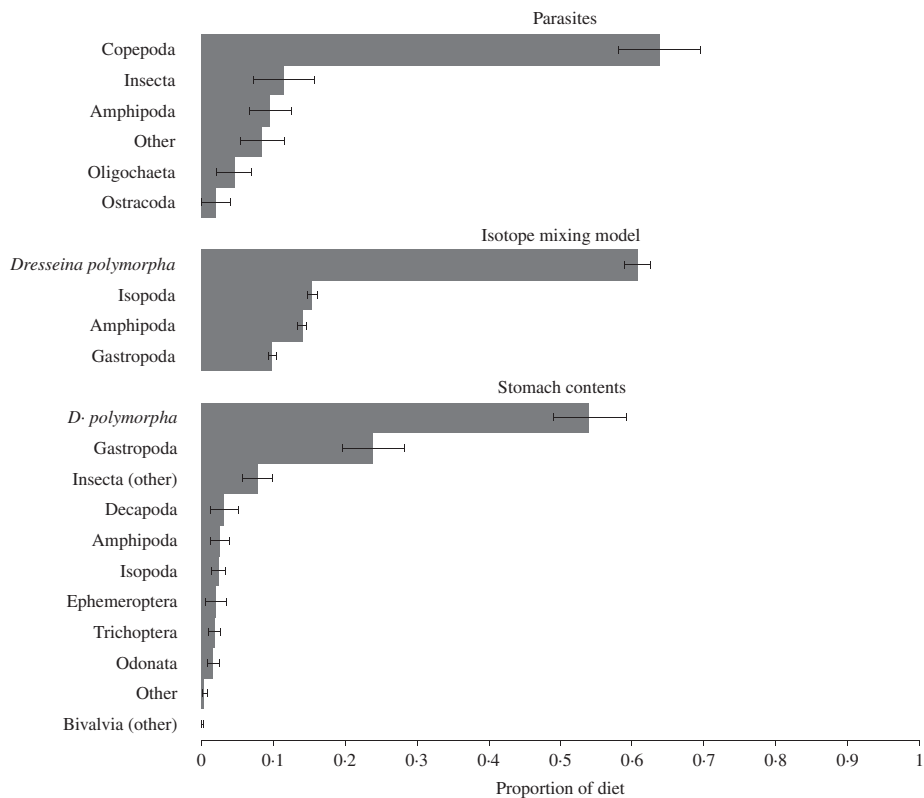

FIG. 3. Proportion of diet (mean ± S.D.) of 13 prey in 64 *Lepomis gibbosus* from Lake Opinicon, Ontario, estimated from trophically transmitted parasites, a mixing model based on stable isotopes of carbon and nitrogen, and stomach contents.
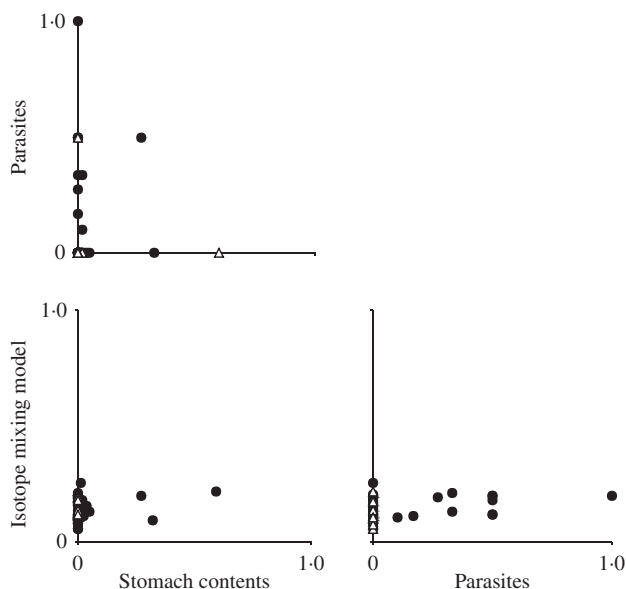
FIG. 4. Comparison of proportion of amphipods in the diet of 64 *Lepomis gibbosus* from Lake Opinicon, Ontario, as estimated from trophically transmitted parasites, a mixing model based on stable isotopes of carbon and nitrogen, and stomach contents. There is no association among any of the variables ($P > 0.05$). Fish with no parasites ($n = 8$) or empty stomachs ($n = 7$) are indicated ($\triangle$).

Proteocephalus sp. in the liver was the only such parasite in 13 fish, and both *C. oxycephalus* and *Proteocephalus* occurred in 10 fish, while other copepod-transmitted parasite species were each found only in a few fish. In other words, most copepod consumption was implied by a relatively short-lived intestinal parasite, while some was also independently indicated by a longer-lived parasite in the liver.

