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Measurement of arthropod body composition using quantitative magnetic resonance

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Abstract. Quantitative magnetic resonance (QMR) is a new technology for measuring the body composition (wet lean mass, fat mass, and total body water mass) of unrestrained and unanesthetized animals. We conducted a validation study using two species of crayfish (mass range 5.5–27 g), American lobsters (680–732 g), and Madagascar hissing cockroaches (6.5–14 g) to assess the utility of QMR for quantifying the body composition of crustaceans and other large arthropods. A comparison of crayfish, lobster, and cockroach wet lean, fat, and body water masses calculated by QMR with those obtained from the traditional chemical extraction method demonstrates that QMR is a valid technology for analysis of wet lean mass and body water. Fat mass could not be accurately predicted, although this might be improved with the use of a QMR analyzer designed specifically for animals of low fat content. QMR analysis allows rapid (<4 min) and non-destructive determination of body composition in field and lab environments, enabling researchers to conduct longitudinal studies and to increase the ethicality and practicality of studying rare or threatened species.

Additional key words: wet lean mass, water content, molt cycle, crustaceans

The body condition of animals has long been of great interest to ecologists because of the information it can provide about the fitness of individuals, populations, and by extension, the productivity of a particular environment (Speakman 2001). Consequently, many methods exist to subdivide the body composition of animals into quantifiable components that provide insight into ecology, physiology, and behavior. For example, body fat mass, wet lean mass (the muscle tissue mass equivalent of all the body parts containing water), or water content can reveal how metabolism and behavior are affected by diet and resource availability (Rikardsen et al. 2006), seasonality (Ewing et al. 1970; O'Farrell & Studier 1976; Barrento et al. 2009), hibernation (Kronfeld-Schor et al. 2000), migration patterns (Schaub et al. 2008), or desiccation (Schimpf et al. 2012).

The "gold standard" for body composition analysis, chemical extraction, is a destructive method. However, this is not ideal because it requires killing the animal, generates chemical waste, and is time consuming. Thus, in many instances, ecologists rely instead on morphometrics (e.g., body size and weight) to compute indices that have predictive value in determining body condition (Hayes & Shonkwiler 2001). Body condition can be estimated non-destructively and more easily with morphometric indices than by chemical extraction; however, such indices can be inappropriately or inconsistently calculated by researchers, leading to erroneous and subjective conclusions (Hayes & Shonkwiler 2001). Furthermore, in some taxa, external morphometrics may not be good indicators of body composition. In arthropods, for example, the possession of a more or less rigid exoskeleton necessitates discontinuous growth. Yet while growth in size occurs only at the molt, the main events of the molt cycle occur internally (Hutchinson et al. 1997). Crustaceans, for instance, undergo substantial alterations in body mass, tissue composition, and water content during the intermolt period (Chang 1995; Musgrove & Geddes 1995). During the next phase of the molting cycle, proecdysis, many species then exhibit significant atrophy of somatic muscle (Mykles & Skinner 1990). Morphometric analysis of crustaceans, which is normally based on changes in carapace length (Seiler & Turner 2004), is therefore a poor method for predicting body condition. Tracking these internal changes without sacrificing specimens, or

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conducting longitudinal studies that require repeated measures of the same individuals, is not possible with standard destructive methods.

Quantitative magnetic resonance (QMR) is a relatively new technology that has been used to provide accurate information on body condition of animals (Taicher et al. 2003). Animals are placed into a Plexiglas holding tube without anesthesia or restraints, and scanned using magnetic resonance for ~3 min (although this scan parameter may be adjusted so that run time is <1 min). Hydrogen nuclei in the specimen exposed to the magnetic field undergo an absorption and emission of energy in the radio frequency of the electromagnetic spectrum. The characteristic release of energy differs between fat, wet lean tissue, and water, and is measured to quantify the amount of the molecular species (or tissue) of interest. This technique for measuring body composition has been validated in many vertebrates, including mice (Taicher et al. 2003), birds (Guglielmo et al. 2011), and bats (McGuire & Guglielmo 2010). Although magnetic resonance imaging (MRI) has been employed to produce images of the organs of crabs (Brouwer et al. 1992; Gardner et al. 1998; Bock et al. 2001), crayfish (Herberholz et al. 2004; Brinkley et al. 2005), freshwater mussels (Holliman et al. 2008), sea urchins (Ziegler et al. 2008), oysters (Davenel et al. 2006), spiders (Pohlmann et al. 2007), and insects (Hart et al. 2003), to our knowledge QMR has not been tested as a tool for determining body composition of invertebrates.

