



Chapter 3.1.

IMPORTANCE OF SAMPLING DESIGN: HOW TO COLLECT DATA ON FISH PARASITES

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Introduction

There is no doubt that the importance of fish parasites is related directly to the importance of fish they may affect (Hoffman 1999). It is well known that fish are an excellent source of complex proteins, they provide an important recreational asset, both for sport fishing and as one of the attractions of nature. In addition, a lot of fish species are also very important for development of various types of aquacultures, and finally, fish and their parasites also represent an important and interesting subject for science including ichthyoparasitology investigating parasites as potential causative agents of various fish diseases and also in ecotoxicology and evolutionary ecology (e.g., Woo 1995; Khalil & Polling 1997; Hoffman 1999; Scholz 1999; Álvarez-Pellitero 2008; Eiras *et al.* 2008a,b; Sitjà-Bobadilla 2008; Buchmann *et al.* 2009; Leatherland & Woo 2010; Woo & Bruno 2011; Woo & Buchmann 2012).

Many years ago, Lester (1984) has reviewed methods for studying the effect of parasites on feral and cultured fish. Before fish parasitic diseases are effectively treated and controlled, the study of fish should follow a logical pattern:

- identify the parasite;
- obtain a thorough knowledge of its life history, which may be simple (direct or monoxenous) or very complicated (indirect or complex);
- learn the ecological requirements of the parasite, such as host specificity, optimum temperature, pH, nutrition, and other metabolic requirements;
- map the geographical range of the parasite;
- determine effect of immunological mechanisms of the host on the parasite, and *vice versa*;
- study control and treatment methods.

Hierarchical structure of parasitology

Parasitology and especially evolutionary ecology of parasites can be studied at three hierarchical levels: (1) organism, (2) population and (3) community (see Fig. 3.1.1). The smallest scale of study in parasite ecology is the individual parasitic organism, but parasitologists also deal with populations of parasite individuals of the same species, and with communities made up of several populations of

different species (e.g., Kennedy 1976; Esch *et al.* 1990; Esch & Fernández 1993; Rohde 2005; Poulin 2007).

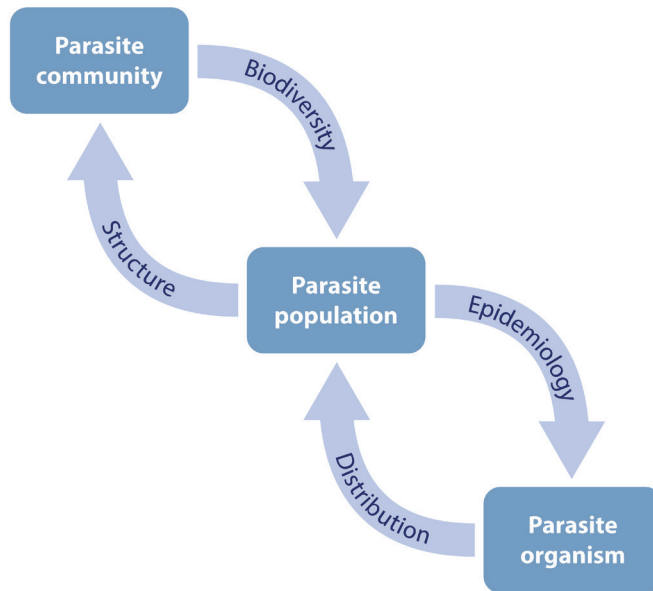


Fig. 3.1.1. A schematic representation for the three hierarchical levels of organisation of parasite-host associations. (Illustration by M. Luo and M. Gelnar.)

Sampling of parasitic organisms

Correct diagnosis is essential not only for parasite species identification but also for effective treatment and control of any fish disease. This means that there needs to be a consensus on the names and terms used in the identification process. Therefore, before we begin to consider a specific parasite, it is necessary to have an understanding of how the taxonomic system works and its relevance to parasitology (e.g., Gussev 1978, 1985; Halton *et al.* 2001; Pugachev *et al.* 2010; Gunn & Pitt 2012). Those who study the classification of organisms are called taxonomists and they arrange organisms into hierarchical categories to reflect their assumed relationships.

