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 ectoparasite, 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.

Note: Supplementary data associated with this article.

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

45 trap types have also been used to first characterize trap sensitivity, then further providing
46 a baseline for comparison of seasonal and geographic counts of a mosquito vector of a
47 livestock virus (Walker, 1977). Dobson et al. (2011) used trap characteristics of multiple
48 drag-trap types to provide a range of estimates of actual density of the Lyme-disease tick
49 on biotic substrate. Similarly, Weeks et al. (2000) used a combination of trapping by
50 suction followed by dye marking, release, and subsequent retrapping by focused suction
51 sampling and by substrate-core removal in a study estimating the ecologically-significant
52 parameter, the rate of dispersal of crop mites (Acari: Penthaleidae, a plant parasite).

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

61 The family Gnathiidae is one of seven marine-parasitic families of the order Isopoda,
62 see Smit et al. (2014). Gnathiids are found in almost all biogeographic zones (Poore and
63 Bruce, 2012) especially temperate (Smit and Davies 2004; Tanaka 2007) and tropical
64 seas (Smit and Basson 2002; Farquharson et al. 2012a, 2012b). From an ecological
65 standpoint, gnathiid-fish interactions in coral reef environments have received the most
66 attention. Gnathiids on coral reefs appear to be host generalists (Jones et al., 2007; Nagel
67 and Grutter, 2007; Coile and Sikkel, 2013) and are therefore highly connected within

68 their communities (for a discussion of measures of connectivity see Ings et al., 2008).
69 These gnathiids participate in cleaning symbioses as the major food item of cleaners
70 (Losey, 1974; Cheney and Côté, 2003; Becker and Grutter, 2004; Clague et al., 2011;
71 Waldie et al., 2011) and appear to influence the interaction between host and cleaners
72 (Grutter, 1999a; Sikkel et al. 2005, 2004). In high numbers, gnathiids can reduce
73 hematocrit and even kill adult fish (Jones and Grutter, 2005; Hayes et al., 2011).
74 Gnathiids are implicated in the spread of disease, notably apicomplexan protozoa (Davies
75 et al., 2004; Curtis et al., 2013). Gnathiids will also feed on settlement-stage reef fish,
76 with as few as one gnathiid capable of causing mortality, and could thus constitute a
77 potential selective pressure influencing choice of settlement habitat (Grutter et al., 2008;
78 Penfold et al., 2008; Sun et al., 2012; Artim et al., 2015). This broad connectedness of
79 coral-reef gnathiids with their associated fish community has led to a recent expansion of
80 studies of gnathiid community interactions, including studies of habitat association
81 (Grutter et al., 2000; Jones and Grutter, 2007; Artim and Sikkel, 2013), host-finding
82 mechanisms (Nagel, 2009; Sikkel et al., 2011), and spatial and temporal patterns of
83 emergence (Grutter and Hendrikz, 1999; Grutter et al., 2000; Chambers and Sikkel, 2002;
84 Sikkel et al., 2006).

85 Emergence traps are one of the most common trap designs used to study gnathiid
86 ecology (Chambers and Sikkel, 2002; Cheney and Côté, 2003; Jones and Grutter, 2007).
87 They are used to quantify the density of gnathiids emerging from a fixed area of substrate
88 and for a fixed time period. An emergence trap contains an area of substrate within a tent-
89 like covering of plankton mesh (Jacoby and Greenwood, 1988). The apex of the trap is an
90 upward-facing funnel acting as a one-way entrance into a sample container. When

91 retrieved the sample, which includes a broad cross-section of small, motile benthic
92 invertebrates, is scanned for gnathiids. The total number of gnathiids retrieved is
93 compared with the sampling period and the area of substrate contained by the trap to
94 determine the rate of emergence of gnathiids from that substrate, unbiased by host
95 attractiveness.

96 While providing an absolute measure of the rate of emergence of gnathiid juveniles,
97 emergence traps suffer two shortcomings: quantitative estimates are only valid when the
98 trap circumference can be sealed and these traps mostly capture unfed juvenile gnathiids.
99 One alternative is to use an open-mesh trap baited with a live fish host. Open-mesh fish-
100 baited traps are simple enclosures, made of plastic or galvanized steel mesh, large enough
101 to allow the bait-fish to turn around in and constructed of an open-weave material that
102 freely passes seawater and parasites yet fine enough that the fish is unable to escape from
103 the trap. These traps determine the relative gnathiid load and are typically used to assess
104 gnathiid load across different habitat (Sikkel et al. in review) or portions of the diel cycle
105 (Grutter, 1999b; Sikkel et al., 2006, 2009). Using open-mesh traps, proportions and total
106 daily loads can be estimated by sampling throughout the diel cycle (Sikkel et al. in
107 review). Fish-baited open-mesh traps are also used to determine relative susceptibility of
108 different fish species to gnathiid micropredation (Coile and Sikkel, 2013; Sikkel et al.,
109 2014).

110 Another variation of trap design is the fish-baited closed-tube design (Sikkel et al.,
111 2011). These sealed traps have one-way funnel inlets that trap all gnathiids collected
112 during the sampling period. As with fish-baited open-mesh traps, these closed-tube traps

113 sample from an open area of substrate thus by themselves provide only relative rates of
114 emergence.