The objective of this study was to assess whether QMR analysis is a valid technique for quantifying the body composition of crustaceans and other large arthropods. We used the crayfish species *Orconectes propinquus* Girard 1852 and *Cambarus robustus* Girard 1852, the American lobster *Homarus americanus* H. Milne Edwards 1837, and the Madagascar hissing cockroach, *Gromphadorhina portentosa* Schaum 1853, as models. Through comparison of fat, wet lean, and body water masses calculated by QMR with those obtained from traditional chemical extraction, it has been possible to draw conclusions as to the suitability of this non-invasive and rapid technology as an alternative to standard methods for destructive body composition analysis.

Methods

Collection and housing of animals

Specimens of *Orconectes propinquus* and *Cambarus robustus* were collected from the local Thames River system in London, Ontario, Canada between 21 September and 1 November 2009. We collected specimens of both species to obtain a greater size range of animals to be scanned. Members of these crayfish species are similar in body form, and because we were not interested in interspecific differences, we pooled data from both species in our analyses. Crayfish specimens were housed in separate 70-L tanks to reduce agonistic interactions. An air stone oxygenated the water, and the crayfish were not fed in the time between being collected and scanned in the QMR body composition analyzer.

Three individuals of *Homarus americanus* were bought alive and kept on ice in a cooler. Males of *Gromphadorhina portentosa* bred in a lab at Western University in London were housed in a container with dry dog food and water.

QMR body composition analysis

Adults of *O. propinquus* (n=14) and *C. robustus* (n=21) with a mean mass of 12.06±1.07 g (mean±SE; range 5.5–27 g), *H. americanus* (n=3) with a mean mass of 697.33±17.33 g (range 680–732 g), and adult males of *G. portentosa* (n=14) with a mean mass of 9.59±0.51 g (range 6.5–14 g) were selected to be scanned. All specimens were scanned within 4 d of collection except the lobsters, which were scanned the day they were purchased. Each individual was dried externally with paper towel, weighed (±0.001 g), and placed in a Ziploc bag immediately before being placed into the Plexiglas holding tube and scanned unanesthetized in the QMR scanner. The lobsters were too large to fit inside the holding tube of our particular scanner; therefore, the claws were removed at their bases and scanned together, independent of the remainder of the body. The claws had a mean mass of 230.74±6.09 g (range 218.87–239.02 g), and the bodies had a mean mass of 389.51±2.91 g (range 383.8–393.4 g).

The scanner used was the Echo-MRI-B (Echo Medical Systems, Houston, TX, USA). It is comprised of a computer (76 cm wide×59 cm deep×76 cm high) and a box that houses the magnet (61 cm W×71 cm D×84 cm H). It can be used in a laboratory and also in field locations when supplied with standard electrical access. A detailed description of the scanner and how the technology works is provided by Guglielmo et al. (2011), and additional specifications may be obtained from the manufacturer. Individuals were scanned three consecutive times at the two-accumulation setting to obtain measures of fat, wet lean, and water masses. A two-accumulation scan takes ~128 s. All specimens were subsequently killed by freezing on dry ice.

Chemical extraction

After completing the QMR scans, we measured the body composition of the specimens using chemical extraction. QMR does not detect mineralized structures such as bone. We therefore reasoned that as the chitin in arthropod exoskeleton is often found in composite forms or combined with calcium carbonate, as in crustaceans, it should likewise not be detected. To confirm that the chitinous structure is not detected and to obtain the most accurate comparison of wet lean masses obtained by QMR with those obtained by chemical extraction, the arthropods' cuticle had to be separated from the tissue prior to extraction. Arthropods must be heat-treated in a hot water bath to make separation of cuticle from tissue possible, which can result in loss of fat or alteration of lipid content. Consequently, so as not to introduce error into the study, fat values for dissected individuals were not included in the analysis. Likewise, total body water values calculated for dissected individuals were excluded from the analysis because water could be gained or lost from tissue during heat-treatment or dissection, respectively. We therefore split the frozen specimens into two groups (both with the same approximate mass range), subsequently referred to as the dissected group (for which wet lean masses were calculated) and non-dissected group (for which wet lean, fat, and body water masses were calculated).