Table 3.1.1. Taxonomic hierarchy with specific reference to the monogenean parasite *Paradiplozoon homoion homoion*

Taxonomic division	Taxon name	Common name
Super kingdom	Opisthokonta	
Kingdom	Animalia	animals
Subkingdom	Bilateria	
Branch	Protostomia	
Phylum	Platyhelminthes Neodermata	flatworms
Class	Monogenea Carus, 1863	
Subclass	Oligonchoinea Bychowsky, 1937	
Order	Mazocreaidea Bychowsky, 1957	
Suborder	Discocotylinea Bychowsky, 1957	
Family	Diplozoidae Palombi, 1949	
Subfamily	Diplozoinae Palombi, 1949	
Genus	<i>Paradiplozoon</i> Akhmerov, 1974	
Species	<i>Paradiplozoon homoion</i> (Reichenbach-Klinke, 1961) Akhmerov, 1974	
Subspecies	<i>Paradiplozoon homoion gracile</i> (Bychowsky et Nagibina, 1959) Akhmerov, 1974	

Note: not all taxonomists agree with the same classification scheme. For example, some specialists prefer to divide the Monogenea (or Monogenoidea according to other authors) into different subclasses:

- Monopisthocotylea (= Polyonchoinea) and Polyopisthocotylea (excluding Polystomatidae and Sphyrnauridae = Oligonchoinea) – Bychowsky (1957)
- Polyonchoinea, Polystomatinea and Oligonchoinea – Lebedev (1989)
- Polyonchoinea and Heterochoinea (including two infra-subclasses Polystomatoinea and Oligonchoinea) – Boeger & Kritsky (2001)

Selection of proper morphometrical characteristics and effective laboratory techniques

There is no doubt that the usage of selected morphological/anatomical characters and some metrical parameters represents the most important step in parasite species identification (e.g., Rubbi 1994; Rizzuto & Fasolato 1998; Lacey 1999).

As an example, the following morpho-anatomical characteristics can be recommended to be used for the identification of monogeneans (Gussev 1978, 1985; Pugachev *et al.* 2010).

- Shape and size of the body and haptor
- Structure of the anterior end; presence or absence of lobes, lappets, suckers and their number
- Structure of the tegument, its thickness and presence or absence of folds, scales or thorns
- Presence or absence of eyes, their number and structure
- Shape, number, arrangement, orientation and size of haptoral structures
- Structure and size of the copulatory organ and vaginal armament
- Structure of the intestine
- Number of testes
- Shape and arrangement of the ovary
- Relative position of the ovary and testes
- Number, shape and position of the gland reservoir of the copulatory organ
- Course of vas deferens and shape of the seminal vesicle
- Position of the genital and vaginal pores, course and armament of the vaginal duct and seminal receptaculum (if present)

It should also be pointed out that correct identification of the fish host is extremely important. Erroneous identification of hosts or infection site may result in misleading conclusions. It is therefore recommended to always take a picture of the host and to fix a small piece of its tissue (fins, liver or muscle) in molecular-grade ethanol for DNA-based identification, or to fix and preserve the entire host specimen as a voucher.

Sampling of parasite populations

Parasite populations vary in size over short and long-time scales and are affected by biotic and abiotic environmental factors. Some of these factors cause changes in parasite numbers, whereas others reduce the amplitude of fluctuations around an equilibrium population size.

Parasite populations are invariably fragmented into as many subgroups as there are infected individuals in a host population. For practical reasons, it is easier to consider only a single parasite life stage, such as adult parasites only, when defining a population (*e.g.*, Esch *et al.* 1990; Esch & Fernández 1993; Hanski 1999; Šimková *et al.* 2002; Poulin 2007). Thus, a parasite population consists of all adult parasites in all individual hosts of a host population; it is subdivided into numerous infrapopulations of unequal size, each inhabiting a different host individual. Infrapopulations are ephemeral groups, lasting no longer than the host's lifespan. Offspring issued from different infrapopulations have the opportunity to mix outside hosts and reassemble in new combinations to form new infrapopulations in new

individual hosts. The infrapopulation fragmentation is thus temporary and changes continually from generation to generation (for a schematic illustration of factors affecting parasite populations, see Fig. 3.1.2).

To date, the population biology of parasites has been investigated on three different fronts (Poulin 2007):

1. The dynamics of parasite populations can be modelled mathematically, usually with a few simplifying assumptions (*epidemiological approach*).
2. Empirical studies of field populations have highlighted the many density-dependent and density-independent mechanisms acting to regulate parasite abundance over time in specific systems (*ecological approach*).
3. Genetic structure among infrapopulations and among populations allows us to determine transmission processes and estimate the frequency of exchange of individuals among populations (*genetic approach*).