115 Light traps have also been used to collect gnathiids (Jones et al., 2007; Hispano et al.,
116 2013). Many motile invertebrates including gnathiid parasites are attracted to light
117 sources at night. One typical implementation of this design features an inward-facing
118 funnel and a light enclosed within the trap and shining out through the inlet funnel. Light
119 traps similar to this are used to capture a wide variety of plankton including larval fish
120 (Artim et al., 2015). Gnathiids and other "plankton" are attracted to the inlet by the
121 interior light and are herded into the sample container by the funnel. This design is
122 typically used in an open configuration that samples from an unlimited area of substrate,
123 though closed configurations sampling from a fixed area of substrate are also practical.
124 Light traps have the advantage in being compact, easy to deploy on or around uneven reef
125 surfaces, and in not requiring the use of live fish as bait. Used in isolation, they suffer
126 from the disadvantage of only providing relative emergence rate measurements. Different
127 gnathiid species and even life cycle stages within a species may respond differently to
128 photo stimulation introducing count bias that must also be accounted for.

129 Attraction to light sources at night likely varies with the varied sensory ecology of
130 different gnathiid species or developmental stages, and counts from light traps may or
131 may not reflect rate of emergence. Gnathiid emergence occurs when gnathiid zuphea
132 (unfed questing juveniles) are present and seeking hosts. Light sources at night attract a
133 cross-section of the gnathiid life-cycle including not only zuphea but also praniza (fed
134 juvenile) and even the occasional adult male (Farquharson et al. 2012a; J M Artim
135 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, Sikkel 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, Smit & Sikkel, 2012, is commonly found throughout the Eastern 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 Sikkel, 2013), and hardiness.

2.1 Multitrap Comparison

Traps of five different designs (Figure 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.

225 Fixed-collection-area traps included conventional (un-baited) and fish-baited
226 emergence traps. The conventional emergence traps were 30 cm base diameter (0.707 m^2
227 base area) conical plankton-mesh traps with a 1L sample container featuring a one-way
228 funnel entrance (see the electronic supplement for design details on all traps used in this
229 study). The fish-baited emergence trap was based on the same 30 cm base diameter trap
230 design but substituted a larger sample container with room for a live fish-host as bait.
231 Both of these fixed-collection-area traps were limited in area-of-collection by the cone of
232 plankton mesh that encloses the substrate beneath them and for the two closed-area traps
233 used in this study, both cover the same area of substrate.

234 The open-area traps included the fish-baited tripod, fish-baited open-mesh, and light-
235 trap designs. Open-area traps were limited in area of collection only by the maximum
236 distance traversed by the gnathiids or by the maximum distance over which the trap's bait
237 attracts gnathiids. The fish-baited tripod employs the same fish-baited sample container
238 used in the fish-baited emergence trap and holds the sample container's one-way opening
239 the same distance above substrate as in the fish-baited emergence trap but without the
240 emergence cone that limits the area from which the trap draws. If the ratio of mean count
241 from the tripod-mounted fish-baited trap to the fish-baited emergence trap exceeds 1.0,
242 then the tripod-mounted fish-baited trap is drawing gnathiids from an area larger than the
243 area of substrate under the fish-baited emergence trap and counts from the fish-baited
244 emergence trap reflect maximum rate of emergence for the enclosed area of substrate (see
245 Figure 1C). The tripod-mounted fish-baited trap is a variation on the closed-tube design
246 used by Sikkel et al., (2011) so the closed-tube design was omitted from this trap-design
247 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. Secondarily, 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 provide 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 multitrap 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 Figure 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 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

314 days—that is, for four days after the last emergence trap sample was retrieved. See Figure
315 2.

316 Because light traps are heavily biased towards praniza and emergence traps are
317 heavily biased towards zuphea, to compare counts from light traps with counts from
318 emergence traps, we combined counts of zuphea and praniza for each stage. Confidence
319 intervals for these counts and for the proportion of zero-count samples were estimated
320 through 10,000 bootstrapping simulations. For *G. marley*, the time between feeding and
321 ecdysis is approximately 5 days for first stage gnathiids and 5-7 days for second and
322 third-stage gnathiids (Sikkel, pers. comm.). Once a third-stage gnathiid morphs into a
323 reproductive adult, an additional 14-16 days elapse before the ovigorous female releases
324 first-stage zuphea. To account for this life history and to better equate counts from the
325 time-series emergence sampling and from the nightly light trap samples, we treated the
326 first 5 days of emergence counts for second and third stage gnathiids as reflective of daily
327 emergence counts while all 10 days of emergence counts were considered reflective of
328 first stage gnathiid daily emergence rates.

329 For each trap sample, gnathiids were counted and photographed on 2 mm grid graph
330 paper. Gnathiid images were later analyzed to measure these gnathiid parameters: total
331 gnathiid length including the extremities of cephalon to telson, maximum body width,
332 width from lateral edge of left eye to lateral edge of right eye, length of blood meal, and
333 length of the long axis of the eye. These measurements were used to classify gnathiids as
334 fed or unfed, to assign gnathiids to juvenile stage (first, second or third), and to estimate
335 volume of blood and plasma in fed gnathiids. Measurements were made using imageJ
336 (Abràmoff 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 Figure 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 praniza measurements are less distinct, but the point clouds can be divided at 1.5 mm between first and second stage praniza and at 2.2 mm between second and third stage praniza.

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 that 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-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 hour versus 24 hours for the emergence trap). Using the 24 h gnathiid load estimates presented in Sikkel et al. (unpublished data), 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 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 praniza 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 Figure 4. The body-length cutoff values shown in Figure 3 and described in Material and Methods (section 2.3, 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 Figure 5.

