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

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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.

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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

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suction sampling and by substrate-core removal in a study estimating the ecologically-significant parameter, the rate of dispersal of crop mites (Acari: Penthalidae, 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 lngs 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 micro-predation (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.

While plate-collection approaches deploy on a considerably-longer time scale than the other methods described here, they are nonetheless powerful approaches to observing community balance and interaction.

Finally, screening of wild-caught fish has been used to estimate changes in intensity of micropredation in relation to time-of-day and host size (Grutter, 1999b; Sikkell et al., 2004; Soares et al., 2007). Fish are netted *in situ* and immediately isolated, for example by placing in a sealed plastic container full of seawater. The fish are transported to the laboratory, placed either in freshwater, seawater and clove oil solution, or simply retained in seawater for several hours until gnathiids complete feeding. All water is filtered and any gnathiids found are counted and measured.

To date, there is extensive collective experience with these many trap designs. Despite this experience, little has been done to compare the relative performance of these trap designs to overcome individual limitations of the traps.

In addition to estimates of overall population densities, an aspect of gnathiid ecology that has been little explored *in situ* are the various population parameters such as brood size or the time between feeding and ecdysis leading into the next life cycle stage. Such life cycle parameters are typically determined in laboratory culture (Grutter, 2003; Smit et al., 2003; Coile et al., 2014).

Here we report the results of a comparative study of multiple trap types, describing the performance characteristics of each. We further use counts from the various trap-designs to derive ecologically-significant gnathiid life cycle parameters.

2. Materials and methods

All work was performed at Virgin Islands Environmental Resource Station (VIERS) on Greater Lameshur Bay on St John, USVI (18°19'04.0"N 64°43'25.7"W). Lameshur Bay is a shallow south-facing bay featuring a mixture of patch reef, rocky rubble, sand flats, seagrass beds and adjacent shoreline mangrove. The multitrap comparison described in 2.1 was performed at Donkey Bight, a sheltered embayment within the larger bay with extensive *Orbicella faveolata* patch reef at its margins surrounded by seagrass bed and sandy bottom. The time-series emergence trap deployment described in 2.2 was performed 50 m south of the VIERS station dock in sand directly adjacent to patch reef. Areas of patch reef within Lameshur Bay feature live coral cover of under 5%, though historically live coral cover ranged up to 40%, particularly at the Donkey Bight site. Both of these sites have historically featured consistently high gnathiid counts. The gnathiid species present at this study site, *Gnathia marleyi* Farquharson et al., 2012b, is commonly found throughout the northeastern Caribbean and to date is the only species identified at this site (Farquharson et al., 2012b).

All fish-baited trap designs used in this study were baited with French grunt, *Heamulon flavolineatum* (Desmarest, 1823). Grunts approximately 150–200 mm SL were caught from Lameshur Bay or other nearby bays and temporarily kept in 600 L rectangular tanks continuously refreshed with seawater drawn from Lameshur Bay. Fish were fed daily until deployed in a trap after which they were released at point of capture. French grunt were chosen because of their relative abundance, susceptibility to gnathiid micropredation (Coile and Sikkell, 2013), and hardiness.

2.1. Multitrap comparison

Traps of five different designs (Fig. 1) were simultaneously set to compare gnathiid counts. All traps were set on similar substrate—sand adjacent to hard reef structure. The location of trap

sets on successive nights was shifted approximately 10 m to avoid sampling from overlapping areas of substrate on successive nights. Two of each of the five trap designs were set each night with the area of trap deployment roughly divided in two and one of each trap design deployed in each half. All traps were set between 15:00 and 17:00 and all traps other than the fish-baited open-mesh traps were retrieved the following afternoon in this same time slot. This included the dusk to dawn peak in gnathiid activity at this site. The open-mesh fish-baited traps were retrieved during the late-night peak in gnathiid activity as explained below. Trap contents were filtered using 160 µm plankton mesh and trap contents were inspected using dissection stereo-microscopes and all gnathiids counted. Immediately after retrieval, fish from the fish-baited open-mesh traps were placed in 20 L buckets half-filled with seawater and allowed to sit overnight so that all gnathiids finished feeding and dislodged from the fish. In the morning, each fish was removed from its bucket and the seawater was then filtered through 160 µm plankton mesh and gnathiids were counted as with other trap designs. All samples were collected in June and July of 2014.

Standard errors of mean counts by trap type and juvenile stage, ratios of mean counts, proportion of zero-count samples, and volume of blood/plasma bolus were estimated using 10,000 bootstrapping iterations. Trap counts were compared using a bootstrapped ANOVA procedure.

One difficulty in estimating ratios based on zero-inflated trap counts is that the denominator in the ratio statistically is likely to drop to zero for some proportion of the Monte Carlo simulations. To avoid divide-by-zero, we substitute an arbitrarily-high value of 1,000,000 for the ratio calculation for that simulation run. This substitution will not affect the confidence-interval estimation provided that the number of simulations that require this substitution does not exceed 250—a proportion of the 10,000 simulations total representing the top half of a 95% confidence interval.

Trap designs varied along two dimensions. The first dimension, method of attraction, included un-baited, fish-baited, and light-baited trap designs. The second dimension, the area sampled, included two trap designs—those with a fixed-collection-area and those that were open.

Fixed-collection-area traps included conventional (un-baited) and fish-baited emergence traps. The conventional emergence traps were 30 cm base diameter (0.707 m² base area) conical plankton-mesh traps with a 1L sample container featuring a one-way funnel entrance (see the electronic supplement for design details on all traps used in this study). The fish-baited emergence trap was based on the same 30 cm base diameter trap design but substituted a larger sample container with room for a live fish-host as bait. Both of these fixed-collection-area traps were limited in area-of-collection by the cone of plankton mesh that encloses the substrate beneath them and for the two closed-area traps used in this study, both cover the same area of substrate.