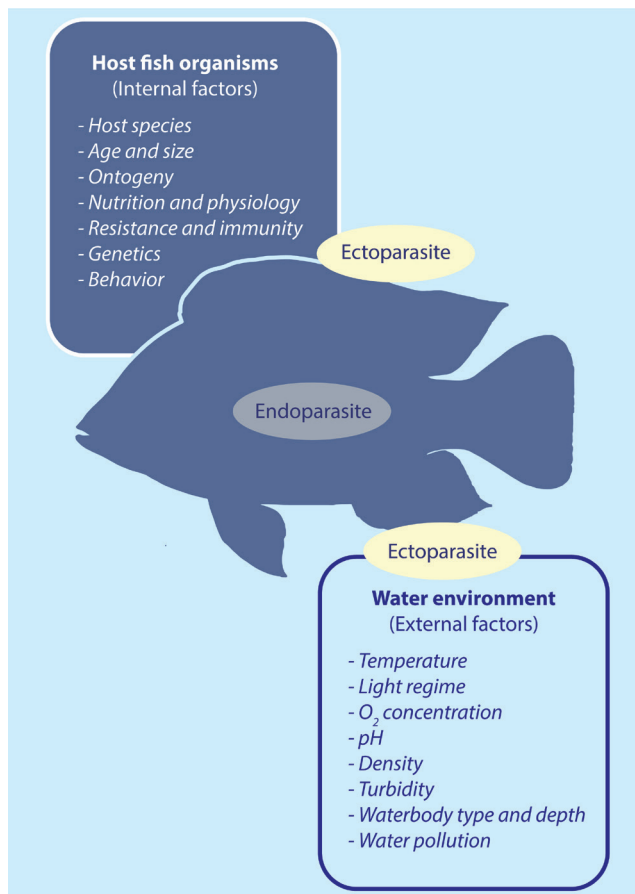


Fig. 3.1.2. A schematic representation of parasite-host interactions in an aquatic environment. (Illustration by M. Luo and M. Gelnar.)

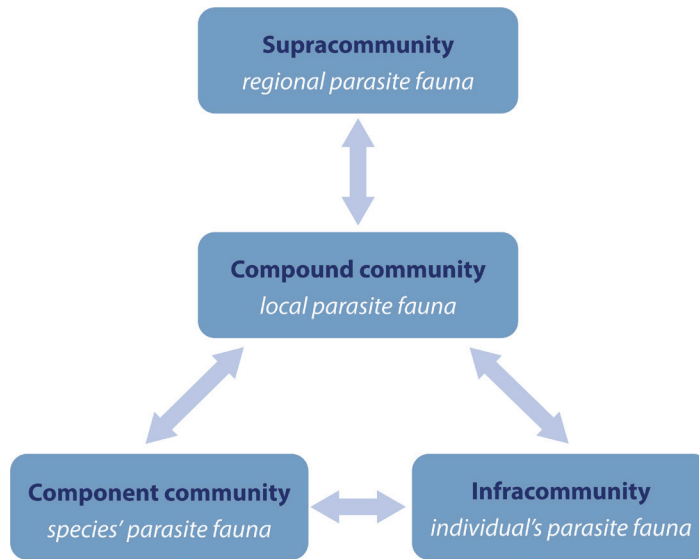


Fig. 3.1.3. A schematic representation of the hierarchical organisation of parasite supracommunity, compound community, component community and infracommunity. (Illustration by M. Luo and M. Gelnar.)

Sampling of parasite communities

The assemblage consisting of all parasites of different species in the same host individual, whether they actually interact or not, forms an infracommunity (e.g., Esch *et al.* 1990; Bush *et al.* 1997). Infracommunities are subsets of the component community, which consists of all parasites exploiting the host population. In theory, infracommunities can range from highly structured and predictable sets of species, to purely stochastic assemblages of species coming together entirely at random (see Fig. 3.1.3 for a schematic illustration of parasite community structure).

Interactions among parasite species are one of the main forces that can shape infracommunity composition and structure and give it a non-random structure. In isolationist parasite communities, where interactions are negligible either because of very narrow niches or small infrapopulation sizes, the co-occurrence of species in hosts is not expected to deviate from that expected by chance (e.g., Esch *et al.* 1990; Esch & Fernández 1993; Rohde 2005; Poulin 2007).

Recommendations for parasite community sampling design

The vast majority of available studies on parasite community ecology are based on the examination of patterns observed in one or a few samples of host individuals, patterns existing among different infracommunities sampled at one point of time. These provide a snapshot of what the parasite infracommunities looked like at the time of sampling, but no information on their development through time, starting from the moment the first parasite arrived on a host. Very few investigations

have attempted a longitudinal survey of parasite infracommunities, beginning with uninfected hosts, either young individuals or animals reared in captivity, that were allowed to recruit parasites under natural conditions (e.g., Poulin 1996a,b; Poulin & Rohde 1997; Bagge & Valtonen 1999; Poulin & Valtonen 2002; Šimková *et al.* 2002, 2004; Vidal-Martínez & Poulin 2003). For hypothetical determinants of parasite community structure in real environmental conditions (see Fig. 3.1.2).

