Review

Can parasites really reveal environmental impact?

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This review assesses the usefulness of parasites as bioindicators of environmental impact. Relevant studies published in the past decade were compiled; factorial meta-analysis demonstrated significant effects and interactions between parasite levels and the presence and concentration of various pollutants and/or environmental stressors. These effects and interactions were also evident in subsets of studies that used different methods such as field surveys or experimental exposure. From this meta-analysis we conclude that parasites are useful bioindicators of environmental impact. Further, by examining aspects of study design, we put forward recommendations for the design of future studies to evaluate anthropogenic impact on host–parasite interactions and increase the efficiency of environmental monitoring programs.

The big issue in environmental parasitology

More than a decade ago Lafferty [1] summarized knowledge of the impact of anthropogenic changes on parasites. He and Kennedy [2] were critical of the sampling methodologies used to evaluate interactions and to examine host responses to different parasites. Where do we stand now? Have we demonstrated that parasites respond to stressors? Have we improved their utility in signaling environmental impacts?

Recent reviews [3–7] have compiled data on parasites as bioindicators of environmental impact. For example, there is increasing evidence that acanthocephalans can act as indicators of bioaccumulation because of their capacity to accumulate heavy metals [8,9]. Changes in parasite abundance, however, cannot be used as a simple bioindicator because they can be influenced by stochastic changes in a population or community [3]. Furthermore, there is conflicting evidence regarding impact on aquatic parasite abundance [9]; depending on the species, numerical or physiological responses to pollutants can be positive, negative, or absent [10]. Therefore, the circumstances under which parasites can be used as indicators of environmental impact have not been demonstrated conclusively. This review evaluates recent research linking parasite

Glossary

Accumulation indicators:: organisms that efficiently take up substances and reach an equilibrium at which the uptake of the respective substance is balanced by its excretion [3].
Before–after/control–impact (BACI):: a method for measuring the potential impact of a discharge, disturbance, or event on organisms, habitats, or ecosystems. The effects need to be analyzed before a planned activity, and then compared to those conditions measured *after* the planned activity [71].
Biomarker:: a functional measure of exposure to environmental stressors, that is usually expressed at the suborganismal level of biological organisation (e.g. an enzyme or a metallothionein) [72].
Effect size:: a measure of the strength of the relationship between two variables.
Effect bioindicators:: organisms that are used to detect environmental impacts through their changes in physiology, chemical composition, behavior, or number [4].
Environmental impact:: a change in environmental quality (the word 'impact' implies that a value judgement has been made on the importance of an environmental effect or change; http://www.icsu-scope.org/).
Environmental quality:: the state of the environment as perceived objectively in terms of measurements of its components (e.g. economic value) or subjectively in terms of its attributes (e.g. beauty and worth; modified from http://www.icsu-scope.org/).
Explicit spatial data:: there are two kinds: (i) cell data, such as those from satellite images, in which the measurement unit is a pixel, and (ii) geo-referenced data that provide the spatial location of the biological or physical measurements [62].
Extent:: the total length, area or volume included in a study [62].
Grain:: in parasitology, the physical dimension at which a measure of parasitism (e.g. prevalence) applies. The sample size used to quantify the parasitism could be considered as the third dimension of space [62].
Heterogeneity:: the variation in study outcomes between studies [13].
Interval distance:: the average distance between neighboring sampling units [62].
Meta-analysis:: a statistical technique that combines the results of several studies addressing a set of related research hypotheses. This is normally done through the identification of a common measure of effect size (http://en.wikipedia.org/) [13].
Pollution:: the anthropogenic activity of damaging the air, water, or land with chemicals or other substances.
Probabilistic sampling design:: a group of methods that attempts to select units such that each has a definable probability of being selected. This approach minimizes the sampling error of the estimates for the most important survey variables, while simultaneously minimizing the time and cost of conducting the survey [59].
Scale:: the spatial or temporal dimension at which an ecological phenomenon occurs.
Spatial analysis:: the analysis of phenomena distributed in space, using distance as a core variable.
Spatial structure:: the tendency of nearby samples to have attribute values that are more similar than samples that are farther apart [62].
Spatial dependence:: the response of organisms to environmental factors or biological processes at a specific spatial or temporal scale. For example, chemical signals from hosts (e.g. snails) are important in determining the parasite settlement. At the ecosystem level, physical factors such as the oceanic current speed are important for parasite transmission.