COMPARISON OF DIETARY RESEMBLANCE (LAKE OPINICON AND ROCK LAKE)

In resemblance-based analyses of fish from both lakes, simple Mantel tests revealed weak associations among raw data obtained with the three different methods of estimating diet. Fish with similar parasite communities tended to have similar stomach contents ($r = 0.11$, $P < 0.05$) and stable isotopes ($r = -0.22$, $P < 0.05$), and those with similar stomach contents had similar stable isotopes ($r = -0.15$, $P < 0.05$).

Data obtained with all three methods were, however, more strongly associated with the distance between collection sites (stomach content $r = -0.29$, parasite $r = -0.32$, isotope $r = 0.66$, all $P < 0.05$) than with each other. In addition, fish that were similar in size tended to have similar stomach contents ($r = -0.18$, $P < 0.05$), parasites ($r = -0.06$, $P = 0.05$) and isotopes ($r = 0.11$, $P < 0.05$). Analysis of similarities showed no sex-related differences in stomach contents (ANOSIM $R = 0.11$, $P > 0.05$), isotopes ($R = 0.04$, $P > 0.05$) or parasites ($R = 0.03$, $P > 0.05$).

The weak associations among data obtained with the three different methods became weaker still, or disappeared, when partial Mantel tests were used to control for spatial autocorrelation and fish size. Controlling for spatial distance, fish

with similar isotopes had an extremely weak tendency to have similar parasites ($r = -0.05$, $P < 0.05$) and fish with similar stomach contents had no tendency to have similar parasites or isotopes ($P > 0.05$).

It was suspected that spatial effects might be driven by inter-lake differences. To control for this aspect of spatial autocorrelation, and further investigate the role of fish size, partial Mantel tests were conducted separately on fish within each lake. There was no relationship among predictors of diet within both lakes, in both simple Mantel tests and partial tests controlling for either size or intra-lake distances ($P > 0.05$). Within both lakes, fish that were similar in size had a weak tendency to have similar stomach contents (Opinicon $r = 0.26$, Rock $r = 0.26$, both $P < 0.05$, controlling for intra-lake spatial separation), and other correlations were extremely weak or non-significant. Fish in Lake Opinicon with similar isotopes tended to be close together ($r = 0.09$, $P < 0.05$, controlling for size; see also Fig. 2) and similar in size ($r = 0.08$, $P < 0.05$, controlling for spatial separation). In Rock Lake, fish of similar size had similar parasites ($r = 0.09$, $P < 0.05$, controlling for spatial separation). Fish of similar size had a weak tendency to be close together in Rock Lake ($r = 0.07$, $P < 0.05$) but not in Lake Opinicon ($P > 0.05$).

DISCUSSION

The stable isotopes, parasites and stomach contents provided nearly unrelated information on the diets of 99 *L. gibbosus* in two lakes with different food webs. There were few points of agreement and only weak correlations between the three different methods of characterizing diet in individual fish, *i.e.* little correspondence in the importance of nearly all prey items. Parasites and isotopes were more strongly associated with each other than with stomach contents, but all associations were extremely weak. When controlling for spatial separation and fish size, fish with similar diets according to one technique showed no tendency to be similar according to others.

The lack of agreement among these methods, all commonly used to characterize fish diet, probably reflects both methodological and biological factors, as well as scale. Reconstructions of diets of individual fish are particularly likely to be influenced by the underlying assumptions of the decisions made. For example, the F_{TE} used here may not have been optimal for comparison among methods. It is also possible that isotope data from additional potential prey items, such as those implicated by parasites, would have led to greater agreement between diet reconstructions (although increasing the number of prey relative to isotopes reduces the performance of mixing models; Parnell *et al.*, 2010). Similarly, other choices in the analysis of stomach contents (*e.g.* volume *v.* abundance of prey, wet *v.* dry mass and stomach fullness) and of parasites (ranks *v.* counts and different assumptions about infections levels in prey) would have led to different reconstructions of individual diets, and possibly more agreement among the three methods. The aim of this study was, however, not to exhaustively characterize the consequences of these and other alternatives. Instead, each method was used to reconstruct individual diets based on a single set of decisions, each based on biologically plausible outcomes, such as would be implemented in a typical investigation.

In this context, it is useful to consider the classification of fish in functional groups, a comparatively simple way of reconstructing diet that relies on minimal assumptions. Both isotopes and stomach contents can be used to assign individual fish to trophic guilds (Paradis *et al*., 2008; Jensen *et al*., 2012) and trophically transmitted parasites can be combined into those implicating benthic *v*. planktonic prey (Johnson *et al*., 2004). Using this approach, stomach contents indicated all fish were purely benthivorous, but parasites indicated that many fish also use a more planktonic feeding strategy (*i.e. Azygia angusticauda* and copepod-transmitted parasites; Table II and Fig. 3).