The open-area traps included the fish-baited tripod, fish-baited open-mesh, and light-trap designs. Open-area traps were limited in area of collection only by the maximum distance traversed by the gnathiids or by the maximum distance over which the trap's bait attracts gnathiids. The fish-baited tripod employs the same fish-baited sample container used in the fish-baited emergence trap and holds the sample container's one-way opening the same distance above substrate as in the fish-baited emergence trap but without the emergence cone that limits the area from which the trap draws. If the ratio of mean count from the tripod-mounted fish-baited trap to the fish-baited emergence trap exceeds 1.0, then the tripod-mounted fish-baited trap is drawing gnathiids from an area larger than the area of substrate under the fish-baited emergence trap and counts from the fish-baited emergence trap

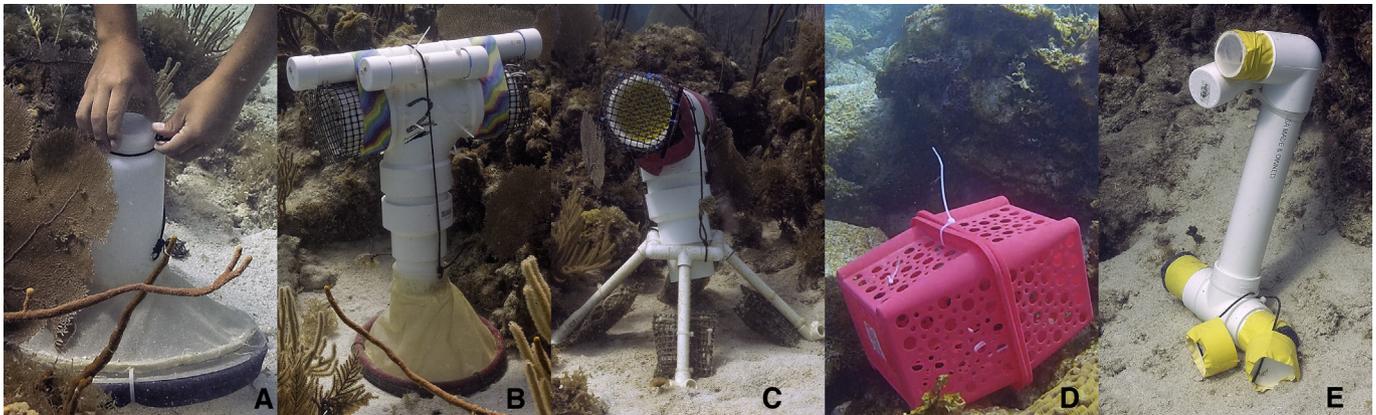


Fig. 1. Traps used in the first study. (A) Small emergence trap, (B) fish-baited emergence trap, (C) fish-baited tripod, (D) open-mesh fish-baited trap and (E) lighted plankton trap. Note that the sample container holding a small French grunt fish for the fish-baited emergence trap (B) and the fish-baited tripod trap (C) are identical units other than the sealed floats attached to the top of the sample container when used with the fish-baited emergence trap.

reflect maximum rate of emergence for the enclosed area of substrate (see Fig. 1C). The tripod-mounted fish-baited trap is a variation on the closed-tube design used by Sikkel et al. (2011) so the closed-tube design was omitted from this trap-design comparison.

The open-mesh fish-baited trap uses plastic mesh to hold a fish in place on substrate while allowing gnathiids to freely enter and leave the trap. This trap design must be retrieved at one of the peaks in gnathiid activity to provide good sensitivity. For this study, the open-mesh fish-baited traps were set along with other trap designs in the late afternoon and retrieved at 22:00 during the late-night peak (Sikkel et al., 2006). All other traps including the light trap whose description follows were set in the late afternoon and retrieved the following afternoon.

The light trap uses light to attract gnathiids and other small motile invertebrates that enter the trap through a one-way funnel opening. The opening for these traps was constructed using PVC T's with two small inward-facing funnels in the arms of the T each facing to the side as the trap rests on the benthos.

The ratio of the median counts from an open-area trap design to a fixed-area trap design provides an estimate of the mean maximum area from which the trap draws gnathiids. Secondly, this ratio can also be used to estimate the maximum distance traveled by gnathiids entering the open-area trap. This estimate is derived by taking the estimate of area of substrate that the open-area is drawing gnathiids from and calculating the maximum path length taken by a gnathiid from substrate into the open-area trap.

The area of the small fixed-area traps used in the first study (0.0707 m^2) and the maximum distance traveled within the fixed-area traps (28 cm) is known. The area from which the open-area trap samples draw gnathiids was estimated using the trap ratios. Using this calculated area, the maximum distance traveled was derived from this area. All traps except the lighted plankton trap were assumed to symmetrically draw from a circular sampling area centered around the trap—see Section 2.2 below.

The ratio of the counts from any of the open-area trap designs to one of the closed-area trap designs also provides a simple metric for the relative sensitivity of the open-area trap design. A comparison to the un-baited emergence trap design provides an estimate of the rate-of-emergence from the substrate.

2.1.1. Supplemental trap comparison

In order to derive an estimate of maximum distance traveled to reach the light trap, we conducted a supplemental trap comparison that compared counts from the previously described light traps

with counts from emergence traps to which we added the same marker lights as used in the light traps. This comparison was conducted immediately after the multitraps comparison was completed with data collected from the same site and over the same type of habitat (sand adjacent to hard reef surfaces). This provided counts from a fixed area of substrate that reflect differences in emergence rate induced by the sensory attraction affect of the light source. We used 10,000 bootstrapping iterations to estimate the ratio of the counts from these traps and the confidence interval for that ratio.

The maximum travel distance estimate assumes a circular sampling pattern, but because of the two outward facing inlet funnels of the light trap design used in this comparison, the pattern of attraction for this light trap design is likely similar to a figure-eight pattern, or more properly, a lemniscate of Bernoulli. The long axis of a lemniscate of Bernoulli pattern is approximately 1.77 times that of the radius of a circle of the same area. We use this correction to approximate maximum travel distance.

2.2. Exhaustive Trapping Study

Nine large emergence traps, each 73 cm in diameter (0.42 m^2), were set on sand abutting hard reef surfaces. These traps are similar to but larger in size than the un-baited emergence traps described in section 2.1 above but with the addition of a 20 cm impermeable coated-nylon skirt surrounding the plankton-mesh collecting cone. Emergence traps were left in position for 10 consecutive days of sampling. The 1 L sample bottle for each trap was removed and replaced once per day throughout the sampling period for a total of 10 samples per trap. All gnathiids found within the sample bottle were photographed on 2 mm grid paper using a Canon DSLR and 60 mm macro lens for later measurement. The 20 cm impermeable coated-nylon skirt surrounding the plankton-mesh collecting cone prevented escape of gnathiids from the trap area, incursion of gnathiids, fish hosts or predators into the trap enclosure, or current-induced gaps in the seal at the trap perimeter throughout the extended sampling period. The maximum distance a gnathiid must travel from substrate to enter the sample bottle was ~83 cm. The large emergence trap and lighted plankton trap designs used in this time-series study can be seen in Fig. 2.