The non-dissected group contained 14 crayfish (five adults of *C. robustus* and nine of *O. propinquus*), one lobster (one body and one set of claws, analyzed separately), and seven cockroaches. These specimens were placed on aluminum weighing dishes and weighed before being put in an oven and dried to constant mass at 70°C. The difference between initial and dry mass was recorded as the mass of total body water. The dried specimens were then ground with a mortar and pestle and each ground specimen was packaged in a cellulose filter paper envelope (Whatman #1, Whatman Ltd., Maidstone, Kent, UK). All filled envelopes were weighed prior to undergoing Soxhlet extraction with petroleum ether. Following the first round of extractions with petroleum ether, the envelopes were dried, weighed, and extracted again with chloroform to maximize the amount of lipids removed. Afterwards, the envelopes were dried and reweighed and the difference from the initial mass was recorded as dry fat mass. The dry fat mass was subtracted from oven-dried mass and the difference was recorded as dry lean mass. Wet lean mass was calculated by adding the mass of body water to the dry lean mass.

The dissected group contained 21 crayfish (15 adults of *C. robustus* and six of *O. propinquus*), two lobsters (two bodies and two sets of claws, analyzed separately), and seven cockroaches. Rather than being extracted whole, as described above, these specimens were first dissected (cuticle separated from tissue). The specimens were heat-treated in water beneath boiling point for ~1 h, or with the crustaceans until the point at which the cuticle turned golden-orange in color. The tissue was then separated from the cuticle, which required ~2 to 2.5 h for each specimen. Cuticle and tissue were placed on separate aluminum weighing dishes and dried, ground, and Soxhlet extracted separately, as with the non-dissected group. Wet lean mass was calculated as for the non-dissected group.

Statistical analysis

We conducted linear regressions in R (version 2.13.2; R Development Core Team 2011) to assess the relationships between dry fat, wet lean, and total water masses obtained by chemical extraction with the values provided by QMR for both crayfish and cockroaches. The triplicate QMR scans of each individual were averaged and the mean values were used in these analyses. Non-dissected and dissected wet lean masses were compared separately against QMR values to examine whether QMR lean mass predicts wet lean mass from chemical extraction better when dissection to remove cuticle is done beforehand. We considered linear regressions with slopes closest to one to be the most accurate, and those with high r^2 to be indicative of high precision. If the 95% confidence intervals of the slope estimates contained the value one, we concluded that the slope was not statistically different from one. Given our small lobster sample size, we did not conduct a linear regression for this species; we did calculate absolute and relative errors for the lobster data.

Absolute and relative errors were calculated for each of the body components. Absolute errors were calculated by taking the mean of the absolute difference between the raw QMR-estimated component mass and the actual mass by chemical extraction for each individual. Relative errors were calculated by taking the mean of the absolute error divided by the actual mass by chemical extraction for each individual and then converting this to a percentage. Following the analysis of the raw data, we used leave-one-out cross-validation to test the predictive ability of our modeling approach. For crayfish and cockroach datasets separately, we retained a single observation for validation and fitted a linear model

to the remainder of the observations. We then predicted the value of the excluded observation and calculated the absolute and relative error between the predicted and observed chemical extraction values. We repeated this procedure for each observation in these datasets and calculated the mean of these errors.

Results

The values from QMR analyses for crayfish wet lean mass and body water were highly correlated with the values obtained by chemical extraction (Fig. 1). The slope of the regression equation for crayfish wet lean mass in the dissected group was near 1 with a high r^2 value (Table 1). In the non-dissected group, the r^2 value was also high; however, QMR underestimated (slope >1) wet lean mass. The slope of the regression equation for crayfish body water was also near one, with a high r^2 value. The total crayfish fat estimate was neither accurate (slope not close to one) nor precise (low r^2). For cockroaches, wet lean values from QMR analyses were accurate and precise, with little difference between the dissected and non-dissected groups. The total fat estimate for cockroaches was more accurate and precise than for crayfish; however, the body water estimate for cockroaches was overestimated by QMR (slope <1).

