

Clownfish metapopulation persistence draft

Introduction

Paragraph 1: Long been a challenge to understand how marine metapopulations -
3 patchy, interconnected populations - are connected and how they persist. Partic-
ularly challenging in the marine environment, where much movement happens at
the larval stage - high mortality, hard to tag/track, can go far b/c of currents. Of
6 key interest, though - both just because we want to understand population dynam-
ics, persistence, and connectivity for basic ecological reasons, and for more applied
reasons, like conservation (establishment of marine protected areas), understanding
9 where to fish.

Lots of theory has been developed about how we would expect populations largely
connected by larval movement to persist, which essentially boils down to replacement.
12 To understand persistence, we need to know 1) whether we see replacement and 2)
the origins of that replacement - self or other patches? Define and distinguish self
persistence vs network persistence. Hastings and Botsford 2006 papers, Bostford
15 2009, some of the White papers (2010?), Burgess et al. 2014. Walk through the

theory, our expectations for persistence, and what we need to know. Might take more than one paragraph.

18 Testing/validating/demonstrating that theory empirically has been challenging
but new technologies in tagging and genetics are making it more feasible. There
have been some recent successes. Cite Johnson et al., Salles et al. the recent paper
21 by someone Will works with (Garavelli or something?). Go through (briefly) what
they did and found.

 We continue this work with our long-ish term data set of clownfish. Like Salles
24 (check) and Johnson (check), we use a tropical reef species blablalbah (maybe study
system description goes in next paragraph). Talk about how we have longer-term
data set (check that this is actually true...) so we can capture longer-term replace-
27 ment, smooth out some of the year-to-year variability. Many years of sampling lets
us assess pop persistence in two ways: 1) from estimated abundance trends and 2)
estimating replacement and persistence metrics. Also, have a portion of the metapop-
30 ulation that is continuous (though might not be quite enough of the metapopulation
to capture the full network). Here, essentially, distinguish what we are doing from
the other studies. Not exactly sure of the flow here - maybe 1 paragraph or maybe
33 2?

 Overview of clownfish and why they are a great system. Here we.....

Methods

36 Study system

We focus on a tropical metapopulation of yellowtail clownfish (*Ampiprion clarkii* in the Philippines. Like many clownfish species, yellowtail clownfish have a mutualistic
39 relationship with anemones, where small colonies of fish live (CITATION). Yellowtail clownfish are protandrous hermaphrodites and maintain a size-structured hierarchy; within an anemone, the largest fish is the breeding female, the next largest is the
42 breeding male, and any smaller fish are non-breeding juveniles (CITATION). The fish on an anemone maintain a strict social and size hierarchy (Buston, 2003), with fish moving up in rank to become breeders only after the larger fish have died. In the
45 tropical patch reef habitat of the Philippines, yellowtail clownfish spawn once per lunar month from November to May, laying clutches benthic eggs that the parents protect and tend (Ochi, 1989). Larvae hatch after about six days (check this) and
48 spend 7-10 days as pelagic larvae before returning to reef habitat to settle in an anemone (Fautin et al., 1992).

Clownfish are particularly well-suited to metapopulation studies due to their lim-
51 ited movement as adults and clearly patchy habitat. Once fish have settled, they tend to stay within close proximity of their anemones (XX meters, CITATION). This makes fish easier to relocate for mark-recapture studies and simplifies the exchange
54 between patches to only the dispersal during the larval phase. Patches, whether considered to be the reef patch or the anemone territory of the fish, are clearly discrete

and easily delineated, which makes determining the spatial structure of the metapop-
57 ultion clear. Additionally, clear patches make it easier to assess how much of the site
has been surveyed. These simplifying characteristics in habitat and fish behavior are
SOMETHING ABOUT CLOWNFISH AND OTHER SIMILAR SPECIES BECOM-
60 ING ALMOST A MODEL SYSTEM FOR METAPOPOPULATION PERSISTENCE
WORK, CITATIONS.

Field data collection

63 We focus on a set of seventeen patch reef sites spanning approximately 30km along
the western coast of Leyte island in the Philippines (MAP FIGURE). The sites
consist of rocky patches of coral reef and are separated by sand flats. Previous work
66 using genetic isolation by distances estimated that yellowtail clownfish larvae have a
dispersal spread of about 10km (Pinsky et al., 2010), so our sites were selected to
cover and exceed that range. On the north edge, the sites are isolated from nearby
69 habitat. with no additional reef habitat for at least 20km.

Since 2012, members of the team have sampled fish and habitat at most of the
sites annually. During sampling, divers using SCUBA and tethered to GPS readers
72 swim the extent of each site. Divers visit each anemone inhabited by yellowtail
clownfish, tagging the anemone to be able to track anemones through time. At each
anemone, the divers attempt to catch all of the yellowtail clownfish 3.5cm and larger,
75 taking a non-lethal tail fin-clip from each for use in genetic analysis, measuring the
fork length, and noting the tail color (as an indicator of life stage). Starting in
the 2015 field season, fish 6.0cm and larger are tagged with a passive integrated

⁷⁸ transponder (PIT) tag, unless already tagged. Divers also looked for eggs around each anemone and measured and photographed any clutches found.

Processing genetic samples

⁸¹ TALK TO KATRINA AND MICHELLE, ADD IN HERE. BRIEF OVERVIEW, WITH CITATIONS TO PAPERS WITH RELEVANT METHODS AND TO KATRINA'S CONNECTIVITY PAPER.

