International Journal for Parasitology: Parasites and Wildlife 5 (2016) 145–157

Contents lists available at ScienceDirect

International Journal for Parasitology: Parasites and Wildlife

journal homepage: www.elsevier.com/locate/ijppaw

Comparison of sampling methodologies and estimation of population parameters for a temporary fish ectoparasite

J.M. Artim [a], P.C. Sikkel [a, b, *]

[a] Department of Biological Sciences and Environmental Sciences Program, Arkansas State University, State University, AR 72467, USA
[b] Water Research Group, Unit for Environmental Sciences and Management, North-West University, Potchefstroom 2520, South Africa

ARTICLE INFO

Article history:
Received 26 March 2016
Received in revised form
19 May 2016
Accepted 20 May 2016

Keywords:
Gnathiid
Tick
Mosquito
Micropredator
Vector
Coral reef
Blood-feeding
Sampling

ABSTRACT

Characterizing spatio-temporal variation in the density of organisms in a community is a crucial part of ecological study. However, doing so for small, motile, cryptic species presents multiple challenges, especially where multiple life history stages are involved. Gnathiid isopods are ecologically important marine ectoparasites, micropredators that live in substrate for most of their lives, emerging only once during each juvenile stage to feed on fish blood. Many gnathiid species are nocturnal and most have distinct substrate preferences. Studies of gnathiid use of habitat, exploitation of hosts, and population dynamics have used various trap designs to estimate rates of gnathiid emergence, study sensory ecology, and identify host susceptibility. In the studies reported here, we compare and contrast the performance of emergence, fish-baited and light trap designs, outline the key features of these traps, and determine some life cycle parameters derived from trap counts for the Eastern Caribbean coral-reef gnathiid, *Gnathia marleyi*. We also used counts from large emergence traps and light traps to estimate additional life cycle parameters, emergence rates, and total gnathiid density on substrate, and to calibrate the light trap design to provide estimates of rate of emergence and total gnathiid density in habitat not amenable to emergence trap deployment.

1. Introduction

A major challenge facing ecologists is the incorporation of parasitic organisms into ecological models of community and trophic dynamics (Hudson et al., 2006; Raffel et al., 2008; Lefèvre et al., 2009; Rudolf and Lafferty, 2011; Dunne et al., 2013; Poulin et al., 2014; Selakovic et al., 2014). A typical characteristic of parasites is that they are substantially smaller than their prey. While ecologists have decades of experience with methodologies characterizing community and trophic interactions of macro organisms, they have much less experience with methods characterizing interactions of small micropredators with their larger prey species.

For large organisms such as elk and wolves, methods focus on counting a substantial fraction of all organisms within a region—for example, the North American Yellowstone Basin (Evans et al., 2006; Vonholdt et al., 2007; Barber-Meyer et al., 2008). But for small organisms such as the ticks that infest them, the focus shifts to sampling small areas within the range and from those counts, estimating density as a function of habitat type and area or species co-occurrence (Lubelczyk et al., 2004; Tack et al., 2012). For ticks this is often done by dragging cloth across the study site to capture active ticks on the vegetative substrate in which they live. This approach is used to estimate potential fitness impacts from the spread of disease (Norman et al., 1999; Randolph, 2001; Curtis et al., 2013) or from loss of blood or hair especially for very young hosts (Grutter, 2008; Bergeron and Pekins, 2014).

A large portion of the parasite literature is devoted to determining sensitivity of detection of blood-feeding arthropods as part of disease prevention programs as with West Nile virus (Farajollahi et al., 2009) and Orbiviruses (Viennet et al., 2011). Multiple trap types have also been used to first characterize trap sensitivity, then further providing a baseline for comparison of seasonal and geographic counts of a mosquito vector of a livestock virus (Walker, 1977). Dobson et al. (2011) used trap characteristics of multiple drag-trap types to provide a range of estimates of actual density of the Lyme-disease tick on biotic substrate. Similarly, Weeks et al. (2000) used a combination of trapping by suction followed by dye marking, release, and subsequent retrapping by focused

* Corresponding author. Department of Biological Sciences and Environmental Sciences Program, Arkansas State University, State University, AR 72467, USA.
E-mail address: psikkel@astate.edu (P.C. Sikkel).

http://dx.doi.org/10.1016/j.ijppaw.2016.05.003

suction sampling and by substrate-core removal in a study estimating the ecologically-significant parameter, the rate of dispersal of crop mites (Acari: Penthaleidae, a plant parasite).

With a life history similar to ticks, gnathiid isopods ("ticks of the sea") are temporary blood parasites on fish hosts. The life cycle of gnathiid parasites includes three juvenile stages. Each juvenile stage has two states: a questing state—called a zuphea—which actively seeks and feeds on the blood of a fish host, and a fed state—called a praniza—which remains on benthic substrate until metamorphosis into the next life cycle stage. The third juvenile praniza stage metamorphs into non-feeding reproductive adults. These reproductive adults also remain on benthic substrate. Female gnathiids are ovigerous. For an overview of gnathiid biology see Smit and Davies (2004).