Lighted plankton traps with large sample-retaining bodies and downward-facing trap openings were set adjacent to the emergence traps on similar reef-adjacent sand substrate. Light trap samples were retrieved at the same time that emergence sample bottles were changed and the traps rinsed and batteries refreshed before placing the traps at new locations adjacent to the emergence



Fig. 2. Traps used in the second study. The lighted plankton trap, in the left foreground, stands on short legs—four large emergence traps can be seen in the middle-ground to the right of the lighted plankton trap. A second lighted plankton trap in the background can be seen towards the center of the frame.

traps. Only two light traps were placed per night and these were placed adjacent to different emergence traps each night. The first light trap was set on the second day the emergence traps were set and this regime continued for 14 days—that is, for four days after the last emergence trap sample was retrieved. See Fig. 2.

Because light traps are heavily biased towards pranizae and emergence traps are heavily biased towards zuphea, to compare counts from light traps with counts from emergence traps, we combined counts of zuphea and pranizae for each stage. Confidence intervals for these counts and for the proportion of zero-count samples were estimated through 10,000 bootstrapping simulations. For *Gnathia marleyi*, the time between feeding and ecdysis is approximately 5 days for first stage gnathiids and 5–7 days for second and third-stage gnathiids (P. C. Sikkell, unpublished data). Once a third-stage gnathiid morphs into a reproductive adult, an additional 14–16 days elapse before the ovigorous female releases first-stage zuphea. To account for this life history and to better equate counts from the time-series emergence sampling and from the nightly light trap samples, we treated the first 5 days of emergence counts for second and third stage gnathiids as reflective of daily emergence rates while all 10 days of emergence counts were considered reflective of first stage gnathiid daily emergence rates.

For each trap sample, gnathiids were counted and photographed on 2 mm grid graph paper. Gnathiid images were later analyzed to measure these gnathiid parameters: total gnathiid length including the extremities of cephalon to telson, maximum body width, width from lateral edge of left eye to lateral edge of right eye, length of blood meal, and length of the long axis of the eye. These measurements were used to classify gnathiids as fed or unfed, to assign gnathiids to juvenile stage (first, second or third), and to estimate volume of blood and plasma in fed gnathiids. Measurements were made using imageJ (Abramoff et al., 2004).

Gnathiids counted in this study were measured and sorted into the three juvenile stages and two phases—zuphea and praniza. To distinguish praniza from zuphea we use the ratio of head width as measured across the eyes to body width as measured at the widest part of the body. Any juvenile whose head-to-body-width ratio was less than or equal to 0.80 was considered a praniza. If this ratio was greater than 0.80 the juvenile was considered a zuphea. Separate scatterplots of gnathiid measurements were used to determine the best morphometric parameter to use to classify gnathiids. Scatterplots of body length and eye length, the two most stage-distinctive morphometric parameters, along with body-length cutoffs by stage are shown in Fig. 3. The point clouds formed by zuphea

measurements are quite distinct with noticeable gaps in the dimension of body length at 1.05 mm between first and second stage zuphea and at 1.50 mm between second and third stage zuphea. The point clouds formed by pranizae measurements are less distinct, but the point clouds can be divided at 1.5 mm between first and second stage pranizae and at 2.2 mm between second and third stage pranizae.

Estimates of the volume of blood and plasma in fed gnathiids were calculated from the measurements of the maximum width of the praniza—used as the length of the minor axes of the blood/plasma bolus—and the length of the blood meal—the length of the major axis of the bolus. These lengths were combined using the formula for an ellipsoidal solid with the two minor dimensions of the ellipsoid equal to the body width at widest point and the major dimension of the ellipsoid equal to the blood meal length. This is the approach used by Grutter (2003, 2008) to estimate feeding volumes.

Gnathiids were stored in molecular-grade ethanol and frozen for a later study. Standard errors of mean counts by trap type and juvenile stage, ratios of mean counts, proportion of zero-count samples, and estimated volume of blood/plasma bolus were estimated using 10,000 bootstrapping iterations.

3. Results

3.1. Multitrap comparison

The median counts and estimates of the 95% confidence interval by trap type as derived by bootstrapping are listed in Table 1. For the un-baited emergence traps and fish-baited emergence and tripod traps, the median count of less than one reflects the substantial number of zero-count samples retrieved for these three trap designs. The proportion of zero-count samples was calculated for each bootstrap sample and the median and 95% confidence intervals are shown in Table 2. For the five trap designs, estimates of the proportion of zero-count samples ranges from 27% for the lighted-plankton traps to 85% for the un-baited-emergence traps.

The results for the bootstrapped ANOVA of the counts indicates at least one trap type was significantly different from other trap types, $F(1,4) = 6.398$, $p = 0.0369$. The ratio of counts of one trap design to another can also be estimated through bootstrapping simulation. The results of bootstrapping the ratio of trap design counts including estimates of the 95% confidence interval are summarized in Table 3. These ratio and confidence interval approximations provide an estimate of the range of variability of the trap counts of one trap design relative to another. We present the ratio of each trap designs' count relative to the un-baited emergence trap counts as well as select additional ratios. These ratios provide a metric of the relative sensitivity of the two designs. Where the ratio is approximately 1.0, both designs exhibit similar sensitivity. When the ratio greatly exceeds 1.0, the numerator design is more sensitive than the denominator (comparison) trap design. For these ratios, the numerator had a value of zero in fewer than 250 out of the 10,000 simulations—95% confidence intervals were therefore computable.

3.1.1. Estimates of distance travelled

Estimates of the maximum travel distance for open-area fish-baited tripod and open-mesh fish-baited traps are found in Table 4. Using the counts from the fish-baited tripod traps and comparing them to the counts from the fish-baited emergence traps, we estimate a maximum travel distance of 42 cm for gnathiids seeking the fish in the tripod trap. Sets from these two trap types are of equal duration making the counts directly equivalent. Comparing the counts from the open-mesh fish-baited traps with the fish-