84 **Estimating inputs from empirical data**

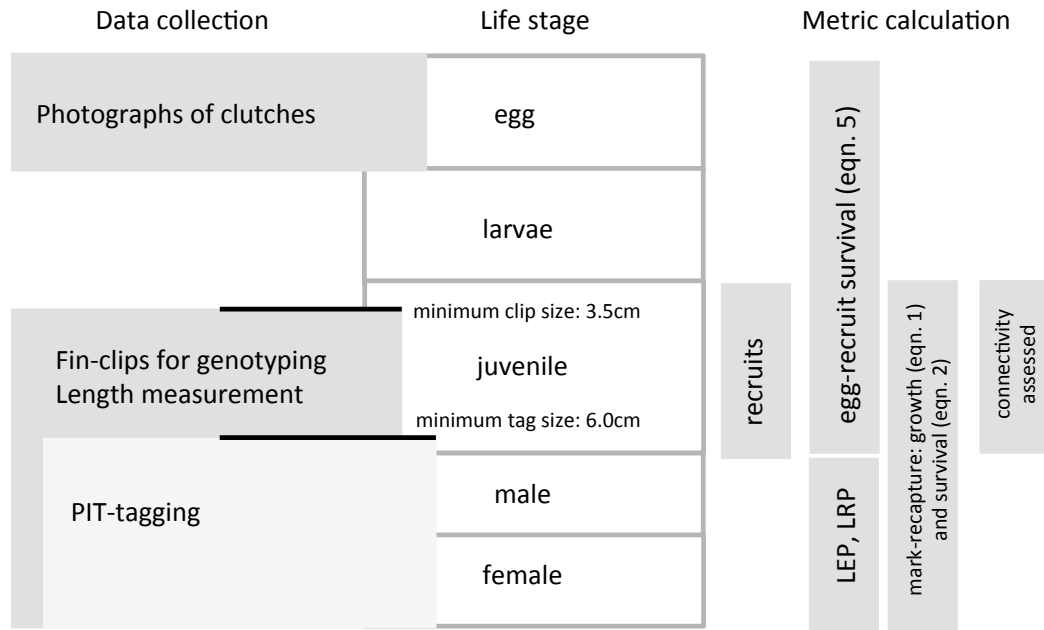


Figure 1: Here, we show the data collected for fish at each life stage (life stage boxes are not scaled by length of stage). We also show how the empirical data fit with the metric calculations.

Growth and survival: mark-recapture analyses

We mark fish through both genetic samples and PIT tags, allowing us to estimate growth and survival through mark-recapture. After matching up recaptures of the

same fish identified by genotype or tag, we have a set of encounters of each recaptured fish that includes size and stage at each capture time.

90 For growth, we estimate the parameters of a von Bertalanffy growth curve (Fabens, 1965) in the growth increment form relating the length at first capture L_t to the length at a later capture L_{t+1} (Hart and Chute, 2009), where L_∞ is the average
93 asymptotic size across the population and K controls the rate of growth:

$$\begin{aligned} L_{t+1} &= L_t + (L_\infty - L_t)[1 - e^{(-K)}] \\ &= e^{(-K)}L_t + L_\infty[1 - e^{(-K)}]. \end{aligned} \tag{1}$$

We see from eqn. 1 that we would expect the first length L_t and the second length L_{t+1} to be related linearly (Hart and Chute, 2009). From the slope $m = e^{(-K)}$ and
96 y-intercept $b = L_\infty[1 - e^{(-K)}]$, we can estimate the von Bertalanffy parameters, such that $K = -\ln m$ and $L_\infty = \frac{b}{(1-m)}$. We use the first and second capture lengths for fish that were recaptured after a year (within 345 to 385 days) to estimate L_∞ and K .
99 We have some fish that were recaptured multiple times so we randomly select only one pair of recaptures from each to use in estimating the parameters, and repeat this process 1000 times to generate a distribution (Fig. 2b, B.1d).

$$p_a = \text{some stuff here.} \tag{2}$$

102 **Fecundity**

We use a size-dependent fecundity relationship, determined using photos of egg clutches and females (Yawdoszyn et al. in prep), where the number of eggs per

105 clutch (E_c) is exponentially related to the length of the female (L) with slope β_l ,
 intercept b , and effect β_e dependent on if the eggs are old enough to have visible eyes:

$$\ln(E_c) = \beta_l \ln(L) + b_e[\text{eyed}] + b. \quad (3)$$

To get total annual fecundity, we multiply the number of eggs per clutch by the
 108 number of clutches per year c_e , using the estimate from Holtswarth et al. (2017).

We only consider reproductive effort once the fish has reached the female stage.
 Though the size at which a fish transitions to become a breeding female L_f will
 111 depend on the size hierarchy in each particular colony (CTIATION), we use the
 average size recaptured fish were first observed as female for the best estimate.

Lifetime egg production

114 We use an integral projection model (IPM) (e.g. Rees et al., 2014) to estimate the
 total number of eggs produced by one individual (lifetime egg production: LEP),
 starting at the recruit stage, when individuals have settled and survived to a size we
 117 can sample.

In an IPM, the state of the population at time t is described by the distribution
 of the population over a continuous trait z , for which we use size: $n(z, t)$. The total
 120 number of individuals in the population at time t is the integral of the size distribution
 over size from the lower size bound L to the upper size bound U : $\int_L^U n(z, t) dz$. The
 population is projected forward with probability density functions, called the kernel,
 123 that describe the survival, growth, and reproductive output of existing individuals
 into the next time step.

We initialize the IPM with one recruit-sized individual $\text{size}_{\text{recruit}}$: $n(t = 0) =$
126 $n(\text{size}_{\text{recruit}}, 0)$, then use a kernel with the size-dependent survival and growth func-
tions described above to project forward for 100 time steps. This gives us the size
distribution at each time step, which represents the probability that the individual
129 has survived and grown into each of the possible size categories. The probability
that the individual is still alive and of any size decreases as the time steps progress;
by using a large number of steps, we are able to avoid arbitrarily setting a maximum
132 age and instead let the probabilities become essentially zero.

We then multiply each size-distribution vector in the matrix by the size-dependent
fecundity function described above (eqn. 3) to get the total number of eggs produced
135 at each time step. To get the total number of of eggs one individual is likely to
produce in its lifetime, we then sum across all time steps in the individual's potential
life.

$$\text{LEP}z = \text{IPM} * \text{Fec.} \quad (4)$$

138 **Survival from egg to recruit**

We estimate survival from egg to recruit S_e using the number of recruited offspring
we can match back to genotyped parents as surviving individuals from genetically
141 "tagged" eggs in a method similar to that in Johnson et al. (2018). We estimate
the number of eggs produced by genotyped parent fish by multiplying the number of
genotyped parents N_g by the expected lifetime egg production of a parent fish LEP_p ,
144 using LEP calculated starting with an individual of 6cm. We make the assumption

that all recruited offspring originating from the genotyped parents end up in one of the sites we sample and estimate the total number of offspring that survive to recruit R_t by dividing the number of offspring matches we find R_m by the proportion of our site habitat we sample P_h and the probability of capturing a fish if we sample an anemone P_c (see B.4 for details on P_h and P_c). Our estimated survival from egg to recruit is the number of tagged recruits divided by the number of tagged eggs produced:

$$S_e = \frac{\frac{R_m}{P_h P_c}}{N_g \text{LEP}_p}. \quad (5)$$

Defining recruit and census stage

When assessing persistence, it is important to consider mortality and reproduction that occurs across the entire life cycle to determine whether an individual is replacing itself with an individual that reaches its same life stage (CITATION?). We define recruit to be a juvenile individual that has settled on the reef the previous year; lifetime egg production assesses how many offspring an individual recruit is likely to produce in its lifetime from that point forward and egg-recruit survival gives us the fraction of those eggs that will survive to reach the recruit stage. In theory, it should not matter exactly how we define recruit so long we use that definition in our calculations of both egg-recruit survival and LEP. In our system it is straightforward to calculate LEP from any point but it is not possible to change our estimate of egg-recruit survival to allow different definitions of recruit: we do not have enough tagged

recruits to reliably estimate survival to different recruit sizes. Instead, we choose the
165 mean size of offspring matched in the parentage study as our best estimate of the
size of a recruit ($\text{size}_{\text{recruit}}$) and test sensitivity to different sizes within the range of
sizes that the recruit stage covers (Table 1).