Combining counts of zuphea and praniza 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 Figure 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 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—gnathiids were attached to planaria (Platyhelminthes: Maricola). We are aware of no other reports of apparent feeding by gnathiids 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 examinee 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

447 second study. There is, however, an upper limit on emergence trap size determined by
448 gnathiid maximum travel distance. Gnathiid studies commonly deploy traps with 1 m by
449 1 m bases (Chambers and Sikkel, 2002; Cheney and Côté, 2003; Jones and Grutter,
450 2007). If we assume a height of 0.75 m, we get a maximum travel distance of 1.2 m, just
451 above our estimate of maximum gnathiid travel distance. Thus, for gnathiids, increasing
452 the size of an emergence trap much beyond 1 m across likely will not increase sensitivity
453 and may yield an underestimate of true density.

454 The sensitivity of the fish-baited tripods and other closed fish-baited traps (see Sikkel
455 et al., 2011) is likely limited by the diffusion of kairomones out of the enclosed fish-and-
456 sample container. Larger fish will presumably emit greater quantities of kairomones but
457 the higher metabolic demands of the larger fish in the enclosed space impose a trade-off
458 of size of fish, period of trap deployment and overall sensitivity. This limit does not apply
459 to open-mesh fish-baited traps, but there are practical limits to collection and use of large
460 fish in these traps. Light trap sensitivity can be manipulated within limits by varying the
461 brightness of the attracting light and the size of the light cone emitted, but these
462 manipulations are constrained by the pragmatics of size of the sample container as well as
463 water flow through it since increases in sensitivity translate to larger plankton volumes in
464 the trap. Larger plankton volumes are more sensitive to the die-off of any one plankton
465 species and also greatly increase processing and counting effort.

466 A caveat concerning light trap design is that collection area is highly dependent on
467 the specifics of the design. Jones and Grutter (2007) deployed light traps with upward
468 facing inlet funnels to capture fed gnathiids returning to substrate. The light traps used in
469 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 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 make 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 taxanomic 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

514 adding suction trapping, Mommertz et al. (1996) used multiple trap types to investigate
515 taxonomic bias of the pit traps, then combined open-area and fixed-area pit traps to
516 provide density measurements for a broad selection of surface-dwelling arthropod taxa
517 while also providing encounter rates with other species. Mommertz et al. conclude by
518 noting that common use of a trap design does not imply adequate information for
519 interpreting counts from that common design advocating the use of trap designs in
520 combination to determine taxonomic bias, area of coverage and other unknown trap
521 parameters. All traps we deployed captured gnathiids and, depending on study goals, all
522 could be appropriately used in certain circumstances.

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

536 The estimates of mean volume of blood and plasma extracted by gnathiid stage
537 presented in Figure 4 are similar to estimates of fed volume by Grutter (2003, 2008) of
538 0.036 µl for first stage, 0.218 µl for second stage, and 1.122 µl for first third stage
539 praniza. Grutter measured engorgement volumes in the laboratory for the mix of
540 gnathiids found on reefs adjacent to Lizard Island on the Great Barrier Reef, which are of
541 a similar size to *G. marleyi*. Our field-derived estimates of extracted volume, when
542 compared with those of Grutter, are somewhat lower for second- and third-stage
543 juveniles. These differences may reflect the proportion of *in situ* gnathiids able to feed to
544 capacity, the time elapsed post-feeding during which gnathiids excrete excess water (and
545 so volume estimates decrease), or differences in species size and feeding volumes
546 between the Pacific species and the Eastern Caribbean species considered here. The
547 estimates of blood and plasma volume extracted by gnathhids provided by Grutter and
548 those reported here are the only such estimates in the literature for this ecologically-
549 important parameter.

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

559 of broods of approximately 30 (10-70) zuphea from individual female gnathiids (Coile et
560 al., 2014). The histograms for first-stage emergence in Figure 6 likely reflect 6 - 8 such
561 events. Note that these large spikes of first-stage gnathiid release increase the confidence
562 interval for first-stage counts.

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

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

575 By combining these calculations with our estimates of maximum travel distance, we
576 can estimate the maximum impact to individual fish. Starting with the estimate of travel
577 distance of 1.04 m (0.47 – 1.80), single fish will attract gnathiids from 3.40 m^2 (0.69 –
578 10.18). Combining the estimate of 3.90 µl (2.60 – 5.70) of fish blood and plasma
579 extracted from fish per square meter per night with this estimate of area from which
580 gnathiids will be attracted we get an estimated maximum extraction of blood and plasma

from individual fish of 13.26 µl (1.79 – 58.03). This corresponds to the exsanguination of a juvenile Yellowtail damselfish with SL ~23 mm long (Marks and Klomp, 2003). More telling, this corresponds to a single-night micropredation by ~70 (15 – 214) gnathiids—a level of micropredation our lab has observed to be fatal in damselfish. The area of patch reef in west Great Lameshur Bay, where we conducted the second study, is ~1000 m^2. On an average night this reef could support ~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

603 substrate and reporting that figure as “prevalence” assumes that zero-count samples
604 represent a true absence of the study organism when many of those zero-count samples
605 simply reflect the sensitivity and variability of the trap design employed.