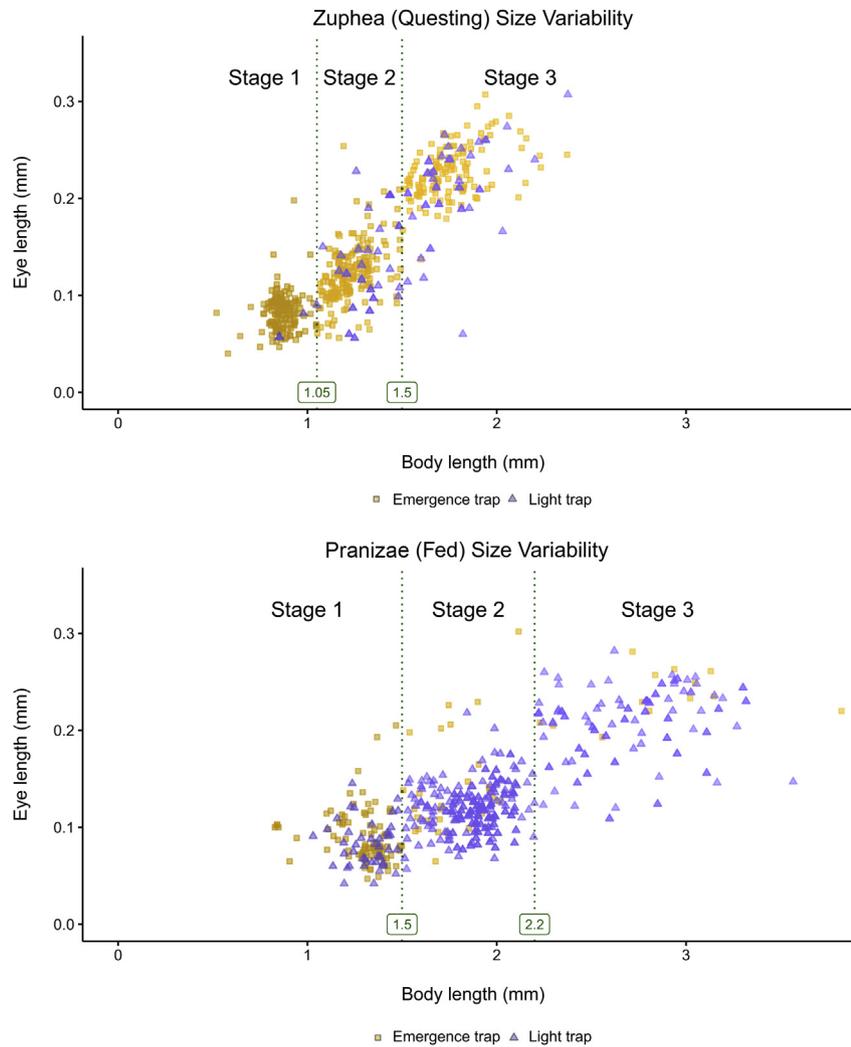


Fig. 3. Scatterplots of total body length in mm plotted against eye length in mm along the long axis. The upper plot shows measurements for *Zuphea* and the lower plot for *Pranizae*. The body length cutoff values separating juvenile stages are shown as a dotted-green line. Gnathiids collected from emergence traps are seen as gold-filled squares and those collected from light traps are presented as purple-filled triangles. Differences in the ontological sampling bias of these two trap designs can be seen by comparing the two scatterplots. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1

Estimates of median count per sample and 95% confidence intervals for each trap design evaluated in the multitrap comparison. Estimates derived from 10,000 bootstrapping iterations drawing with replacement from the 26 sample counts for each trap type.

Trap type	Median count	95% CI	
		Lower	Upper
Un-baited Emergence	0.31	0.04	0.81
Fish-baited Emergence	0.42	0.19	0.69
Fish-baited Tripod	0.92	0.46	1.46
Open-mesh Fish-baited	1.50	0.35	3.54
Lighted Plankton Trap	5.69	2.69	9.58

Table 2

Estimates of the proportion of zero count samples and 95% confidence limits based on the 10,000 bootstrap simulations drawn with replacement from sets of 26 sample counts for each trap type.

Trap type	Median proportion of zero counts	95% CI	
		Lower	Upper
Un-baited Emergence	0.85	0.69	0.96
Fish-baited Emergence	0.65	0.46	0.85
Fish-baited Tripod	0.58	0.38	0.77
Open-mesh Fish-baited	0.65	0.46	0.85
Lighted Plankton Trap	0.27	0.12	0.46

baited emergence trap, the uncorrected estimate for maximum travel distance was 29 cm for gnathiids seeking the fish surrounded only by the open mesh. But this last estimate does not take into account the shorter time period over which gnathiid load was accumulated (approximately 1 h versus 24 h for the emergence trap). Using the 24 h gnathiid load estimates presented in Sikkel et al. (in press), open-mesh traps retrieved at 22:00 represent about 27% of the total daily gnathiid load. Adjusting the open-mesh

travel distance estimates we get 104 cm.

3.1.2. Supplemental trap comparison

The ratio of light trap counts to lighted emergence trap counts was 0.84—see Table 3. This yields an estimated maximum travel distance of 13 cm. Applying the radius correction factor for a lemniscate of Bernoulli of 1.77, the estimate of the maximum travel distance for the lighted plankton trap was 23 cm. Note that this much shorter estimate reflects the light cone pattern for the trap

Table 3

Estimates of the ratio of counts and the estimates of standard error and 95% confidence interval based on 10,000 bootstrapping iterations drawing with replacement from the 26 sample counts for the respective trap types. In cases of divide-by-zero, a ratio of 1,000,000 was substituted for that individual simulation. Fish-baited emergence counts provide an estimate of emergence rate per square area for other trap types. The ratio of Lighted Plankton Trap counts to fish-baited open-mesh counts provides a metric for the relative sensitivity of these two trap designs. See text for discussion of inflation of upper-bounds estimates.

Count ratio	95% CI		
	Median ratio	Lower	Upper
Fish-baited Emergence to Un-baited Emergence	1.29	0.40	12.00
Fish-baited Tripod to Fish-baited Emergence	2.18	0.92	5.17
Open-mesh Fish-baited to Un-baited Emergence	4.75	0.80	47.01
Open-mesh Fish-baited to Fish-baited Emergence	3.63	0.79	11.25
Lighted Plankton Trap to Un-baited Emergence	17.70	5.29	160.00
Lighted Plankton Trap to Fish-baited Emergence	13.79	5.64	34.20
Lighted Plankton Trap to Fish-baited Open-mesh	3.78	1.23	17.33
Lighted Plankton Trap to Lighted Emergence	0.84	0.32	1.76

Table 4

Estimates of the maximum distance traveled by juvenile gnathiids to reach a fish-host. These estimates are discussed in sections 3.1.1 and 3.1.2. The estimates shown in boldface are adjusted values - see footnotes associated with those rows.

Estimate basis		Median maximum travel distance	95% confidence interval	
Open trap	Closed trap		Lower	Upper
Fish-baited Tripod	Fish-baited Emergence	0.42 m	0.34 m	0.55 m
Fish-baited Open-mesh	Fish-baited Emergence	0.29 m	0.13 m	0.50 m
Fish-baited Open-mesh	Fish-baited Emergence	1.04 m	0.47 m^a	1.80 m^a
Adjusted for 24 h emergence load^a				
Lighted Plankton Trap	Lighted Emergence Trap	0.13 m	0.09 m	0.20 m
Lighted Plankton Trap	Lighted Emergence Trap	0.23 m	0.16 m^b	0.35 m^b
Adjusted for light cone shape^b				

^a The proportional adjustment is derived from data presented in Sikkel et al. (in press)—see the text for details.