168 **Probability of dispersal**

We use a distance-based dispersal kernel, estimated in other work using parent-
offspring matches from our genetic data (Catalano et al. in prep) using the method
171 described in Bode et al. (2018). The relative dispersal is a function of distance d as
measured in kilometers and parameters θ and k_d , which control the shape and scale
of the kernel:

$$p(d) = e^k e^{-(e^k d)^\theta}. \quad (6)$$

174 The dispersal kernel is estimated using fish that have already recruited to a
population and survived to be sampled so it gives the relative amount of dispersal
given that a fish recruits somewhere, not the probability that a released larvae will
177 travel a particular distance. To find the probability of fish dispersing among our
sites, we calculate the distance between the middle of each site to the closest and
farthest edge of each other site, then use the distances as upper and lower bounds
180 when integrating eqn. 6, which we do numerically. For example, the probability of
dispersal from site A to B, where d_1 is the distance from the middle of A to the
closest edge of B and d_2 is the distance from the middle of A to the far edge of B, is:

$$p_{A,B}(d) = \int_{d_1}^{d_2} e^k e^{-(e^k d)^\theta} dd. \quad (7)$$

183 **Persistence metrics**

For a metapopulation to persist, at least one patch needs to achieve replacement, where the number of individuals entering the population balances those lost to mor-
 186 tality or emmigration (CITATION). In our focal system, adults do not move among patches so we do not need to consider emmigration and only need to assess whether fish produce enough offspring that survive to recruitment to be able to replace them-
 189 selves and where those offspring travel within the metapopulation. We consider three primary metrics to assess whether and how the population is persistent: 1) lifetime production of recruits, to assess whether the population has enough surviving off-
 192 spring to achieve replacement 2) self-persistence, to assess whether any individual patches would be able to persist in isolation without any input from other patches, and 3) network persistence, to assess whether the metapopulation is persistent as a
 195 connected unit. We explain each metric below in detail.

Lifetime production of recruits

To assess whether individuals at our focal patches produce enough offspring that
 198 survive to become recruits themselves, we find the estimated number of recruits an individual recruit will produce over its lifetime (lifetime recruit production: LRP) by multiplying LEP by the estimated survival from egg to recruit S_e :

$$\text{LRP} = \text{LEP} * S_e. \quad (8)$$

201 If $LRP \geq 1$, the population has the possibility for replacement; individuals produce
 enough surviving offspring, before taking into account the probability of dispersal
 and settlement. If $LRP < 1$, the individuals are not replacing themselves and the
 204 population cannot persist without input from outside patches .

Self-persistence

A patch is able to persist in isolation (self-persistent) if individuals produce enough
 207 offspring (LEP) that disperse back to the natal patch and survive to recruitment
 to be able to replace themselves (LR): $LEP \times LR \geq 1$ (Burgess et al., 2014). Our
 dispersal kernel represents the probability that a recruit disperses a distance given
 210 that it recruits somewhere, rather than the probability of a larvae dispersing and
 recruiting to a particular patch, which implicitly encompasses mortality from egg to
 recruitment. We modify the equation to fit our data and include survival from egg
 213 to recruit to whether a particular patch i is self-persistent:

$$SP_i = LEP \times \frac{\text{recruits}}{\text{egg}} \times \frac{p_{i,i} \times \# \text{ recruits from site}}{\frac{\text{recruits}}{\text{egg}} \times \# \text{ eggs produced by patch } i} \quad (9)$$

$$SP_i = LEP \times S_e \times p_{i,i}.$$

A patch is self-persistent if $SP \geq 1$. If at least one patch is self-persistent, the
 metapopulation as a whole is persistent as well (CTITATION).

216 Realized connectivity matrix and network persistence

We find the probabilities of a recruit dispersing between each set of sites ($p_{i,j}$) by
 integrating the dispersal kernel (eqn. 6 over the distance between each set of sites. We

219 then create a realized connectivity matrix C by multiplying the dispersal probabilities
 by the expected number of recruits an individual produces: $C_{i,j} = \text{LRP} \times p_{i,j}$ (Burgess
 et al., 2014). The diagonal entries of C , where the origin and destination are the
 222 same sites, are the values of self-persistence we calculate above.

Network persistence requires that the largest real eigenvalue of the realized con-
 nectivity matrix be λ_C be greater than 1: $\text{NP} = \lambda_C > 1$ (e.g. Hastings and Botsford,
 225 2006; White et al., 2010; Burgess et al., 2014).

Incorporating uncertainty

To represent the uncertainty in our estimates of the parameters that go into cal-
 228 culating our persistence metrics, we calculate each metric 1000 times, pulling each
 parameter from a distribution. In our results, we show the range of values of each
 persistence metric as well as the value with our best estimate of each parameter.