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

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

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839

840 **Table 1. Estimates of median count per sample and 95% confidence intervals for each trap design evaluated**
841 **in the multitrap comparison. Estimates derived from 10,000 bootstrapping iterations drawing with replacement**
842 **from the 26 sample counts for each trap type.**

Trap Type	Median Count	95% CI Lower	95% CI 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

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844 **Table 2. Estimates of the proportion of zero count samples and 95% confidence limits based on the 10,000**
845 **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	95% CI 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

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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	Median Ratio	95% CI Lower	95% CI 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

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862 **Table 4. Estimates of the maximum distance traveled by juvenile gnathiids to reach a fish-host. These**

863 **estimates are discussed in sections 3.1.1 and 3.1.2.**

Estimate Basis		Median	95% Confidence Interval	
Open Trap	**Closed Trap**	**Maximum Travel Distance**	**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***	**1.80 m***
Adjusted for 24 hr emergence load*				
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**	**0.35 m**
Adjusted for light cone shape†				

* The proportional adjustment is derived from data presented in Sikkel et al. (in press)—see the text for details.
† The shape of the light cone was taken to be a lemniscate of Bernoulli—see the text for details.

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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 praniza 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

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 m^2. 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

884

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

890

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

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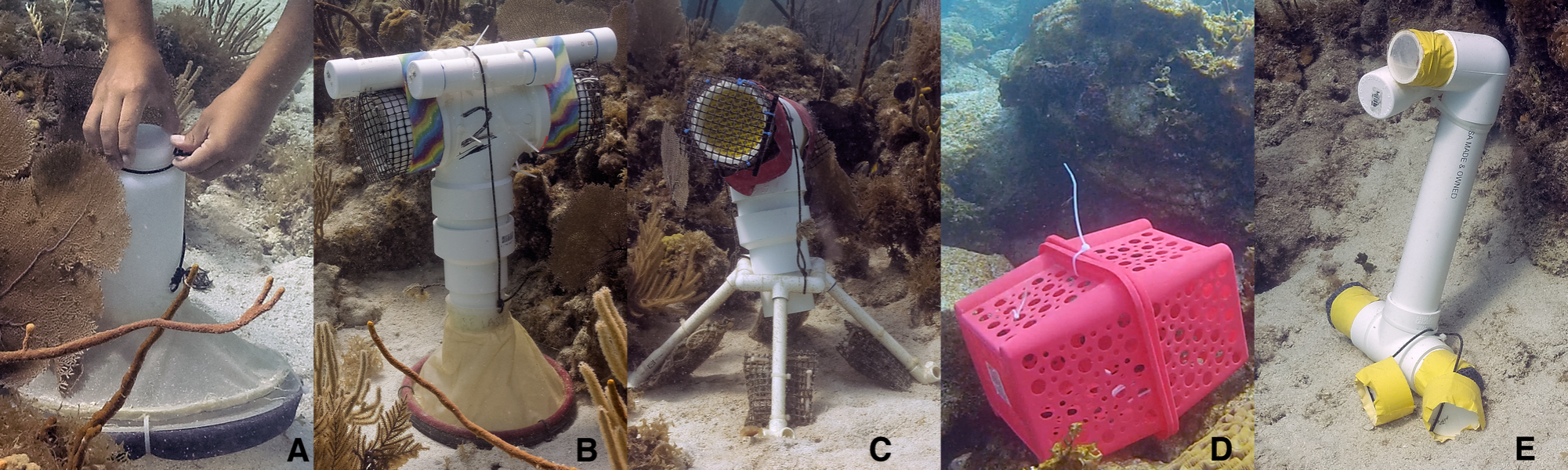
Figure 3. Scatterplots of total body length in mm plotted against eye length in mm along the long axis. The upper plot shows measurements for zuphea and the lower plot for praniza. The body length cutoff values separating juvenile stages are shown as a dotted-green line. Gnathiids collected from emergence 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.

Figure 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.

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

Figure 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.