It is also important to note that lack of agreement among methods emerged from the fundamentally different, resemblance-based analyses, in which no attempt was made to reconstruct diet, and involving no corresponding assumptions. Employing only the raw data from each method (or transformations thereof), these analyses showed that fish that were similar according to one technique are not similar according to either of the other two. The differences among the methods are therefore not attributable entirely to methodological issues; they also reflect biological processes. The different temporal scales of stomach contents, parasites and isotopes comprise one source of potential discrepancy. The dietary history provided by stomach contents is clearly shorter than that of both trophically transmitted parasites and isotopes, but it is difficult to compare the temporal scale of data acquired from the last two methods. Isotopes of carbon and nitrogen have been found to reflect dietary conditions dating back as long as 1·5 years, although this interval varies markedly with tissue, size and growth rate (Hesslein *et al*., 1993; Herzka, 2005). Similarly, shorter lived parasites reflect only recent prey while others, particularly extra-enteric parasites, can remain alive in hosts for years (McDaniel & Bailey, 1974). Long-lived parasites could partly explain one of the more striking inconsistent implications of the present results, namely whether or not *L. gibbosus* consumed copepods, and when. Copepods are seldom observed in the stomachs of adult *L. gibbosus*, yet parasites transmitted by copepods are common in both adult and sub-adult *L. gibbosus* (Keast, 1968, 1978*a*, *b*; Keast & Welsh, 1968; Cone & Anderson, 1977; Godinho *et al*., 1997; Hoffman, 1999; Locke *et al*., 2013). In Lake Opinicon and other systems, copepods occurred almost exclusively in stomachs of *L. gibbosus* <100 mm L_T, with small numbers occasionally observed in larger fish feeding nocturnally. This suggests that some of the parasites in older fish may reflect copepods consumed by juveniles. The L_T of the fish sampled in this study indicate that they were at least 5 years old (Scott & Crossman, 1973; Keast, 1978*a*), while those in which copepods are commonly observed are <2 years old (Cone & Anderson, 1977; Keast, 1978*a*; Godinho *et al*., 1997). Thus, some of the copepod-derived infections observed here may be ≥3 years old. This scenario is plausible for plerocercoids of *Proteocephalus* sp. in the liver, which may be long-lived, but less so for *C. oxycephalus* in the intestine, which probably live *c*. 1 year in the host (McDaniel & Bailey, 1974; Bailey, 1984; Anderson, 2000; Steinauer & Font, 2003). An interestingly similar observation was recorded by Valtonen *et al*. (2010) in the parasites and stomach contents of almost 7000 fishes belonging to 31 species in Bothnian Bay. The largest discrepancy in diets implied by trophically transmitted parasites compared to stomach contents was that parasites indicated much more copepod consumption (Valtonen *et al*., 2010).

Other possible reasons why copepods are seldom observed in the stomachs of adult *L. gibbosus* are that they may be digested more rapidly than other food items,

or harder to find or identify among the larger prey of adult fish compared with juveniles (Kitchella & Windella, 1968; Hyslop, 1980). Similarly, it was assumed that no parasites were acquired from fish prey because fishes were not observed in *L. gibbosus* stomachs. Small fishes are, however, rapidly digested (Legler *et al.*, 2010) and fish remains have occasionally been observed in the stomachs of *L. gibbosus* in other systems (Godinho *et al.*, 1997). The difficulty of distinguishing species of *Phyllodistomum* (Gibson, 1996; Rosas-Valdez *et al.*, 2011) and the multiple transmission pathways used by species in this genus also make the identity of implied prey less certain.

The foregoing considerations, arising both from methodology and biology, make conclusions about the importance of many prey in individual diets elusive even with the three different methods used here. The distinct temporal scales of these data will be differentially influenced by ontogenetic and seasonal shifts in diet and different digestion rates. It is also relevant that isotopes are based on assimilated biomass, while stomach content analysis (as performed here) and parasites reflect the abundance of prey ingested. Finally, the observed lack of correspondence in methods at the scale of individual fish probably does not apply to fish populations, which are longer lived entities occupying a greater area. Regardless of cause, the lack of association among these methods in individual fish suggests that a multidisciplinary approach to diet inference may be most productive, similar to what has been proposed for discrimination of marine fish stocks and construction of food webs (Begg & Waldman, 1999; Marcogliese, 2008; Valtonen *et al.*, 2010). These results also emphasize the importance of taking fish size and spatial origins into account when these methods are used, even at small spatial scales and in populations of similarly sized individuals. It is interesting that the use of parasites, stomach contents and isotopes as indicators of fish diet is common enough that each technique has been reviewed a number of times (Hyslop, 1980; Williams *et al.*, 1992; Michener & Kaufman, 2007), but no such synthesis considers all methods together, and few original studies employ all three techniques (Johnson *et al.*, 2004; Dick *et al.*, 2009), particularly at the scale of the individual fish.

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