The family Gnathiidae is one of seven marine-parasitic families of the order Isopoda, see Smit et al. (2014). Gnathiids are found in almost all biogeographic zones (Poore and Bruce, 2012) especially temperate (Smit and Davies, 2004; Tanaka, 2007) and tropical seas (Smit and Basson, 2002; Farquharson et al., 2012a, 2012b). From an ecological standpoint, gnathiid-fish interactions in coral reef environments have received the most attention. Gnathiids on coral reefs appear to be host generalists (Jones et al., 2007; Nagel and Grutter, 2007; Coile and Sikkel, 2013) and are therefore highly connected within their communities (for a discussion of measures of connectivity see Ings et al., 2008). These gnathiids participate in cleaning symbioses as the major food item of cleaners (Losey, 1974; Cheney and Côté, 2003; Becker and Grutter, 2004; Clague et al., 2011; Waldie et al., 2011) and appear to influence the interaction between host and cleaners (Grutter, 1999a; Sikkel et al., 2004, 2005). In high numbers, gnathiids can reduce hematocrit and even kill adult fish (Jones and Grutter, 2005; Hayes et al., 2011). Gnathiids are implicated in the spread of potential disease-causing organisms, notably apicomplexan protozoa (Davies et al., 2004; Curtis et al., 2013). Gnathiids will also feed on settlement-stage reef fish, with as few as one gnathiid capable of causing mortality, and could thus constitute a potential selective pressure influencing choice of settlement habitat (Grutter et al., 2008; Penfold et al., 2008; Sun et al., 2012; Artim et al., 2015). This broad connectedness of coral-reef gnathiids with their associated fish community has led to a recent expansion of studies of gnathiid community interactions, including studies of habitat association (Grutter et al., 2000; Jones and Grutter, 2007; Artim and Sikkel, 2013), host-finding mechanisms (Nagel, 2009; Sikkel et al., 2011), and spatial and temporal patterns of emergence (Grutter and Hendrikz, 1999; Grutter et al., 2000; Chambers and Sikkel, 2002; Sikkel et al., 2006).

Emergence traps are one of the most common trap designs used to study gnathiid ecology (Chambers and Sikkel, 2002; Cheney and Côté, 2003; Jones and Grutter, 2007). They are used to quantify the density of gnathiids emerging from a fixed area of substrate and for a fixed time period. An emergence trap contains an area of substrate within a tent-like covering of plankton mesh (Jacoby and Greenwood, 1988). The apex of the trap is an upward-facing funnel acting as a one-way entrance into a sample container. When retrieved the sample, which includes a broad cross-section of small, motile benthic invertebrates, is scanned for gnathiids. The total number of gnathiids retrieved is compared with the sampling period and the area of substrate contained by the trap to determine the rate of emergence of gnathiids from that substrate, unbiased by host attractiveness.

While providing an absolute measure of the rate of emergence of gnathiid juveniles, emergence traps suffer two shortcomings: quantitative estimates are only valid when the trap circumference can be sealed and these traps mostly capture unfed juvenile gnathiids. One alternative is to use an open-mesh trap baited with a live fish host. Open-mesh fish-baited traps are simple enclosures, made of plastic or galvanized steel mesh, large enough to allow the bait-fish to turn around in and constructed of an open-weave material that freely passes seawater and parasites yet fine enough that the fish is unable to escape from the trap. These traps determine the relative gnathiid load and are typically used to assess gnathiid load across different habitat (Sikkel et al. in press) or portions of the diel cycle (Grutter, 1999b; Sikkel et al., 2006, 2009). Using open-mesh traps, proportions and total daily loads can be estimated by sampling throughout the diel cycle (Sikkel et al. in press). Fish-baited open-mesh traps are also used to determine relative susceptibility of different fish species to gnathiid micropredation (Coile and Sikkel, 2013; Sikkel et al., 2014).

Another variation of trap design is the fish-baited closed-tube design (Sikkel et al., 2011). These sealed traps have one-way funnel inlets that trap all gnathiids collected during the sampling period. As with fish-baited open-mesh traps, these closed-tube traps sample from an open area of substrate thus by themselves provide only relative rates of emergence.

Light traps have also been used to collect gnathiids (Jones et al., 2007; Hispano et al., 2013). Many motile invertebrates including gnathiid isopods are attracted to light sources at night. One typical implementation of this design features an inward-facing funnel and a light enclosed within the trap and shining out through the inlet funnel. Light traps similar to this are used to capture a wide variety of plankton including larval fish (Artim et al., 2015). Gnathiids and other "plankton" are attracted to the inlet by the interior light and are herded into the sample container by the funnel. This design is typically used in an open configuration that samples from an unlimited area of substrate, though closed configurations sampling from a fixed area of substrate are also practical. Light traps have the advantage in being compact, easy to deploy on or around uneven reef surfaces, and in not requiring the use of live fish as bait. Used in isolation, they suffer from the disadvantage of only providing relative emergence rate measurements. Different gnathiid species and even life cycle stages within a species may respond differently to photo stimulation, introducing count bias that must also be accounted for.

Attraction to light sources at night likely varies with the varied sensory ecology of different gnathiid species or developmental stages, and counts from light traps may or may not reflect rate of emergence. Gnathiid emergence occurs when gnathiid zuphea (unfed questing juveniles) are present and seeking hosts. Light sources at night attract a cross-section of the gnathiid life-cycle including not only zuphea but also pranizae (fed juvenile) and even the occasional adult male (Farquharson et al., 2012a; J M Artim personal observation).

There are some additional sampling techniques that should be considered. Suction trapping is an effective method of removing gnathiids and other small benthic invertebrates from substrate (Purcell, 1996; Kramer et al., 2012; Hispano et al., 2014; Wetzer, 2015). Unlit suction traps may reduce sampling bias due to sensory cues such as ambient light level. Another technique is to remove samples of substrate and immerse these in fresh or brackish water or an ethanol and water mixture to flush out gnathiids from the substrate sample (Wetzer, 2015). The effectiveness of both of these trapping approaches—that is, the proportion of gnathiids originally present on substrate before the sample was taken that are successfully removed—likely varies by substrate and gnathiid species, making these trapping approaches much more valuable in biodiversity surveys and less desirable for quantitative assessment. Long-term monitoring studies such as the Smithsonian's Tennenbaum Marine Observation Network (Lefcheck et al., 2016) also make use of flat-plate and stacked-plate (ARMS) collection methods to assess invertebrate diversity and abundance.