231 Not sure where to put this, or if it should exist, but seems like it might be
 helpful:

Parameter	Description	Best estimate	Range in uncertainty runs	Notes

k_d	scale parameter in dispersal kernel	-1.36	-2.03 to -0.96	estimated using methods in Bode et al. (2018) in Catalano et al. (in prep)
θ	shape parameter in dispersal kernel	0.5	NA	estimated using methods in Bode et al. (2018) in Catalano et al. (in prep)
size _{recruit}	size (cm) of re- cruited offspring	mean of size of off- spring in parentage analysis = 4.4cm	3.5 - 6.0cm	
S_e	egg-recruit sur- vival			
E_c	eggs per clutch	depends on female size (eqn. 3)		relationship from Yawdoszyn et al. (in prep)
b_e	coefficient for eyed eggs	-0.608		Yawdoszyn et al. (in prep)

b_l	slope in eggs-per-clutch relationship	2.39		Yawdoszyn et al. (in prep)
b	intercept in eggs-per-clutch relationship	1.17		Yawdoszyn et al. (in prep)
L_f	size at transition to female	9.32cm	5.2 - 12.7cm	

Table 1:

17 sites along a 30km stretch of coastline in Leyte in the Philippines fairly distant
234 from other habitat to the north (20km away), previous estimates of clownfish larval
dispersal have been put at 10km so expect a lot of connectivity among these patches

More info about clownfish either on this slide or one before tag anemones tag
237 adult clownfish fin clip adults and sub-adults photograph eggs

The Pinsky lab has been following these populations for several years, marking
anemones, taking fin clips and putting in pit tags for the fish for parentage analyses
240 and mark-recapture studies, measuring fish sizes, take pictures of eggs to estimate
fecundity.

Can we tell if the patches and overall population are persistent? survival and pop-
243 ulation size from mark/recap does input (recruits x surv to adult) = adult mortality
Are any patches self-persistent? At what scales do groups of patches show network

persistence? replacement achieved with increasing number of patches? analyses with

246 realized connectivity matrices

–size likely matters b/c of the hierarchy –tag shedding

Many marine populations are spatially-structured, with different patch popula-
249 tions that are connected through movement and exchange of individuals, like these
population patches on a coastline here. One challenge that can be particularly pro-
nounced in the marine environment is understanding how spatially-structured popu-
252 lations are connected how many individuals they exchange how often and what that
means for their structure, dynamics, and persistence.

For understanding, the consequences of connectivity, we have to take a step back
255 to think about what it means for a population to persist. Essentially, for a population
to persist, on average adults need to replace themselves each generation, meaning
on average, they each needs to produce one offspring that survives to become a
258 reproductive adult. This concept goes by different names, depending on the context,
sometimes mentioned as R_0 , replacement, lifetime egg production, or a growth rate
 $\lambda = 1$. It can also be thought of as equilibrium flow (

261 Results

Figure 1: map of study sites, picture of clownfish

Any figure summarizing the data? How many fish captured, sequenced, etc?

264 Could go in the appendix?

Figure 2: schematic figure

Figure 2: abundance trends through time with some sort of time series analysis

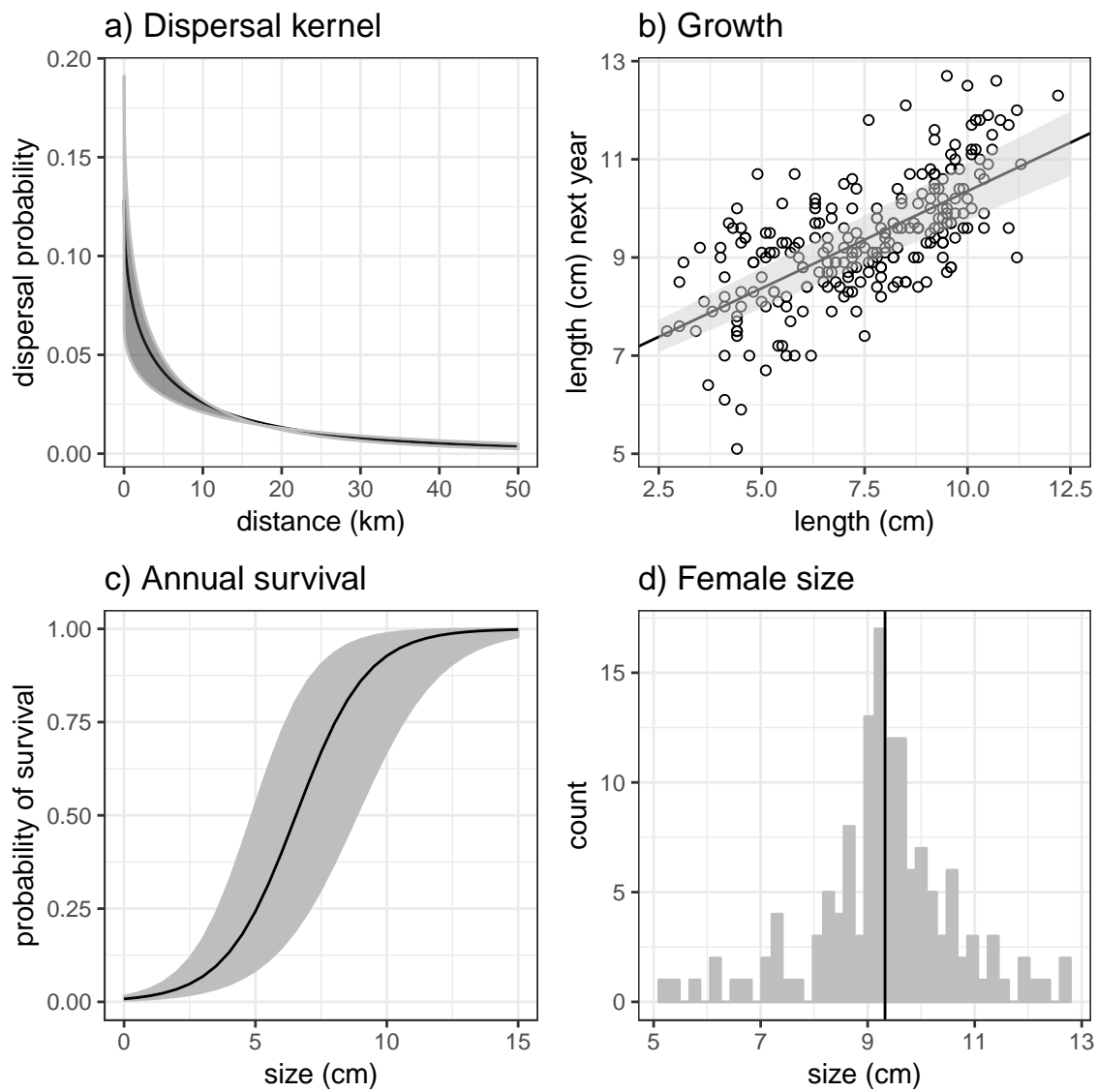


Figure 2: WRITE A CAPTION!