A
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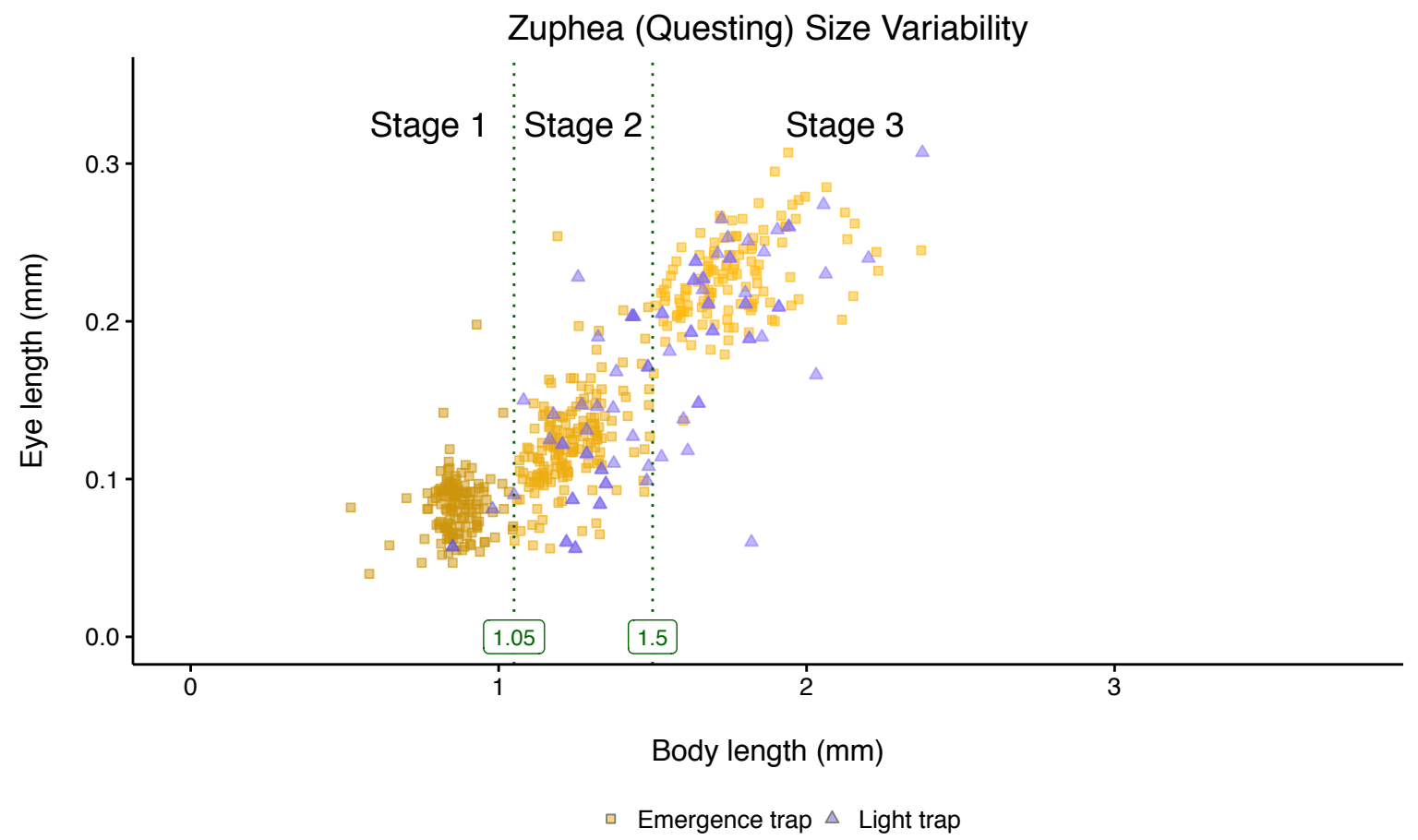