^b The shape of the light cone was taken to be a lemniscate of Bernoulli—see the text for details.

design and not a shorter estimate of actual distance traveled by gnathiids. The mean count from lighted emergence traps was 4.70 gnathiids per trap per night, considerably greater than the mean count of 0.31 gnathiids per trap per night for the unlit emergence traps reported in section 3.1.1. The mean counts for the light traps in both comparisons were similar—5.69 gnathiids per night per trap versus 3.67 gnathiids per night per trap. Counts from the lighted emergence trap included numerous pranizae while the unlit emergence trap primarily contained zuphea.

3.2. Time-series emergence study

Praniza body length versus estimated volume of blood and plasma is shown in Fig. 4. The body-length cutoff values shown in Fig. 3 and described in Material and Methods (Section 2.2, Exhaustive Trapping Study) were used to classify all gnathiids counted in this study. The resulting emergence trap counts by day-of-deployment for each juvenile state and stage are summarized as histograms in Fig. 5.

Combining counts of zuphea and pranizae by juvenile stage yielded 90 emergence samples for first-stage gnathiids (9 traps by 10 sample days) and 45 emergence samples each for second- and third-stage gnathiids (9 traps by 5 sample days) and 28 light trap samples for each gnathiid stage (2 traps by 14 sample days), see Fig. 6. These mean counts, proportion of zero-count samples, and confidence intervals are shown in Table 5.

Using these data from Table 5, we are able to calibrate the light trap counts. That is, we can use the ratio of the mean light trap count to the mean emergence trap count to provide a scale factor

used to estimate actual rate of emergence in the area immediately surrounding a light trap. Table 6 provides these scale factors, one

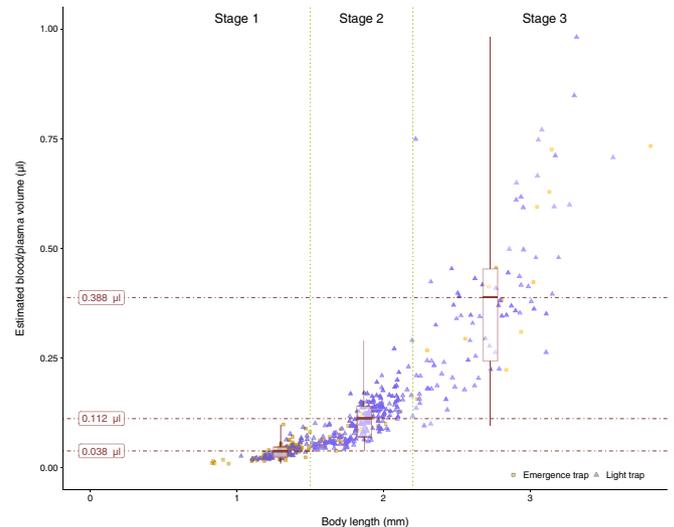


Fig. 4. Scatterplot showing total body length in mm versus estimated volume of blood and plasma extracted in µl. The box-and-whisker plots are centered on the mean body length for each of the three juvenile stages. The box edges are placed at the 2nd and 3rd quartiles for volume estimates and the whiskers show extreme minimum and maximum volumes. The mean estimate of extracted volume by juvenile stage is shown as a labeled dashed-red horizontal line. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

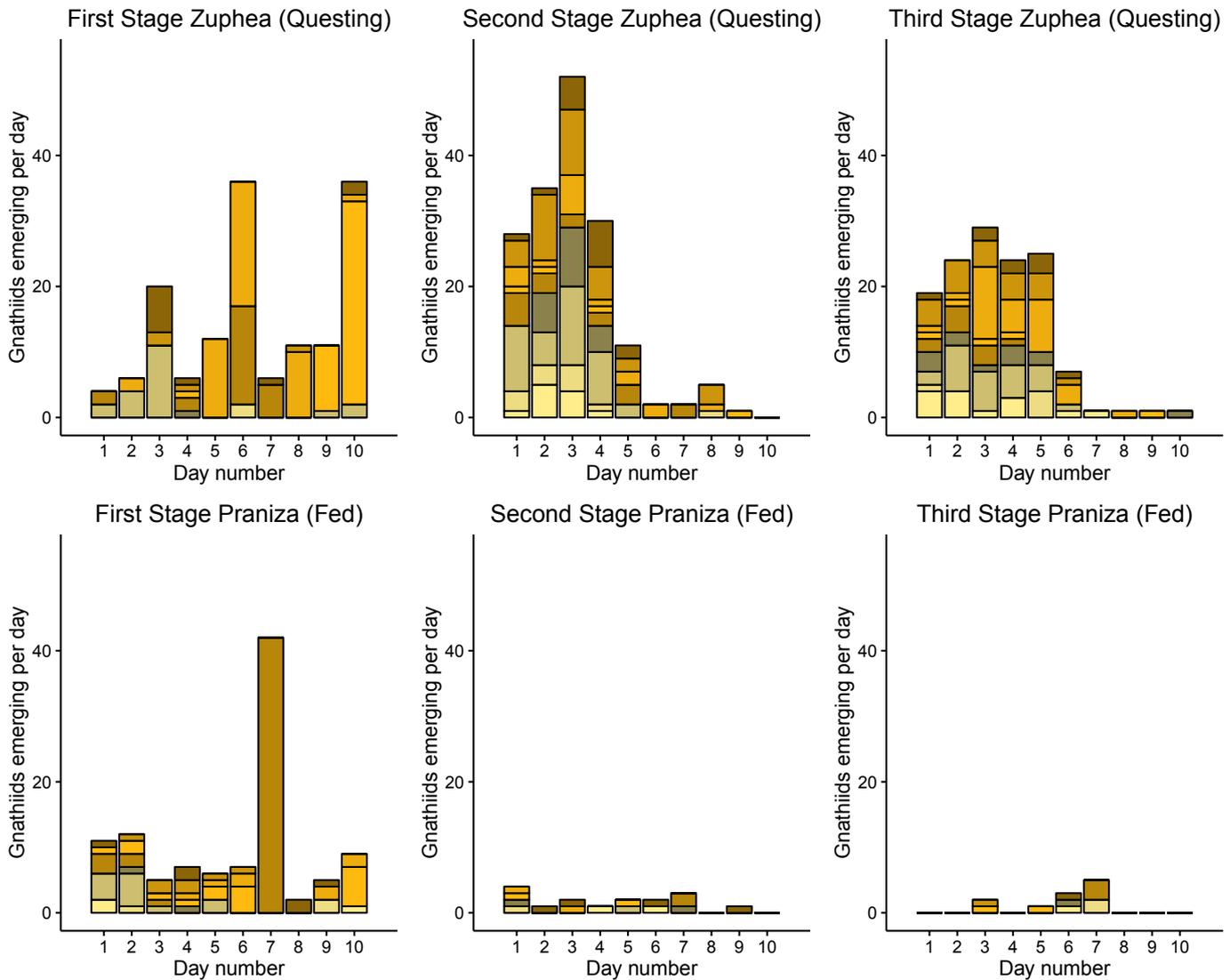


Fig. 5. Histograms of emergence counts from the time-series emergence traps. Count bars for each day are subdivided by individual trap.

for each gnathiid stage, as well as 95% confidence intervals.