Absolute and relative errors obtained by QMR analysis for dissected wet lean mass and for body water (Table 2) were reasonable, in that they fall within the bounds of error reported by QMR validation studies for other taxa (McGuire & Guglielmo 2010). Errors for crayfish and lobster claws and bodies were lower in the non-dissected group, although higher error was found for cockroach wet lean mass from the non-dissected group. Fat was not accurately predicted, but errors were lower for lobsters. When using the cross-validated equations, the predicted errors for crayfish and cockroach body components were reduced (Table 2).

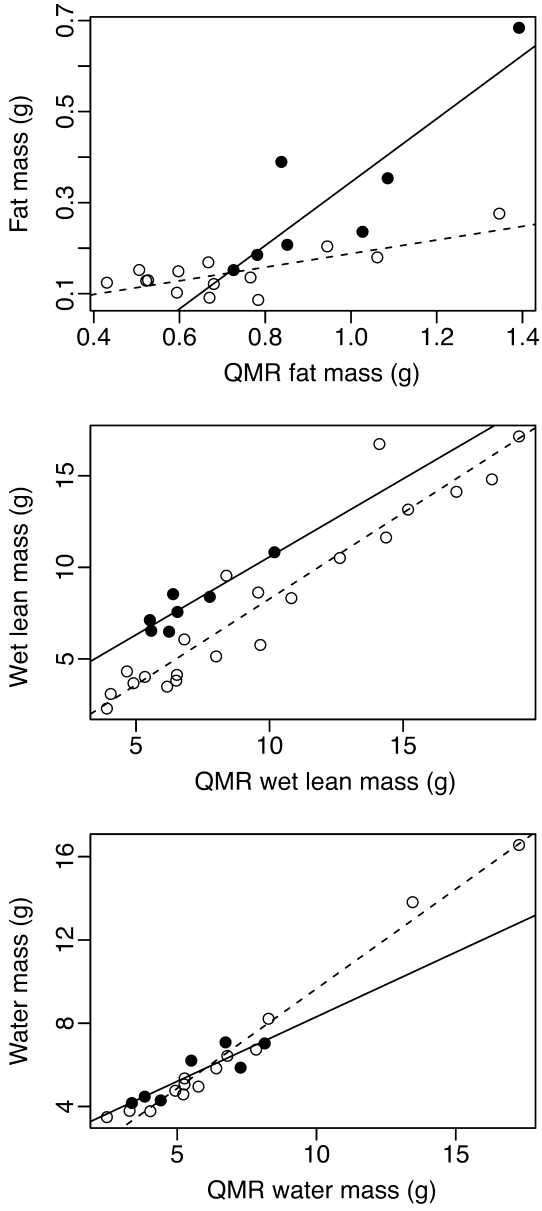

Fig. 1. Regressions of body composition values obtained from chemical extraction (dissected wet lean mass, fat mass, body water mass) against values obtained from quantitative magnetic resonance (QMR) for crayfish (○, dotted line) and cockroaches (●, solid line).

Discussion

QMR analysis is a safe and easily used technology that provides rapid estimates of dry fat, wet lean, and body water masses. It has many advantages over standard destructive methods for determining body composition, and although it has been employed with a variety of vertebrate species, ours is the first study to investigate whether it may be used with crustaceans and other large arthropods. QMR analysis provided accurate and precise measurements of total body water and wet lean mass for crayfish, lobsters, and cockroaches. It did not provide accurate and precise estimates of fat mass.

Crustacean edible tissue (muscle+gonads+hepatopancreas) normally has a fat content of ~5% (Barrento et al. 2009, 2010), and crayfish edible tissue contains <5% fat (Stanek et al. 2011). The mean percentage fat content we measured by chemical extraction is in line with these studies, with lobster fat content at 1.63% and mean crayfish fat content

Table 1. Parameters from regressions of body composition values obtained from chemical extraction against values obtained from quantitative magnetic resonance analysis for crayfish (*Orconectes propinquus* and *Cambarus robustus*) and Madagascar hissing cockroach (*Gromphadorhina portentosa*).