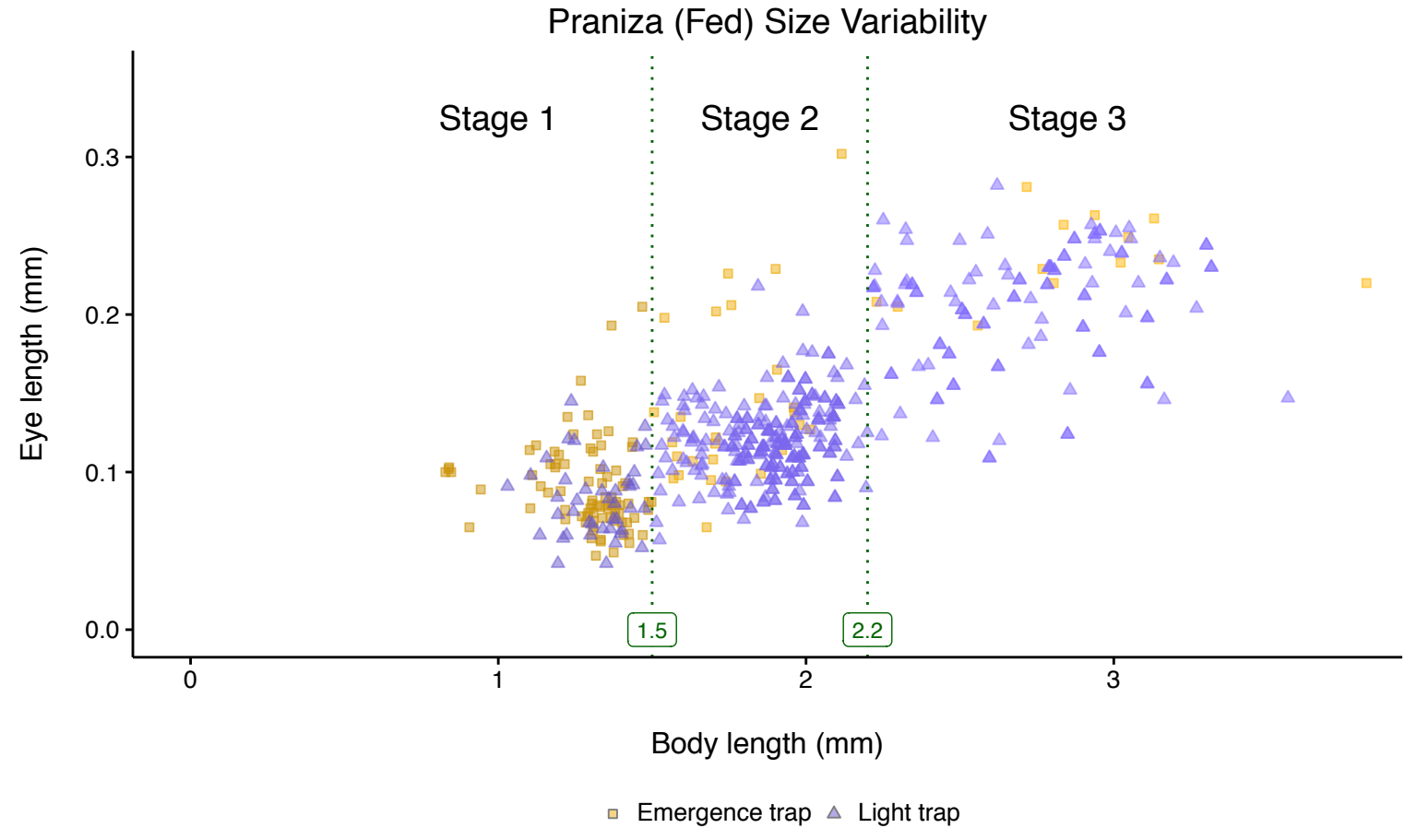
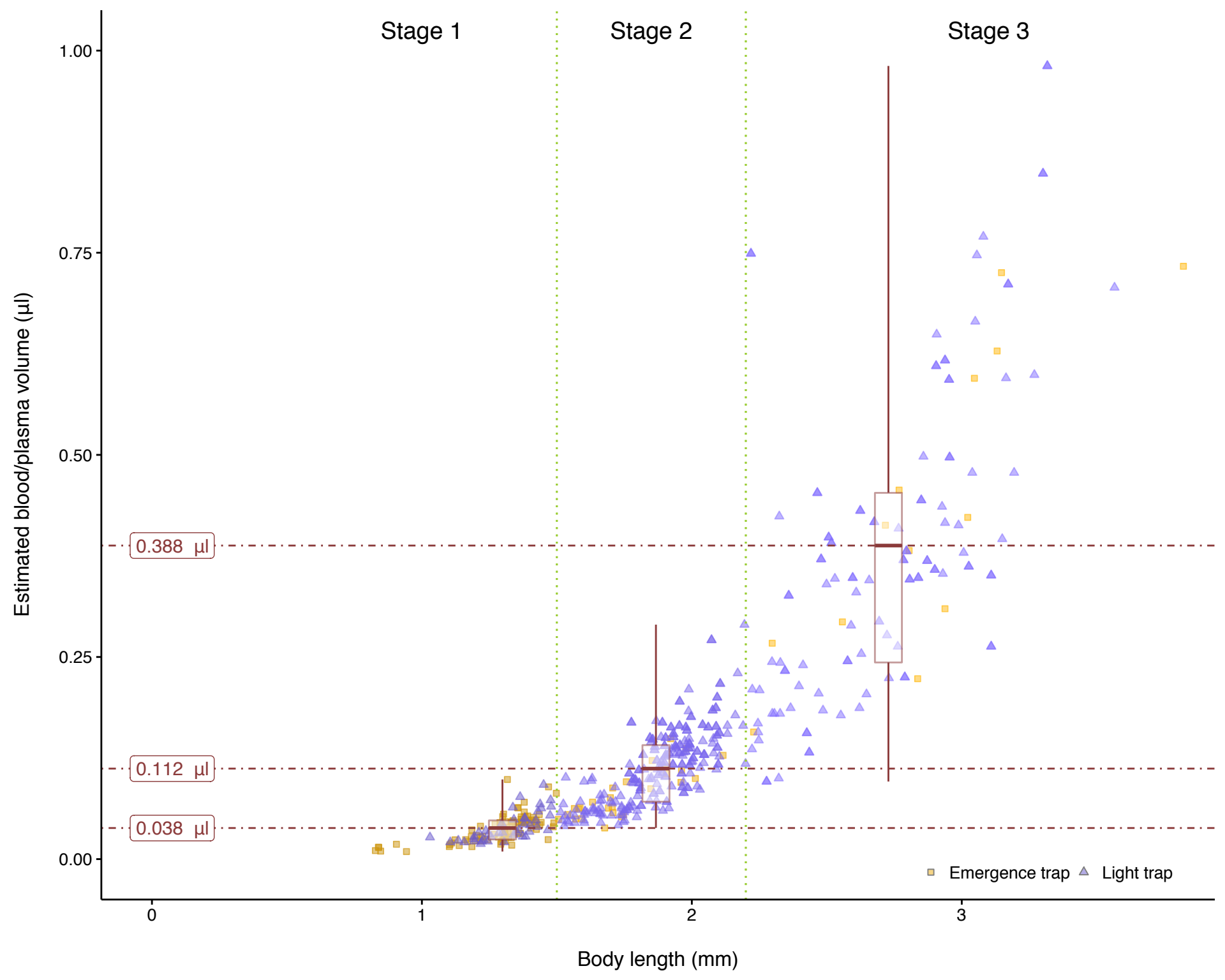
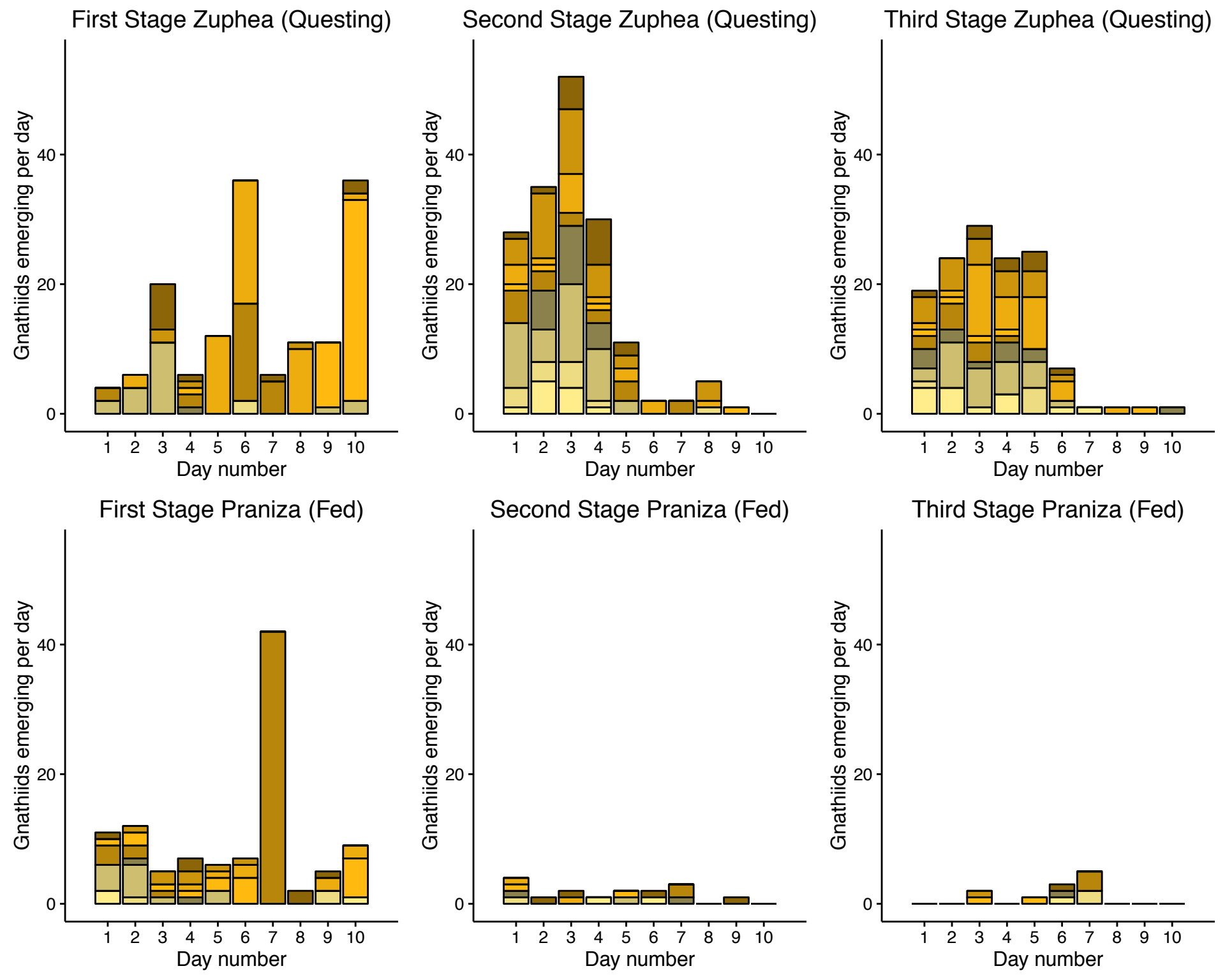