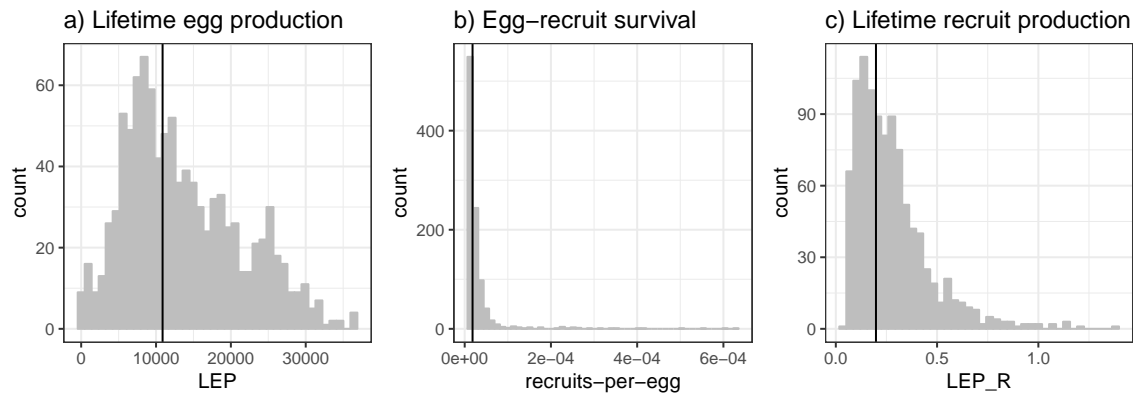


Figure 3: Metrics with best estimate (using recruit size of mean of offspring size) and uncertainty. WRITE A CAPTION!

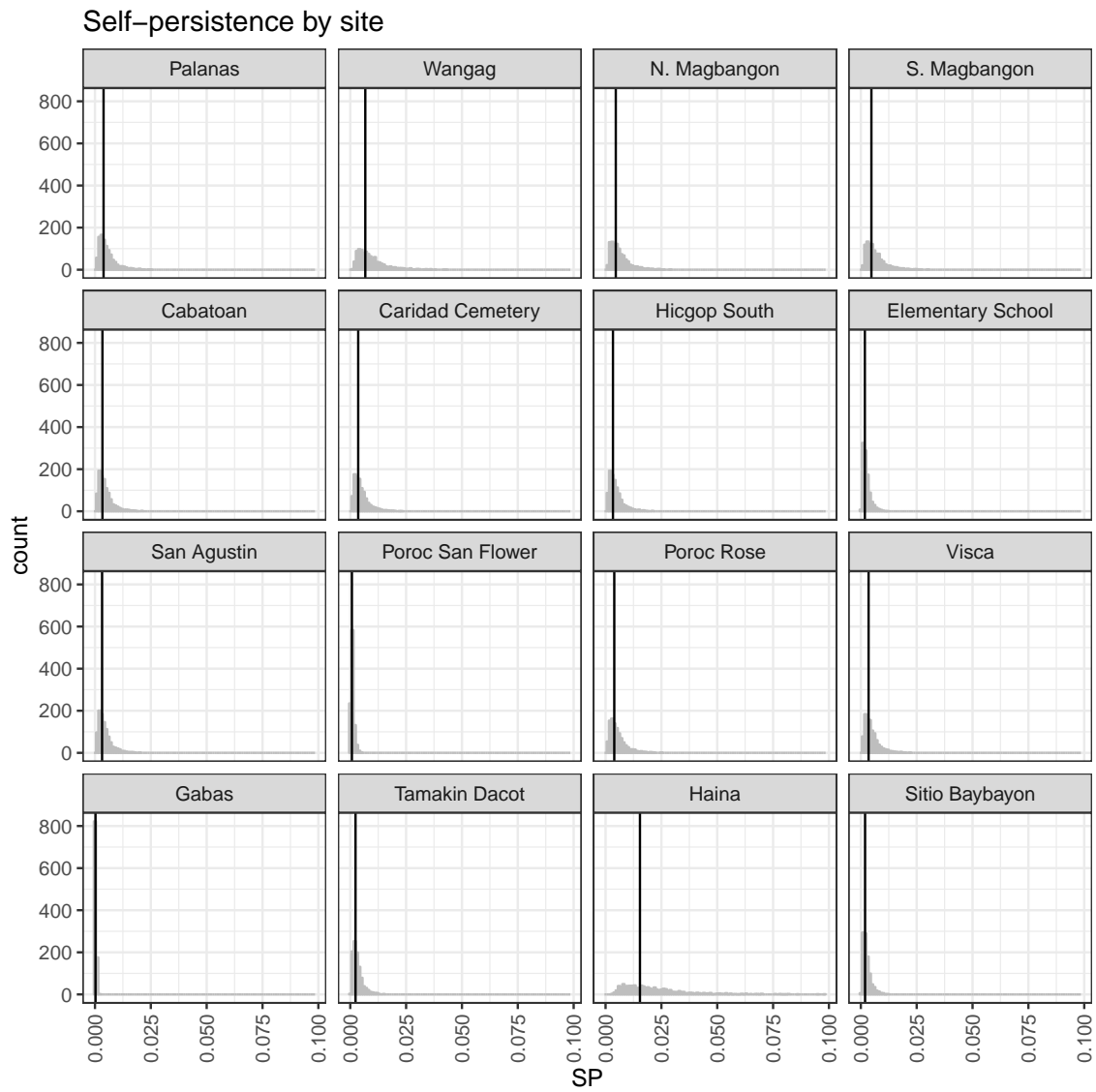


Figure 4: Metrics with best estimate (using recruit size of mean of offspring size) and uncertainty. WRITE A CAPTION!