From three of the time-series emergence samples we recovered a gnathiid attached to an invertebrate host. On one occasion a second-stage gnathiid was attached to a cumacean shrimp (Arthropoda: Cumacea), and on two occasions—once by two first-stage juveniles and once by a third-stage juvenile—gnathiiids were attached to planaria (Platyhelminthes: Maricola). We are aware of no other reports of apparent feeding by gnathiiids on invertebrate hosts.

4. Discussion

The work reported here compared and contrasted counts from six variations on emergence, light and fish-baited gnathiid trap designs to determine their relative sensitivity and to derive estimates of various ecologically-relevant gnathiid life-history parameters. We also examined temporal aspects of gnathiid emergence and measured additional gnathiid population parameters *in situ* using fixed-position emergence traps which, when sampled daily, provided a measure of temporal variability from the same area of substrate.

4.1. Trap design and sensitivity for ecological inquiry

The confidence intervals for the traps considered in the first study overlap, as seen in Table 1. Small emergence traps show the least sensitivity but emergence trap sensitivity was dependent on diameter of the trap, as seen in the larger emergence traps in the second study. There is, however, an upper limit on emergence trap size determined by gnathiid maximum travel distance. Gnathiid studies commonly deploy traps with 1 m bases (Chambers and Sikkel, 2002; Cheney and Côté, 2003; Jones and Grutter, 2007). If we assume a height of 0.75 m, we get a maximum travel distance of 1.2 m, just above our estimate of maximum gnathiid travel distance. Thus, for gnathiiids, increasing the size of an emergence trap much beyond 1 m across likely will not increase sensitivity and may yield an underestimate of true density.

The sensitivity of the fish-baited tripods and other closed fish-baited traps (see Sikkel et al., 2011) is likely limited by the diffusion of kairomones out of the enclosed fish-and-sample container. Larger fish will presumably emit greater quantities of kairomones but the higher metabolic demands of the larger fish in the enclosed space impose a trade-off of size of fish, period of trap deployment and overall sensitivity. This limit does not apply to open-mesh fish-

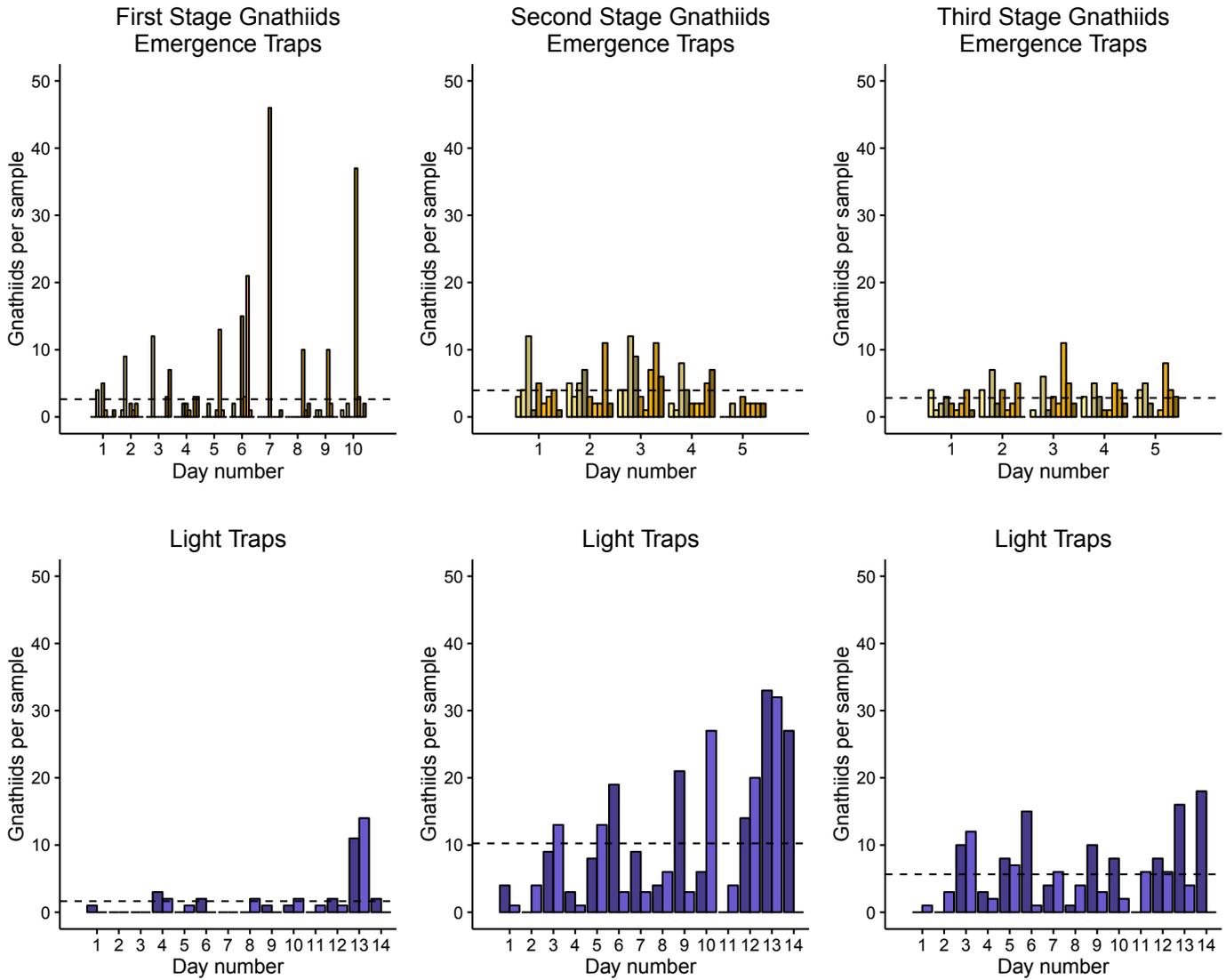


Fig. 6. Histograms of trap counts by sample day and juvenile stage. The upper histograms show counts from emergence traps and the lower histograms show counts from light traps. Mean count for each histogram is shown as a dashed horizontal line. See text for an explanation of the number of sampling days shown in each plot.