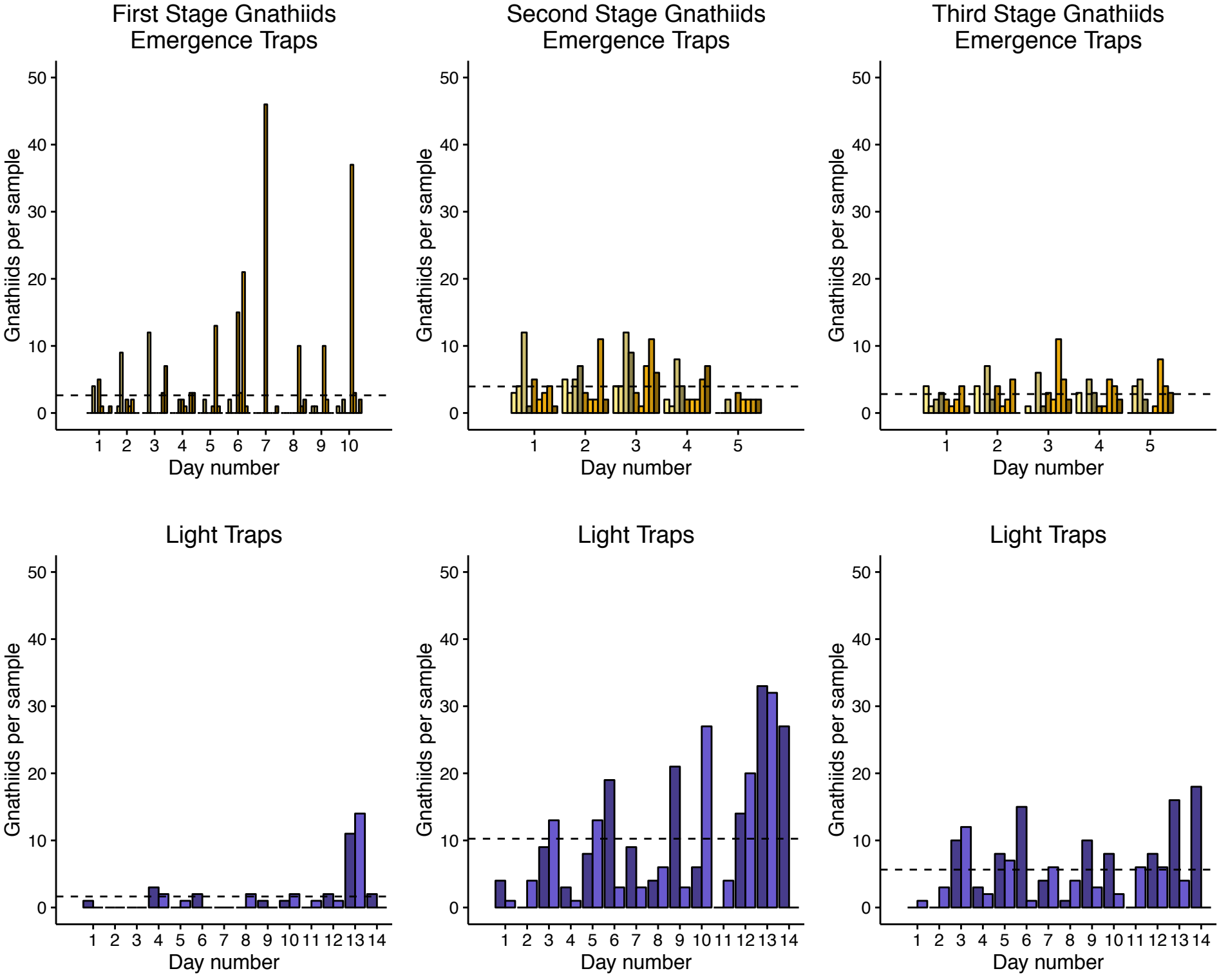
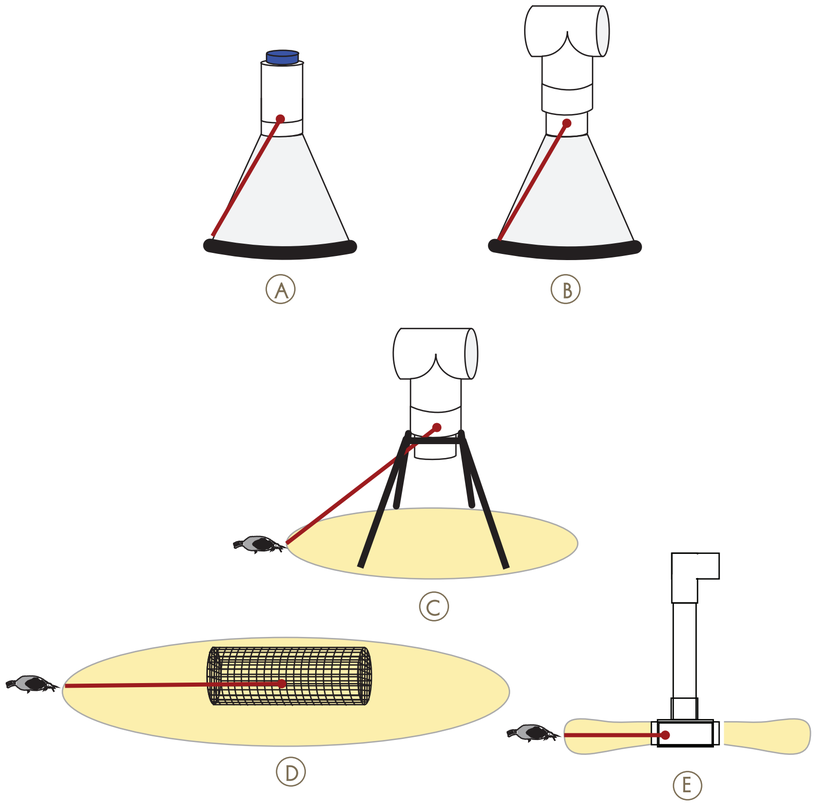