	Intercept	Slope	Slope 95% CI	r^2	F	p
Dissected wet lean						
Crayfish	−1.09*	0.94	(0.97, 1.08)	0.899	$F_{1,19}$=179.2	<0.0001
Cockroach	2.06*	0.85	(0.43, 1.27)	0.812	$F_{1,5}$=26.95	<0.05
Non-dissected wet lean						
Crayfish	−0.855	1.40	(1.30, 1.50)	0.986	$F_{1,12}$=908.6	<0.0001
Cockroach	2.71*	0.96	(0.35, 1.57)	0.720	$F_{1,5}$=16.4	<0.05
Fat						
Crayfish	0.04*	0.15	(0.07, 0.23)	0.525	$F_{1,12}$=15.36	<0.05
Cockroach	−0.35*	0.70	(0.24, 1.15)	0.710	$F_{1,5}$=15.72	<0.05
Water						
Crayfish	0.08*	0.96	(0.87, 1.04)	0.979	$F_{1,12}$=601.4	<0.0001
Cockroach	2.1	0.62	(0.26, 0.98)	0.75	$F_{1,5}$=19.4	<0.05

*, Not significant (p>0.05).

at 2.18±0.25% (±SE). However, the QMR scanner measured these as 3.99% and 10.6±0.98%, respectively. The overestimation of fat content, particularly in crayfish, could be due to limitations of the Echo-MRI-B scanner that we used. The scanner was custom designed in consultation with CGG for analysis of body composition of small birds and bats and has been shown to accurately measure fat and wet lean mass in bats with mean total body mass as little as 7.44±0.20 g (mean±SE; range 5.77–9.64 g) (McGuire & Guglielmo 2010). Although the mean body mass of crayfish (12.06±1.07 g) was greater than this, the bats had a greater mean fat content (0.94±0.12 g [±SE]; range 0.3–2.51 g) than did the crayfish (0.15±0.01 g; range 0.09–0.28 g). The crayfish fat masses might therefore have been inaccurately measured by the scanner because they were very small. This may also be related to the QMR scanner providing better measures of fat in cockroaches than in crayfish; the cockroaches, which store fat in the relatively large insect organ called the fat body, on average contained greater fat reserves than crayfish (0.32±0.07 g in cockroaches, vs. 0.15±0.01 g in the crayfish). For studies concerned with the accuracy of fat measurements, more tests should be performed. It may be that effective fat measurements could be made with crustaceans or other arthropods of higher fat content, or using a scanner designed for samples of low tissue mass.

Except for fat mass, in most cases, we were satisfied with the raw errors. The QMR analyzer predicted dissected wet lean mass with a raw error of ±2.07 and ±1.03 g and body water with raw error of ±0.49 and ±0.73 g, respectively, for crayfish and cockroaches. Lobster body component masses were predicted with the lowest relative errors of the three taxa, except for dissected wet lean mass. Crayfish (6.67±1.03 g) or lobster body (267.73 g) and claws (149.12 g) have higher mean water content than cockroaches (5.59±0.48 g). As with fat content, it may be that the improved errors and slope (0.96 for crayfish vs. 0.62 for cockroaches) are partly explained by increased mass of the molecular species of interest.

Using calibration equations (as generated by the cross-validation) reduces the error of dissected wet lean mass to ±1.12 and ±0.59 g, and slightly improves body water error to ±0.48 and ±0.55 g, respectively, for crayfish and cockroaches. Cross-validation provides a more realistic estimate of the error incurred from predicting an out-of-sample datapoint. Error is reduced because fitting a regression line through cross-validation allows the relationship between QMR values and chemical extraction values to deviate from the one-to-one line (which is the assumed relationship when calculating raw errors). Thus, whereas the QMR scanner might not predict chemical extraction values exactly, the model that describes the relationship between the two can have high predictive ability. Calibrating for the taxon of study is therefore important. One caveat, however, is that in these calculations, we assume that chemical extraction is an error-free method, when in actuality it is likely not.

The underestimation by QMR of wet lean mass from non-dissected individuals is consistent with the expectation that QMR does not detect exoskeleton, keratin, and other non-fat body components typically included in wet lean mass in chemical

Table 2. Mean absolute and relative errors (±SD) of quantitative magnetic resonance (QMR) measurements of body composition components for crayfish (n=21 for dissected wet lean, n=14 for non-dissected wet lean, fat, and body water), lobster body and claws (n=2 for dissected wet lean, n=1 for non-dissected wet lean, fat, and body water), and cockroaches (n=7). Results are presented for raw values (comparison of QMR values and chemical extraction values) and cross-validated values (see text).