Table 5

Estimates of the range of sample counts by gnathiid stage for emergence and lighted plankton traps. A total of 10,000 bootstrap simulations were run. For each stage, counts of zuphea and pranizae were combined. For an explanation of sample size, see the text.

Trap type	Stage	Sample size	Mean count trap ⁻¹ day ⁻¹	95% lower	95% upper	Mean	95% lower	95% upper
Emergence	1	90	2.63	1.40	4.19	0.56	0.46	0.66
Emergence	2	45	3.96	3.09	4.89	0.07	0.00	0.16
Emergence	3	45	2.82	2.18	3.51	0.13	0.04	0.24
Emergence	All	45	9.41	6.69	10.36	0.02	0.00	0.07
Light Trap	1	28	1.64	0.68	2.96	0.46	0.29	0.64
Light Trap	2	28	10.25	6.71	14.04	0.11	0.00	0.21
Light Trap	3	28	5.64	3.89	7.54	0.14	0.04	0.29
Light Trap	All	28	17.53	11.93	23.71	0.11	0.00	0.21

baited traps, but there are practical limits to collection and use of large fish in these traps. Light trap sensitivity can be manipulated within limits by varying the brightness of the attracting light and the size of the light cone emitted, but these manipulations are constrained by the pragmatics of size of the sample container as well as water flow through it since increases in sensitivity translate to larger plankton volumes in the trap. Larger plankton volumes are more sensitive to the die-off of any one plankton species and also

greatly increase processing and counting effort.

A caveat concerning light trap design is that collection area is highly dependent on the specifics of the design. Jones and Grutter (2007) deployed light traps with upward facing inlet funnels to capture fed gnathiids returning to substrate. The light traps used in our first study employed side-facing inlets resting on the benthos. The design we used for the second study used a downward-facing inlet funnel whose light cone covered an area of substrate

Table 6

Estimates of the ratio and 95% confidence interval of light trap to emergence trap counts from the time-series emergence study normalized to 1 m². Dividing the count of the appropriate gnathiid stage from a light trap sample by the appropriate ratio from this table yields an approximation of gnathiid emergence rate for that gnathiid stage at the sampling location. The ratio and confidence interval estimates are based on 10,000 bootstrapping simulations.

Gnathiid stage	Ratio	95% lower	95% upper
1	0.25	0.10	0.62
2	1.09	0.68	1.64
3	0.84	0.54	1.24

approximately 40 cm in diameter. Because these various light trap designs vary considerably in the area of substrate from which they sample and because light sources vary in intensity, counts from each design must be individually calibrated.

One other light trap design caveat mentioned previously is that light traps to some degree attract gnathiids at all points in their life cycle while true emergence rate reflects only those gnathiids emerging to seek and feed on a host. Light trap counts can be used to estimate emergence rate only if the light trap design employed has been calibrated. Light traps can be calibrated against emergence traps in order to provide estimates of emergence rate.

The estimates of proportion of zero-count samples shown in Table 2 illustrate the relationship between trap sensitivity and ability to detect patchiness. There are two influences on the proportion of zero-count samples: (1) the true proportion of substrate from which the study animal is absent and (2) the sensitivity of the trap. Traps with very low sensitivity—that is, traps whose mean count is less than one, will have a very high proportion of zero-count samples. Only for the lighted plankton trap design is the 95% confidence limit for proportion of zero-count samples entirely under 0.5. For the other designs the high proportion of zero-count samples makes it very difficult for these traps to reveal patterns of spatial patchiness. Caution is therefore warranted when reporting the proportion of zero-count samples as a reflection of prevalence in cases where mean counts are near or below one.

Using multiple trap types to estimate organismal or community parameters is not limited to gnathiid isopods or to aquatic organisms. Mommertz et al. (1996) compared counts of soil-dwelling arthropods (Arthropoda) from suction traps and from fenced and unfenced pitfall traps to evaluate taxonomic bias in trap performance. They then used counts from fenced pitfall traps with their fixed area of collection to calibrate counts from unfenced pitfall traps to estimate actual emergence rates. This use of fenced and unfenced pitfall traps for calibration of counts has been validated using mark-recapture (Holland and Smith, 1999). Holland and Smith found linear relationships between counts for fixed- and open-area traps for many but not all taxa they examined.

The ecological study of epigeal terrestrial arthropods and of benthic/demersal aquatic arthropods both focus on habitat and community interactions on a surface. While the flightless epigeal arthropods such as Carabid beetles and Lycosid spiders are constrained to mostly two-dimensional interactions along the surface, many marine demersal arthropods—including gnathiids—travel and interact in a three-dimensional volume. While ecological analyses of organisms living in and on substrate focus on the surface where they live, these differences in motility must be kept in mind during study design and analysis.

4.2. Estimates of gnathiid life history parameters

Comparisons of counts from water, pitfall and malaise traps have been used by non-parasite ecologists to evaluate trap

taxonomic specificity for true flies (Arthropoda: Diptera) (Disney et al., 1982). Using a combination of fenced and unfenced pitfall traps and mark-recapture techniques, Holland and Smith (1999) provided *in situ* density measurements for individual species across various surface-dwelling arthropod taxa. By adding suction trapping, Mommertz et al. (1996) used multiple trap types to investigate taxonomic bias of the pit traps, then combined open-area and fixed-area pit traps to provide density measurements for a broad selection of surface-dwelling arthropod taxa while also providing encounter rates with other species. Mommertz et al. conclude by noting that common use of a trap design does not imply adequate information for interpreting counts from that common design advocating the use of trap designs in combination to determine taxonomic bias, area of coverage and other unknown trap parameters. All traps we deployed captured gnathiids and, depending on study goals, all could be appropriately used in certain circumstances.

Estimates of travel distance (Table 4) are based on the assumption that the fish-baited emergence trap is small enough in area that all available gnathiids will seek the fish host and enter the trap. The sensitivity of fish-baited traps varies depending on the type of fish used to bait the trap and the availability of other, possibly preferred, hosts in the area in which the trap is set. Bootstrapped confidence intervals provided limited evidence that counts in the fish-baited tripod exceeded those from the fish-baited emergence trap. Estimates of maximum distance traveled to seek a host fish, while preliminary, for the first time provide measurements of this critical gnathiid life-history parameter. However, this estimate of travel distance could and should be experimentally tested in the lab. Comparison of counts between trap types has been similarly used to estimate, in the field, life cycle parameters such as dispersal rates for other parasitic and predatory arthropods, for example mites (Acari: Penthalpidae; Weeks et al., 2000) and ladybugs (Coleoptera: Coccinellidae; van der Werf et al., 2000).