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Spatial heterogeneity:: the variability of a system property in a given space (e.g. the distribution of parasites within a host population).
Spatial autocorrelation:: the degree of association of a variable with itself due to the closeness of the sampling points from which the variable has been recorded [62].
Two-tailed test:: a statistical test in which the null hypothesis will be rejected when the value of the statistic falls into either tail of its sampling distribution (e.g. the normal distribution).
Temporal structure:: the tendency of periods that are close in time to have attribute values more similar than periods that are farther apart [62].

responses to environmental impact. We address study design and make recommendations for future studies to minimize sampling effort and maximize useful biomonitoring information.

Bioindicators and the data

Bioindicators are species that reflect environmental impact because they respond to habitat alterations with changes in physiology or chemical composition. Importantly, bioindicators can be either accumulation or effect indicators (see Glossary). Accumulation indicators must be efficient in accumulating substances from their environment without showing adverse effects; effect indicators can range from functional molecular or physiological changes to changes in population size or status. These bioindicators measure exposure to pollutants at different levels of organization, from the subcellular to the ecosystem level, and are useful for effect indication. This review focuses on potential indicators using helminths, crustaceans, and protozoans (mainly ciliates and myxozoans) of both terrestrial and aquatic environments, hereafter called 'parasites.'

From the Web of Science we obtained 98 peer-reviewed papers, published between 1997 and 2008, using the keywords 'environmental impact', 'pollution', 'parasites', 'sampling design', 'heavy metals', 'polychlorine biphenyls', 'polyaromatic hydrocarbons', and 'pesticides' (see Table S1 in the supplementary material online). However, data analysis requirements further limited studies based on additional criteria: an alleged effect of environmental impact on parasites, quantitative data on parasites and host population sizes for control and impacted localities and/or treatments, and pollutant accumulation for parasites and hosts. These criteria yielded a dataset of 52 studies (see Reference List S1 in the supplementary material online) with 242 total associations between environmental variables and parasites.

We used a 2×2 factorial meta-analysis to determine possible statistical interactions between environmental impact variables and parasites [11]. Briefly, this is similar to a 2×2 factorial ANOVA with two factors tested simultaneously: environmental impact variables and natural variation. Natural variation is implicit in all the studies considered and includes aspects such as seasonality, spatial variation or parasite aggregation in the host population [12]. Outcomes are estimates of the overall effects for each factor and interactions. The null hypothesis was that no overall effects or interactions would be significant. For a full explanation of calculations refer to Ref. [11]. Effect sizes for each study were expressed using Hedges' d. This measures the difference between experimental and control means, divided by a pooled standard deviation and multiplied by a sample size correction factor [11]. Because