	Raw		Cross-validated	
	Absolute error (g)	Relative error (%)	Absolute error (g)	Relative error (%)
Dissected wet lean				
Crayfish	2.07±0.95	36.37±23.01	1.12±1.15	16.35±12.81
Lobster body	89.92±14.63	37.20±7.61		
Lobster claws	69.22±3.14	59.38±5.74		
Cockroach	1.03±0.64	13.44±8.22	0.59±0.41	7.61±5.63
Non-dissected wet lean				
Crayfish	2.07±1.55	26.45±4.59	0.54±0.52	6.04±5.05
Lobster body	42.44	11.32		
Lobster claws	24.49	10.74		
Cockroach	2.46±0.85	29.23±8.75	1.01±0.56	11.05±5.07
Fat				
Crayfish	0.57±0.21	430.22±160.06	0.03±0.02	25.86±21.27
Lobster body	12.87	155.41		
Lobster claws	0.82	68.14		
Cockroach	0.64±0.11	255.90±111.07	0.07±0.08	27.62±24.20
Water				
Crayfish	0.49±0.33	8.95±7.85	0.48±0.40	9.00±9.30
Lobster body	11.13	4.16		
Lobster claws	8.05	5.40		
Cockroach	0.73±0.44	13.69±7.54	0.55±0.44	9.47±7.49

extraction. In this way, QMR provides a more functionally relevant measurement of wet lean mass than traditional chemical extraction. Dissection did not overly improve the r^2 or slope for crayfish wet lean mass, although it did for cockroach wet lean mass. Additionally, crayfish and lobster dissected wet lean mass relative errors are greater than non-dissected wet lean mass relative errors. This is most likely explained by the fact that any increase in accuracy in wet lean mass as a result of removing the exoskeleton is negated by additional error incurred through the dissection process. Water can be gained by the specimens when heat-treated in water if the cuticle cracks open and, conversely, can be lost from the tissue during dissection.

QMR body composition analysis has, in recent years, provided researchers working with a variety of vertebrates (EchoMRI^{TM} 2012) with the means to test hypotheses that would have been impossible or difficult to investigate using standard destructive methods. For example, QMR has been used to provide before and after analysis of dry fat, wet lean, and water masses of birds flown under different atmospheric humidity treatments, leading to new insights into avian metabolic strategies during migratory flights (Gerson & Guglielmo 2011), and has enabled longitudinal monitoring of fat mass to determine whether physical activity levels early in life predict future adiposity in rats, which has implications for human obesity and health (Teske et al. 2012). It similarly has many potential applications in research on arthropods. In addition to tracking internal changes throughout the molt cycle without sacrificing specimens, QMR might be used to quantify differences in functional wet lean mass between individuals to improve understanding of biomechanical or physiological factors on competitive performance. For instance, male crayfish engage in agonistic interactions, and their claws are important in establishing dominance within social hierarchies (Tierney et al. 2000). Thus, quantifying claw strength is important for predicting individual success in competition. We have demonstrated that it is possible to scan crustacean claws separately from the body. However, they must be detached from the body to do so, which means that repeated measures of the claws alone (e.g., measuring claw muscle mass before and after exposing the specimen to some experimental

treatment) cannot be done. QMR analysis could also be of great value to the crustacean aquaculture industry, where there is currently a large interest in developing low cost diets (Catacutan 2002), by allowing rapid assessment of body composition of crustaceans with formulated diets varying in levels of protein and lipid.

The field portability of the Echo-MRI-B^{TM} can also make it suitable for carrying out studies in natural environments. The lack of methods to measure *in situ* growth of individuals has been recognized as an impediment to studying crayfish (Olsson et al. 2008), which, like other aquatic arthropods, play a fundamental role in shaping community structure (Giller & Malmqvist 1998). Crayfish are keystone omnivores (Lodge et al. 1994; Nystrom et al. 1996; Whitledge & Rabeni 1997), and as wet lean tissue growth is greatly dependent on diet and habitat productivity, measuring body composition provides a good indicator of the quality of these factors (Speakman 2001). In this way, QMR analysis could be a useful tool when attempting to evaluate the health of freshwater systems.

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