The estimates of mean volume of blood and plasma extracted by gnathiid stage presented in Fig. 4 are similar to estimates of fed volume by Grutter (2003, 2008) of 0.036 μ l for first stage, 0.218 μ l for second stage, and 1.122 μ l for third stage pranziae. Grutter measured engorgement volumes in the laboratory for the mix of gnathiids found on reefs adjacent to Lizard Island on the Great Barrier Reef, which are of a similar size to *G. marleyi*. Our field-derived estimates of extracted volume, when compared with those of Grutter, are somewhat lower for second- and third-stage juveniles. These differences may reflect the proportion of *in situ* gnathiids able to feed to capacity, the time elapsed post-feeding during which gnathiids excrete excess water (and so volume estimates decrease), or differences in species size and feeding volumes between the Pacific species and the Eastern Caribbean species considered here. The estimates of blood and plasma volume extracted by gnathiids provided by Grutter and those reported here are the only such estimates in the literature for this ecologically-important parameter.

Life history parameters are revealed in the time-series emergence data, as well. The five-day emergence time-course for second- and third-stage gnathiids seen in Fig. 6 is consistent with results of our laboratory culturing experience for *G. marleyi*. The full time-course for first-stage gnathiid emergence should be 21–23 days, with duration probably dependent on temperature and possibly host availability. Thus we would not expect to see a change in first-stage rate-of-emergence over the course of a 10-day time-series. The constant rate of emergence over the 10-day period is consistent with *G. marleyi* development as seen in laboratory culture. The large spikes in first-stage gnathiid counts seen in the emergence trap samples likely reflect the highly-synchronous release of broods of approximately 30 (10–70) zuphea from

individual female gnathiids (Coile et al., 2014). The histograms for first-stage emergence in Fig. 6 likely reflect 6–8 such events. Note that these large spikes of first-stage gnathiid release increase the confidence interval for first-stage counts.

By dividing the daily trap count estimates from Table 5 by the area of the emergence traps (0.42 m^2), we estimate a first-stage emergence rate of $6.2 \text{ gnathiids m}^{-2} \text{ night}^{-1}$, a second-stage emergence rate of $9.4 \text{ gnathiids m}^{-2} \text{ night}^{-1}$ and a third-stage emergence rate of $6.2 \text{ gnathiids m}^{-2} \text{ night}^{-1}$. This yields an estimate of total emergence rate of $22.4 \text{ gnathiids m}^{-2} \text{ night}^{-1}$ ($15.9\text{--}30.0$).

Using these nightly emergence rate estimates and the mean pranzia blood/plasma meal size by stage, we can estimate the total amount of fish blood and plasma extracted per square meter of substrate. For first-stage gnathiids the estimated total volume is $0.25 \text{ } \mu\text{l m}^{-2} \text{ night}^{-1}$ ($0.12\text{--}0.45$). For second-stage gnathiids the estimated total volume is $1.10 \text{ } \mu\text{l m}^{-2} \text{ night}^{-1}$ ($0.75\text{--}1.50$). For third-stage gnathiids the estimated total volume is $2.50 \text{ } \mu\text{l m}^{-2} \text{ night}^{-1}$ ($1.70\text{--}3.70$). In total, we estimate $3.90 \text{ } \mu\text{l}$ ($2.60\text{--}5.70$) of fish blood and plasma are extracted from fish per square meter of substrate every night.

By combining these calculations with our estimates of maximum travel distance, we can estimate the maximum impact to individual fish. Starting with the estimate of travel distance of 1.04 m ($0.47\text{--}1.80$), single fish will attract gnathiids from 3.40 m^2 ($0.69\text{--}10.18$). Combining the estimate of $3.90 \text{ } \mu\text{l}$ ($2.60\text{--}5.70$) of fish blood and plasma extracted from fish per square meter per night with this estimate of area from which gnathiids will be attracted we get an estimated maximum extraction of blood and plasma from individual fish of $13.26 \text{ } \mu\text{l}$ ($1.79\text{--}58.03$). This corresponds to the exsanguination of a juvenile Yellowtail damselfish with SL $\sim 23 \text{ mm}$ long (Marks and Klomp, 2003). More telling, this corresponds to a single-night micropredation by ~ 70 ($15\text{--}214$) gnathiids—a level of micropredation our lab has observed to be fatal in some adult *Stegastes* damselfish. The area of patch reef in west Great Lameshur Bay, where we conducted the second study, is $\sim 1000 \text{ m}^2$. On an average night this reef could support over 100 such events each and every night.

4.3. Assessing community interaction

Some trapping techniques, notably un-baited emergence traps, suction sampling and collection plates, are better suited to sampling across gnathiid species. Studies aimed at comparison of gnathiid species populations should make use of one or more of these trapping approaches to calibrate counts of different species against one another. Long-term monitoring efforts such as the Smithsonian's Tennenbaum Marine Monitoring Network (Lefcheck et al., 2016) aimed at tracking community balance within marine systems also present a unique opportunity to monitor gnathiid population dynamics relative to other community guilds.

5. Conclusions

Deploying multiple ectoparasite trap designs in combination can yield field measurements of ecologically-relevant parameters including estimates of travel distance and rates of emergence as well as provide a direct comparison of trap design performance for highly motile ectoparasites with benthic life history stages. Trap design and power analysis are complementary tools during study design and trap sensitivity must be considered when interpreting count data. Simply taking the proportion of non-zero-count substrate and reporting that figure as "prevalence" assumes that zero-count samples represent a true absence of the study organism when many of those zero-count samples simply reflect the

sensitivity and variability of the trap design employed.

The unexpected finding of gnathiids attached to invertebrates raises the intriguing possibility that gnathiids may be able to feed on invertebrates. This was observed in the middle of the emergence time-series sampling when zuphea might be expected to be running low on energy reserves and without fish hosts. The ability to shift host choice in response to contextual needs is well documented in mosquitos with the females of most mosquito species shifting between feeding on plant fluids and taking blood meals from animal hosts in response to as-yet incompletely-understood ecological forces on these haematophages (Stone and Foster, 2013; Takken and Verhulst, 2013). Follow-up study of this observation is warranted.

The role of micropredators (temporary ectoparasites) in communities and ecosystems remains understudied. Information derived from the comparison of counts from multiple trap designs can provide some of the ecological measurement needed to better integrate parasites into descriptions of community interaction and food webs.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.ijppaw.2016.05.003>.

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