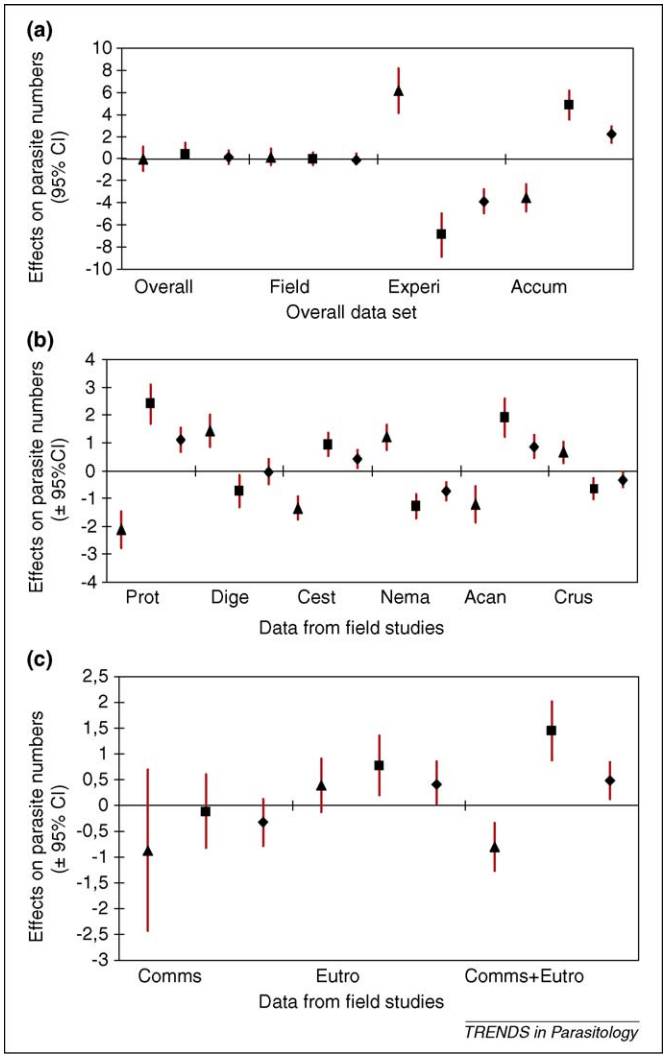

Figure 1. Average effect sizes (± 95% confidence intervals) of natural variation (▲), environmental impact variables (■), and their interaction (◆) on parasite numbers for the whole dataset **(a)**, for subsets of different parasite taxonomic groups **(b)**, and for communities and eutrophication **(c)**. Hedges' d values for overall effects and interactions are significantly different from zero if the confidence interval (CI) does not overlap zero. Abbreviations: Accum, accumulation data (number of associations involved $n = 62$); Acan, Acanthocephala ($n = 10$); Cesto, Cestoda ($n = 12$); Comms, component communities ($n = 15$); Crus, Crustacea ($n = 9$); Comms+Eutro, component communities and eutrophication ($n = 17$); Dige, Digenea ($n = 59$); Eutro, eutrophication ($n = 43$); Experi, experimental data (n = 31); Field ($n = 149$); Nema, Nematoda ($n = 17$); Overall, field, experimental and accumulation data together ($n = 242$); Prot, protozoans and Myxosporidia ($n = 21$). Protozoans and Myxosporidia have been summarized together because there were only four studies for Myxosporidia.

Hedges' d is a parametric estimator of effect size [13] we transformed our data to natural logarithms, ln (x + 1), and used the standard error as a measure of the precision of the sample mean. In Figures 1 and 2 we illustrate the mean values of both overall effects and the interaction terms that were judged to be significantly different from zero if their confidence interval (CI) does not overlap zero. Following Cohen's scale [14] the thresholds for small, medium, and large effects are 0.2, 0.5, and 0.8, respectively. The individual effect estimates from the studies were combined in a mixed study effects model and the Q statistic was used to calculate heterogeneity [11]. This indicates whether the variation between studies is sufficiently large to reject a homogeneity assumption [15]. We partitioned the dataset