Zuphea (Questing) Size Variability
Stage 1
Stage 2
Stage 3
Eye length (mm)
0.0
0.1
0.2
0.3
1.05
1.5
0
1
2
3
Body length (mm)
Emergence trap
Light trap


Praniza (Fed) Size Variability
Stage 1
Stage 2
Stage 3
Eye length (mm)
0.0
0.1
0.2
0.3
1.5
2.2
0
1
2
3
Body length (mm)
Emergence trap
Light trap

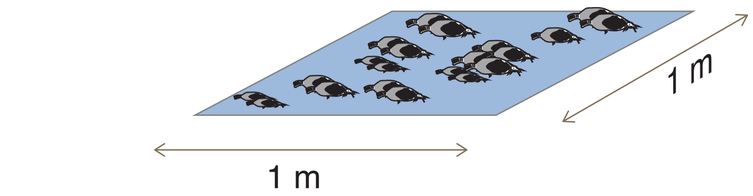

Stage 1
Stage 2
Stage 3
1.00
0.75
0.50
0.25
0.00
Estimated blood/plasma volume (µl)
0.388 µl
0.112 µl
0.038 µl
Emergence trap
Light trap
0
1
2
3
Body length (mm)


First Stage Zuphea (Questing)
Second Stage Zuphea (Questing)
Third Stage Zuphea (Questing)
First Stage Praniza (Fed)
Second Stage Praniza (Fed)
Third Stage Praniza (Fed)
Gnathiids emerging per day
Day number
0
20
40
1 2 3 4 5 6 7 8 9 10


First Stage Gnathiids
Emergence Traps
Second Stage Gnathiids
Emergence Traps
Third Stage Gnathiids
Emergence Traps
Light Traps
Light Traps
Light Traps
Gnathiids per sample
Day number
0
10
20
30
40
50
1 2 3 4 5 6 7 8 9 10
1 2 3 4 5
1 2 3 4 5 6 7 8 9 10 11 12 13 14



Gnathiid Travel Distance



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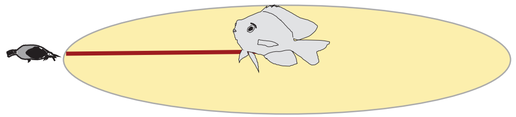

Fish Micropredation Load