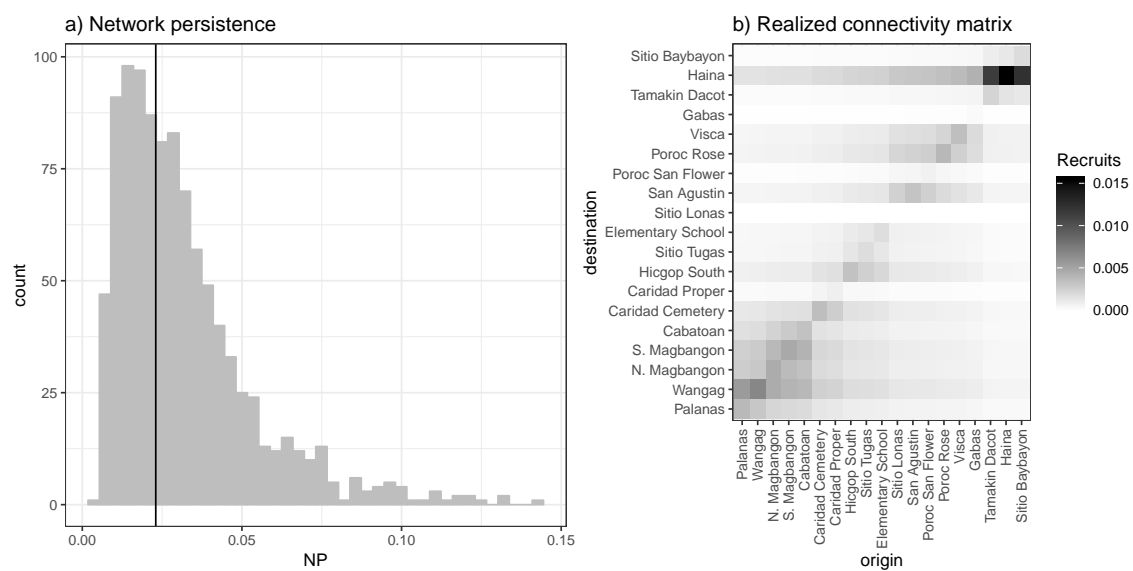


Figure 5: Metrics with best estimate (using recruit size of mean of offspring size) and uncertainty. WRITE A CAPTION!

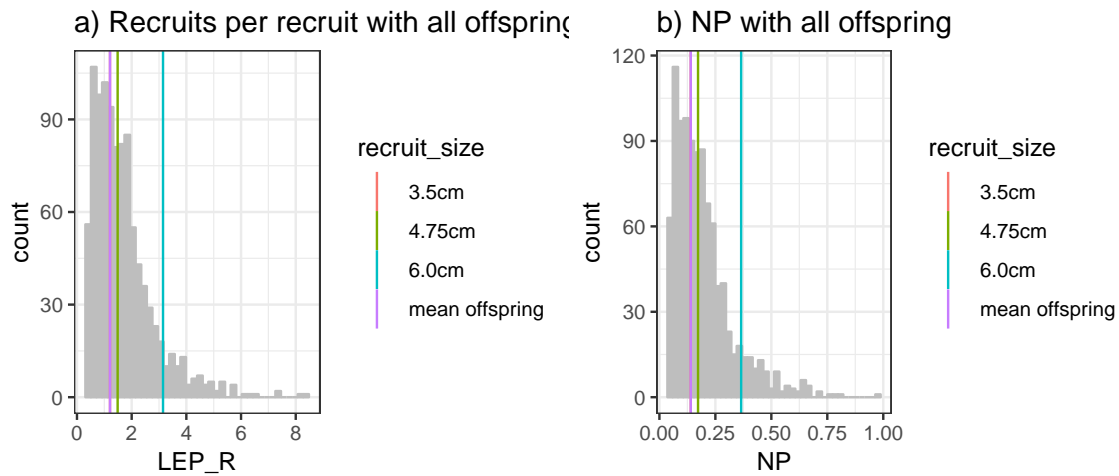


Figure 6: Range of parameter inputs for uncertainty runs with all uncertainty included. Census size is the size at fish are considered to have recruited, such that egg-recruit survival ends. Female transition is the size at which fish transition from male to female and their reproductive output is included in the estimate of lifetime egg production (LEP). FINISH LISTING PARAMS!

267 Discussion

previous research suggests 10km dispersal kernel spread for yellowtail clownfish (Pinsky et al., 2010)