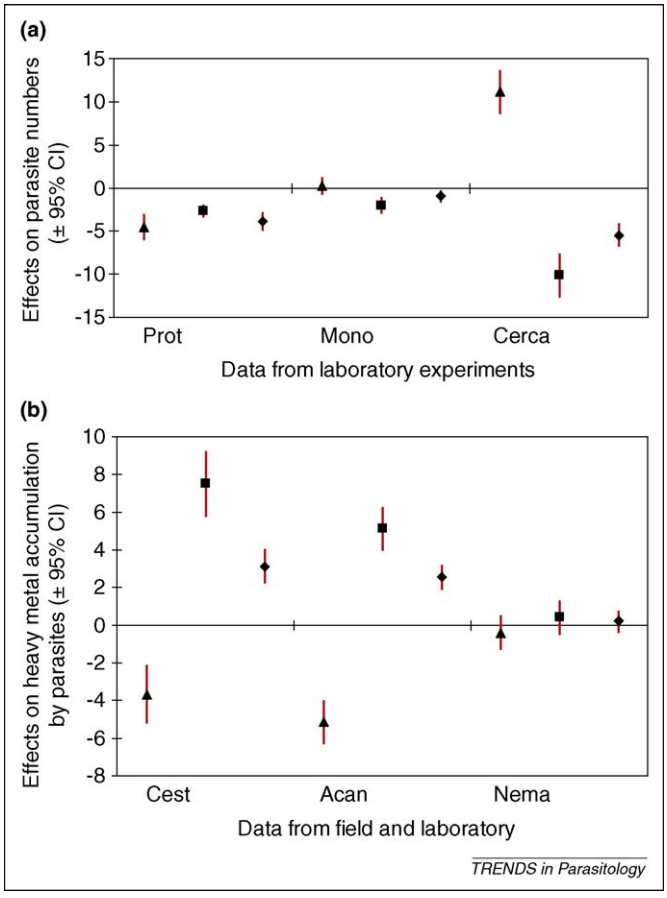

Figure 2. Average effect sizes (± 95% confidence intervals) of natural variation (▲), environmental impact variables (■), and their interaction (◆), on parasite numbers for **(a)** subsets of different parasite taxonomic groups, and **(b)** heavy metal accumulation by parasites. Hedges' *d* values for overall effects and interactions are significantly different from zero if the confidence interval (CI) does not overlap zero. Abbreviations: Acan, Acanthocephala (number of associations involved $n = 27$); Cerca, cercariae ($n = 18$), Cest, Cestoda ($n = 21$); Mono, Monogenea ($n = 11$); Nema, Nematoda ($n = 9$); Prot, protozoans ($n = 4$).

into three method subsets (field, experimental and accumulation studies) to determine variation within and between study approaches. Within each subset we studied the overall effects and interaction terms of each taxonomic group.

Results of the meta-analysis

Meta-analysis for the 52 studies and their 242 comparisons, as well as the field studies subset, revealed no significant (i.e. $d \approx 0$) overall effects or interactions (Figure 1a). The experimental and accumulation studies subsets showed significant overall effect sizes for both factors and the interaction (Figure 2). Figures 1 and 2 show significant effects for both factors and also show interaction terms for different taxonomic groups of parasites within the three study subsets.

All parasite taxonomic groups presented significant overall effects and interaction terms for at least one of the three subsets in Figures 1 and 2. The magnitudes and directions of significant effects and interaction terms varied widely between parasite taxa for both field and experimental subsets (Figure 1b). Communities showed a positive interaction term due to eutrophication (Figure 1c).

For the accumulation data, effects are related specifically to the higher uptake of heavy metals by parasites than by their hosts. Interestingly, cestodes and acanthocephalans (but not nematodes) showed similar medium effect and interaction values (Figure 2). Furthermore, the effect sizes for comparisons between terrestrial hosts and their parasites for bioconcentration of heavy metals were non-significant in all cases for cestodes, nematodes and digeneans (data not shown). Full heterogeneity data are shown in Table S2 (supplementary material online). Heterogeneity measures for the whole dataset varied widely (Q = 31–42981). However, other meta-analyses in ecology with Q values >1000 have yielded valuable interpretations of the data [11].

Table 1 summarizes significant interactions between the identified environmental impact variables and the parasite taxa. Several overall effects and interactions support Lafferty's view regarding responses for specific groups such as protozoans and acanthocephalans; however, important points emerge from the meta-analysis. First, communities have a positive effect and appear to be a promising indicator of responses to eutrophication. Second, the type of study (field or laboratory) can influence the effect of environmental impact on parasites (e.g. protozoans). Third, results of the meta-analysis suggest that each group of parasites provides distinct information (Figures 1 and 2) and, except for eutrophication effects on communities, groups should not be pooled for analysis.