Collection of data

Parasitologists, like ecologists and other biologists, collect data to be used for testing hypotheses or describing nature. Modern science including parasitology proceeds by conjecture and refutation, by hypothesis and test, by ideas and data, and it also proceeds by obtaining good descriptions of ecological events. Parasitology like ecology is an empirical science that cannot be done solely on the blackboard or on the computer; it requires data from the real world. However, ecological data on parasites do not say everything about ecology of parasites.

Data represent only one half of this science; ecoparasitological hypotheses are the other half. Some evolutionary parasitologists even feel that hypotheses are more important than data themselves, while others argue the contrary. The central tenet of modern empirical science is that both are necessary. Hypotheses without data are not very useful, and data without hypotheses are wasted (e.g., Krebs 1999; Henderson 2003). One problem that all research fields face is: what to measure? So selection of good, relevant and correct data is essential for the study and understanding of ecological or parasitological systems.

Host fish as habitat and sampling unit

Selection of a suitable and proper habitat unit is among the key questions in sampling design in the ecology of free living animals. In the case of parasites, a host organism represents the environment colonised and inhabited by parasites and due to that host organism, infrapopulation and infracommunity or local host population, metapopulation and component community can be conceptually identical to the concept of habitat and sampling units for free-living animals, respectively (see Fig. 3.1.3).

At the outset, a scientist must be sure about the problem he/she is proposing to investigate. As it is normally impossible to count and identify all the animals in a habitat, it is necessary to estimate data on the population or community by sampling. Naturally, these estimates should have the highest possible accuracy in relation to the effort spent. This requires a plan that includes a sampling program stipulating the number of samples, their distribution and their size. For example, the number of hosts is typically seen as sufficient to characterise a population at a given point in time. The importance of careful formulation of hypotheses to be tested cannot be overstressed (e.g., Southwood & Henderson 2000; Sutherland 2006).

Sampling design and field work

In community studies, preliminary work should explore species richness and potential problems with species identification. The appropriate degree of taxonomic discrimination must be decided as it is important to maintain a consistent taxonomy. Sample sorting and species identification are often the most labour-intensive parts of a study and it may be useful to carry out a pilot trial to assess the effort required. Planning of the timing requires knowledge of life cycles. Preliminary work will be necessary to gain some knowledge of the occurrence of parasites to be studied.

The first decision concerns the scale of the environment to be sampled. A correct definition of the target population or community is essential: if too small, it may not produce results representative of the structure as a whole; if too large, it will waste resources. The second decision must be to define the accuracy or precision of the population estimates required. These decisions must be taken by considering both the objectives of the study and the variability of the system under study.

According to Henderson (2003), the following elements should be considered in any preliminary sampling design for populations of a host fish and for populations and communities of its parasite species.

- The need for sampling
- The scale of the study
- Safety
- Care for the environment and animal welfare
- Taxonomy
- Recording, labelling and noting down observations
- Data security and processing
- Effect of the time of year on sampling
- Effect of the time of day on sampling
- Size of population and community estimate
- Definition of the habitat unit
- Proper selection of unit area for sampling
- Subdivision of the habitat unit
- Statistical considerations

The selection of habitat and sampling unit for parasite ecology research

In general, the criteria for sample unit selection are, for parasites, broadly those of Morris (1955), where the term 'habitat unit' is identical with the term metapopulation of the parasites on a local metapopulation of host fish and the term 'sample unit' is identical with infrapopulation/infracommunity of fish parasites infecting the above mentioned metapopulation of host fish (e.g., Krebs 1999; Southwood & Henderson 2000; Henderson 2003).

- All units of the environment must have an equal chance of sampling.
- It must have environmental stability.
- The proportion of the population using the sample unit as a habitat must remain constant.
- The sampling unit must lend itself to conversion to unit areas.
- The sampling unit must be easily delineated in the field.
- The sampling unit should be of such a size as to provide a reasonable balance between the variance and the cost.
- The sampling unit must not be too small in relation to the animal's size, as this would have edge-effect errors.
- The sampling unit for mobile animals should approximate the average ambit of an individual.

Conclusions – Top 10 golden rules

- Not everything that can be measured should be.
- Find a problem and state your objective clearly.
- Collect data that will help achieve your objective and make a statistician happy.
- Some ecological questions are impossible to answer at the present time.
- With continuous data, save time and money by deciding on the number of significant Figures in the data before you start field work/an experiment.
- Never report an ecological estimate without some measure of its possible error.
- Be sceptical about the results of statistical tests of significance.
- Never confuse statistical significance with biological significance.
- Code all your ecological data and enter it on a computer in some machine-readable format.
- Garbage in, garbage out.

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