A Method details

Proportion of habitat sampled

273 B Uncertainty details

B.1 Sensitivity to parameters

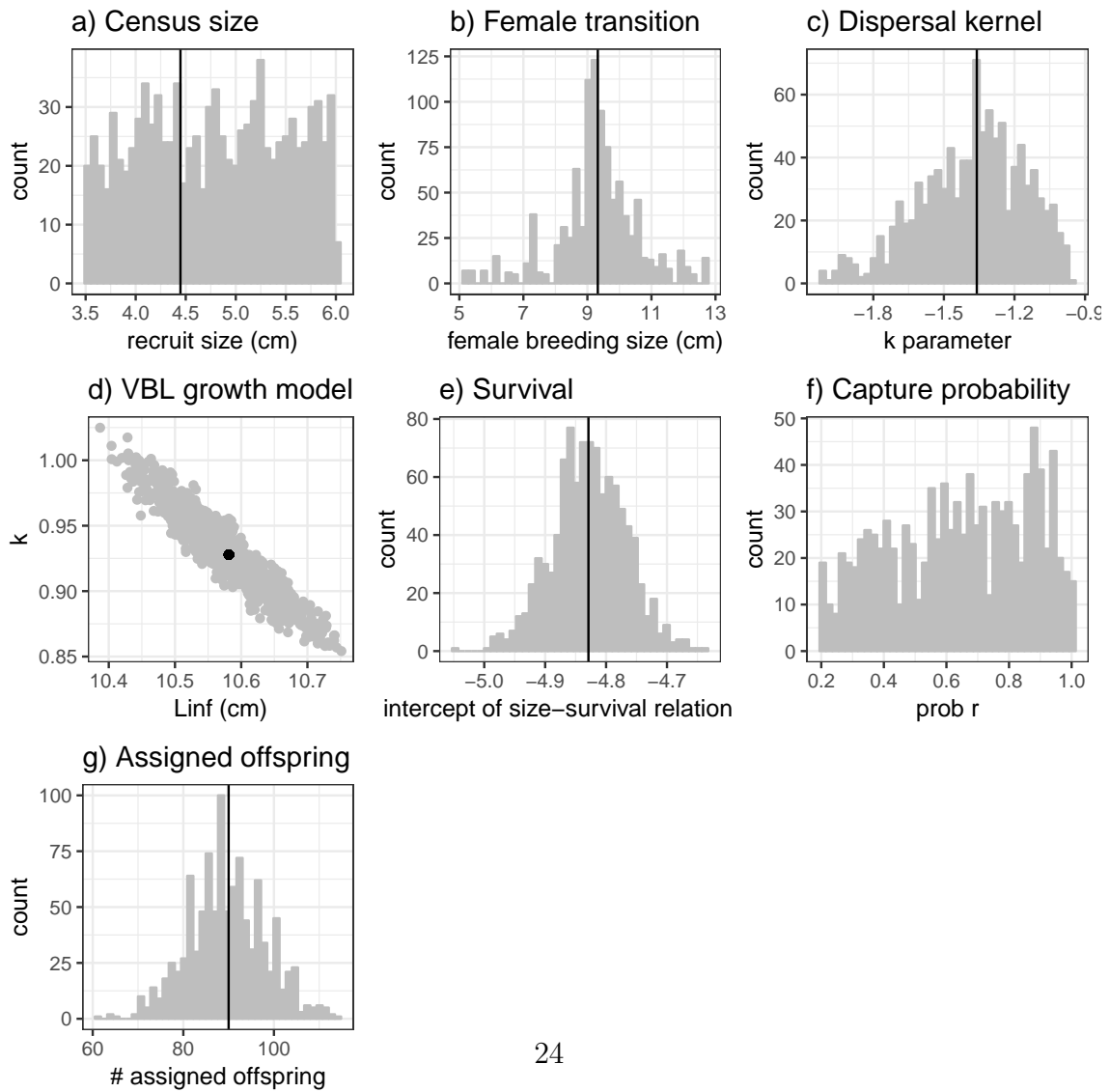


Figure B.1: Range of parameter inputs for uncertainty runs with all uncertainty included. Census size is the size at fish are considered to have recruited, such that egg-recruit survival ends. Female transition is the size at which fish transition from

B.2 Effects of different types of uncertainty on metrics

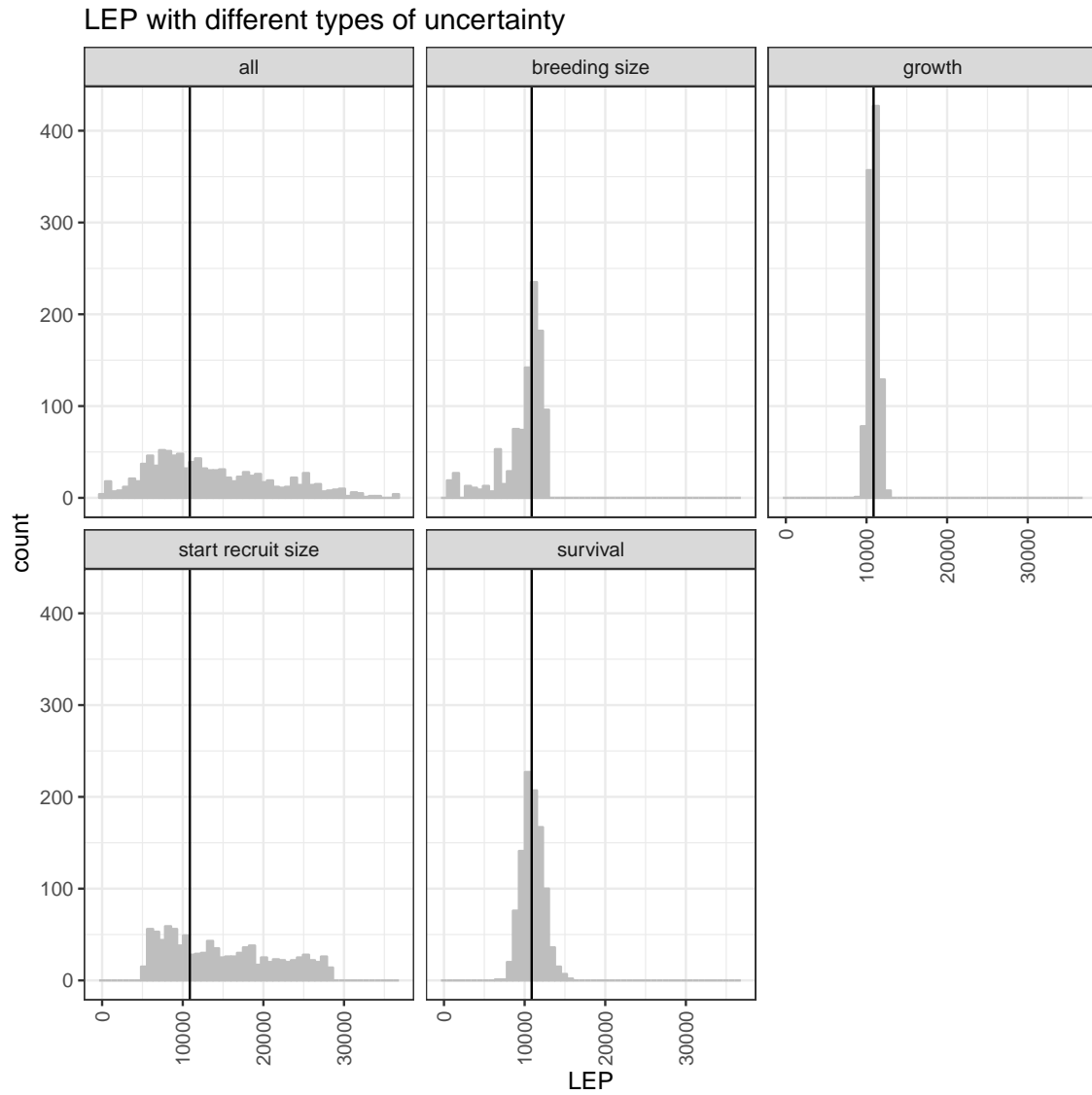


Figure B.2: WRITE A CAPTION!