Lafferty [1] attributed the overall weak association between environmental impact and parasite abundance to the wide range of stressor intensities, as well as to the inherent variability of parasite responses. As with other organisms, parasites have a stochastic response to stress, and this uncertainty is at least partly irreducible. However, it is possible to control for the degree of 'effect' for environmental impact variables on parasites by applying ecological assessment concepts such as toxicological hazard indices [16]. This is difficult for field exposure studies because they often involve complex mixtures [17]. Undoubtedly, experimental exposure of hosts and parasites to specific pollutants will continue to be necessary for understanding effects. A compromise might be to increase bioassay approaches involving long-term exposures to contaminated media from polluted and control sampling points [18,19]. Another problem highlighted by Lafferty [1] was that most studies are not well replicated. This seems to have been adequately addressed in that more recent studies [19–48] have used larger effect sizes and adequate controls.

Another meta-analysis recently conducted by Blanar *et al*. on the effect of pollutants on aquatic parasites [49] reinforces the view that parasites respond to environmental impact. From the biological standpoint most of their results [49] concur with those of the present meta-analysis. However, in our analysis nematodes, acanthocephalans and crustaceans had significant interactions with environmental impact (Table 1), whereas Blanar *et al*. did not detect interactions. There are three additional biological distinctions between the studies: (i) the dataset for meta-analysis is highly heterogeneous and therefore requires subdivision (field, laboratory, accumulation), (ii) our study

Table 1. Environmental impact variables reported for the parasite taxonomic groups with significant interaction terms in the 2 × 2 factorial meta-analysis[a]

Parasite group	Eutrophication	Pulp-mill effluents	Crude oil	PCBs	Pesticides	Heavy metals
Protozoans and Myxosporidia	F+	F+/L−	=	F+	F+/L−	F+/L−
Digenean (adults and metacercariae)	F+	F−	F−	F−	F−	F−
Digenean (cercariae)	=	=	=	=	=	L−
Monogeneans	=	=	L−	=	=	L−
Nematoda	F+	F−	F−	F−	F−	=
Cestoda	F+	=	=	F−	F−	F−/A+
Acanthocephala	F+	F+	=	=	F−	F+/A+
Crustacea	F−	F−	=	F−	F−	F−

[a]Parasite groups are arranged in phylogenetic order. PCBs are polychlorinated biphenyls. The symbols are as follows: +, positive interaction term between natural variation and the environmental impact variable; −, negative interaction term; F, field study; L, laboratory study; A, accumulation study; =, no significant interaction.

considers accumulation by acanthocephalans, and (iii) the Blanar *et al.* analysis considers the effect of pollutants on parasites but not the actual interaction between this factor and the number of parasites or pollutant concentrations in parasites.

From the statistical standpoint there are additional differences. Blanar *et al*. implemented a sign test, and this procedure has been criticized because it sacrifices power and dichotomizes the information contained in the *P* values [50]. In addition, Blanar *et al*. did not weight the effect sizes for each study because they implemented a nonparametric Wilcoxon test. Finally, their implementation did not adjust the *P* values, a necessary correction because multiple, non-independent tests were performed.

Methodological issues related to environmental parasitological surveys

Confounding issues related to using parasites as indicators of environmental impact, including inconsistencies in design, measurement, and interpretation of the underlying surveys, present additional uncertainties. These can also include selection of environmental variables, the measurement methods employed, and the statistical methods used to account for external sources of correlations (e.g. spatial or temporal processes).

The identification and counting of parasites are challenging tasks in environmental parasitology, although new technologies such as image analysis are improving the capability for taxonomic identification [51]. Another possibility is bar coding [52,53] that requires minimal extra effort during sampling but allows for molecular identification. Molecular tools also provide opportunities to overcome the time-consuming process of counting individuals: quantitative PCR is rapidly improving the process, as shown by Hung and Remais [54], and non-destructive sampling – used for studying shrimp, lobsters and other aquatic animals – is also a possibility [55].

Parasites in an environmentally compromised setting also face changes to the host's immune response. We therefore suggest that, during parasitological surveys, variables related to the host's immune response and biomarkers should also be studied. Cytochrome P450 1A, ethoxyresorufin-O-deethylase activity [56], histological data [25], and lipid peroxidation [57] can be useful when analyzing data for significant correlations.

Field observations of host and parasite abundance are collected at specific spatial locations. There are several viable sampling strategies for determining spatial structure. A suite of probability-based sampling designs – variations on random, systematic, stratified, and cluster sampling [58] – allows calculation of the uncertainty associated with estimates and the application of statistical inference techniques. Traditionally, random or systematic designs are applied when no prior knowledge of a spatial correlation exists. However, the presence of spatial correlation in the stressor and/or in biological response can cause problems for correlation measures, hypothesis testing [59], and host sample-size calculations [60], by inflating the degree of correlation or by affecting the power of significance tests [61]. Methods exist to 'correct' for such correlation, but these have not been used for examining possible perturbation of host–parasite interactions by environmental stressors.

Identifying the spatial scale at which the environmental impact occurred is crucial in estimating how parasite populations and community dynamics are affected. Were these impacts on the same scale as the variability in parasite abundance? Similar questions can be addressed if spatially explicit data are considered [62,63]. Scale has emerged as an important factor in ecology, and the processes and factors operational at a specific scale might not translate to other scales (Figure 3) [62]. Therefore, a key question in environmental parasitology is scalar resolution

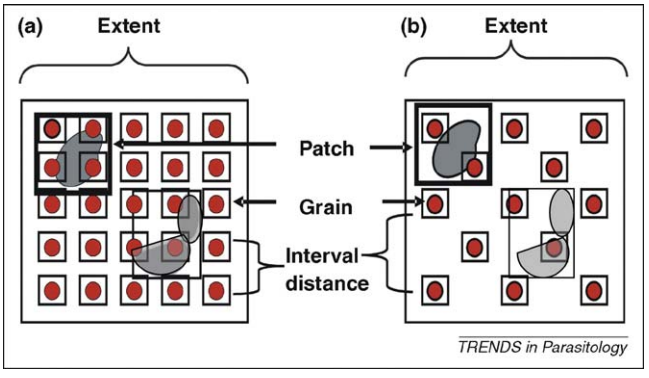

Figure 3. Spatial sampling design for the detection of a possible spatial structure. A spatial structure can be manifested as the spatial dependence of individuals, populations, or communities on a specific number of environmental variables (e.g. oxygen concentration, prey number, or shelter) in a defined area or patch (grey shading). There are three key concepts: the extent, in other words the total length, area, or volume included in the study; the grain size – the number of hosts examined per sampling site; and the sampling interval, that refers to the average distance between neighboring sampling units. The spatial structure in panel **(a)** would be more readily detected than in panel **(b)** because of the closer interval distance used in **(a)**. The closer interval distance used in **(a)** results in a higher probability of detecting the spatial structure. This does not mean that the interval distance used in **(b)** is wrong, but that the spatial structure of the data will be detected and explained with a lower degree of precision. Figure modified with permission from Ref. [62].

Box 1. Recommendations and cautions for study design in environmental parasitology

Recommendation

- Select the target area considering the spatial scales of the pollutant and of the response variable.
- Select a probability sampling design.
- Select an appropriate statistical analysis for the data.
- Seek professional judgement before applying the definitive sampling protocol [12].

Caution: this should be done before applying the definitive sampling protocol. Adequate knowledge of the biology and life cycles of parasites and hosts is required.

Recommendation: when the spatial scale of the process is unknown, a scale-oriented sampling design is suggested [62].

Caution: this involves collecting large quantities of data, and this is not always possible.

Recommendation: the denser the lattice of sampling points, the better the accounting of the variability of the phenomena under observation.

Caution: an increase in the number of lattice points produces fine-scale information; use of spatial statistics is then important to detect the effect of large-scale processes.

Recommendation: make the degree of precision in the observed patterns explicit by determining the percentage of the variance conveyed in the scale scenario used [62].

Caution: the use of statistics based on explicit spatial data requires the use of an experimental variogram.

Recommendation: consider that pollutant effects are not the same for the different parasite life stages.

Caution: this is time-consuming because different sampling and statistical designs need to be considered as a function of ecological characteristic of the parasites. In addition, the field or laboratory approaches can influence the perceived effect.

of parasite population/community characteristics and the environmental impact. In these instances a sampling design that accounts for discernable spatial structure is recommended (see Box 1).

Individual hosts as sampling units

Increases in sampling density are often necessary to characterize spatial or temporal phenomena adequately. Probabilistic sampling designs (see e.g. Ref. [64]), where all potential sampling sites have a non-zero probability of being selected for sampling, afford a practical approach and allow for a range of techniques to characterize uncertainty and optimize sample sizes. Each individual host is a 'sampler' of its environment, in terms of parasites and stressor exposures, and a limited number of infracommunities [65] can represent that location. This is especially applicable to sessile hosts. However, host behavior and other biological characteristics can modify their efficiency as samplers and impact the sampling strategy. Infracommunities allow for full use of the statistical power represented by each individual host harboring a community of parasites. The minimum number of infracommunities representative of a location can be determined by using species accumulation curves [66]. An advantage of probabilistic sample design is that it accommodates increased sampling points in areas of interest. Therefore, when working with sessile organisms, one can use species accumulation curves to determine the minimum number of infracommunities needed to characterize a sampling point, although sampling density could have to be increased for mobile organisms (Figure 4). Ultimately, however, the overall interpretation of the (effectively biased) sample

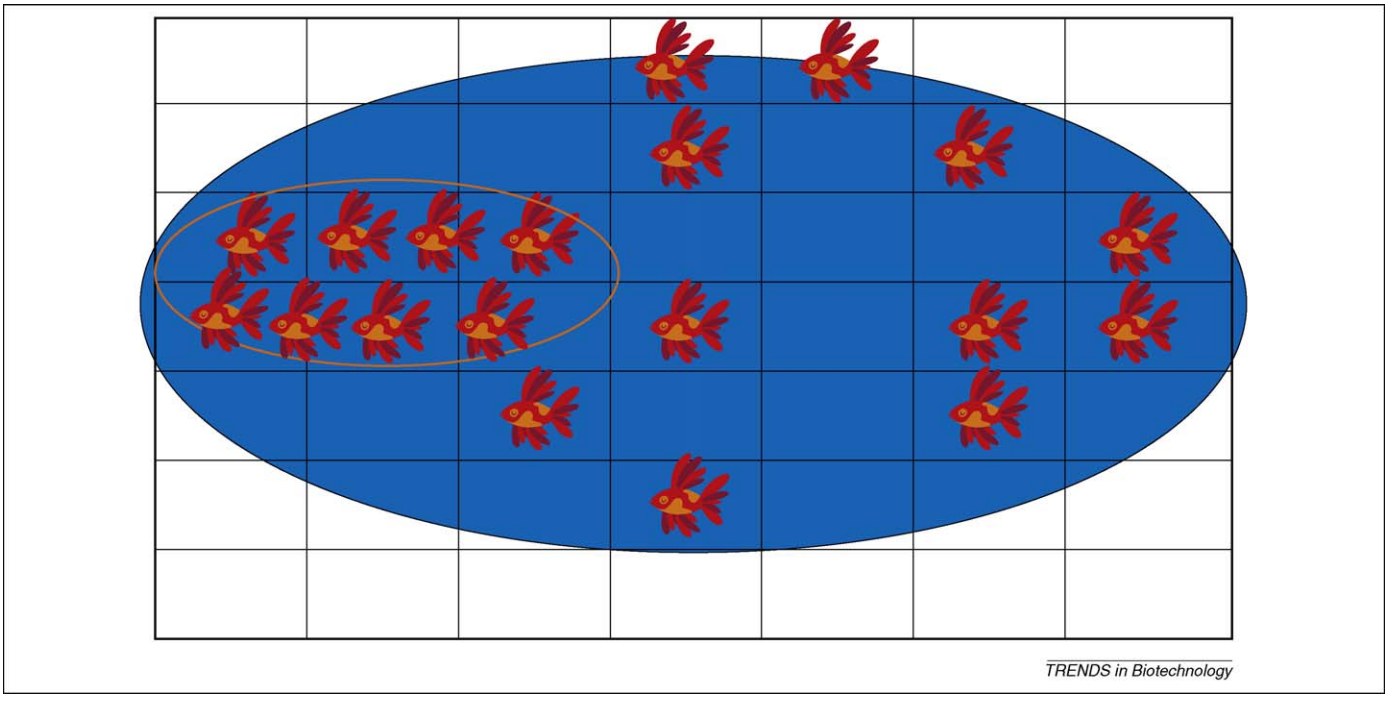

Figure 4. A hypothetical representation of a Generalized Random Tessellation Stratified (GRTS) spatially balanced survey design over a lake, illustrating the use of infracommunities as sampling units. The orange ellipse is an impacted area in the lake. If the fish are vagile, one can increase the number of sampling points within the area of concern so as to increase the probability of catching the required number of hosts. The increase in the number of sampling points within that area can be included within the sampling design by using unequal probability of selection. This procedure is fully described on the website of the US Environmental Protection Agency (http://www.epa.gov/).

design can still be accomplished [64]. A further recommendation is to identify intermediate hosts (e.g. snails) that would allow the study of exposure of intra-molluscan larval stages to sediments from the sampling point(s). This technique can provide information on host behavior and on the sensitivity of parasite transmission stages to each environmental impact.

Temporal patterns

Mounting evidence from temporal data suggests that parasites are good bioindicators of environmental impact [67,68], although thresholds for when specific environmental impacts cause parasite abundance changes are still undetermined. For example, no significant changes were found in the composition of the parasite species infecting spottail shiners following exposure to urban Montreal effluents over the course of three years [29]. One explanation is that the level of pollution in the St. Lawrence River was only moderate and, in consequence, parasite communities did not display large changes in species composition. By contrast, parasites of flounders were found to be useful for monitoring in the more contaminated waters of the German Bight [33]. However, only part of the picture is seen if we are restricted to field data; our recommendation, therefore, is that field and experimental data should be simultaneously obtained. This strategy has been applied successfully [18,69].

Several examples show the usefulness of parasite abundance as a bioindicator of the recovery of impacted habitats [70]. During a Finnish lake survey, parasite abundance in lakes that were highly polluted with pulp and paper effluents (PPE) was lower than in lakes with lower PPE levels [70]. After environmental conditions in the lakes improved, parasites returned to levels similar to those in less polluted lakes. A Newfoundland study, by contrast, showed that the numbers of *Cryptocotyle lingua* metacercariae infecting winter flounders were increased in the most polluted localities versus control sites [25]. Therefore, different types and degrees of environmental impacts can produce different physiological or numerical responses of both hosts and parasites, but we identified only three studies [20–22] that included both fieldwork and laboratory bioassays.

Huspeni and Lafferty [67] studied larval digenean infections of snails by applying a BACI (before–after/control–impact) design with replicated controls. Here, parasites were used as bioindicators of environmental recovery in salt marshes. After six years the number of infected snails in the impacted zones recovered to values at least as high as in control zones. Apart from BACI, a time series from the impacted site can be used [12,71]. It should be noted that all sampling designs suggested in this review also apply to temporal studies. For long-term recovery studies, therefore, parasites appear to be useful bioindicators when rigorous sampling designs are applied.

Conclusion

This meta-analysis suggests that environmental impacts have significant effects on parasites, and is the first to determine quantitatively the interaction terms of these factors. Rather than simply demonstrating the weakness of the data, the observed level of heterogeneity opens the door to novel hypothesis-testing and causation studies. We suggest that such investigations should use hybrid strategies such as field surveys combined with bioassays. Further, we consider it necessary to estimate the threshold at which parasites respond to environmental insults. Finally, we suggest a sampling strategy for surveys that optimizes both the number of sampling points and the number of hosts that need to be collected. Such sampling designs are more likely to be replicated over time to produce datasets that account for spatiotemporal system variability.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.pt.2009.11.001.

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