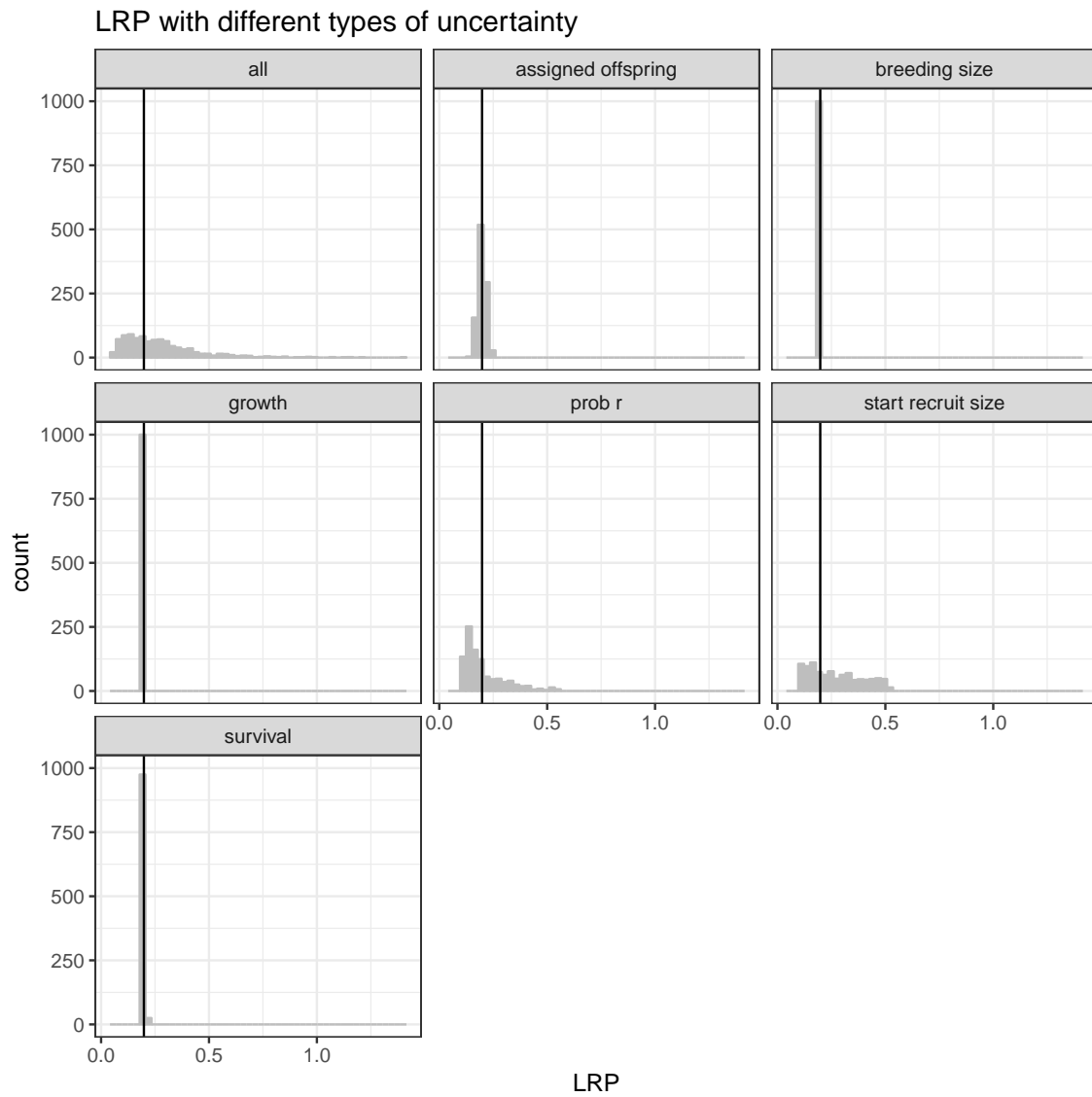


Figure B.3: WRITE A CAPTION!

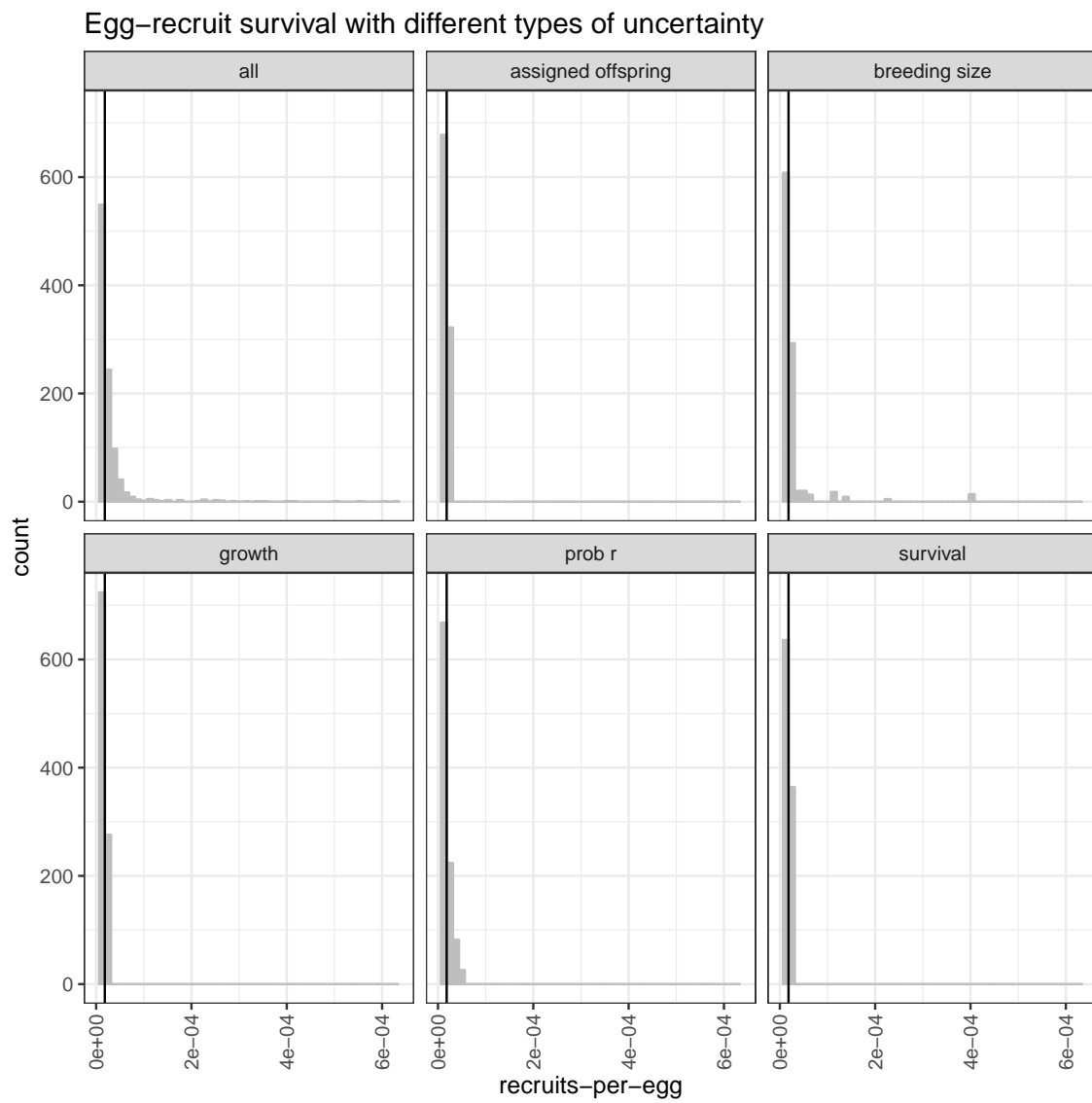


Figure B.4: WRITE A CAPTION!

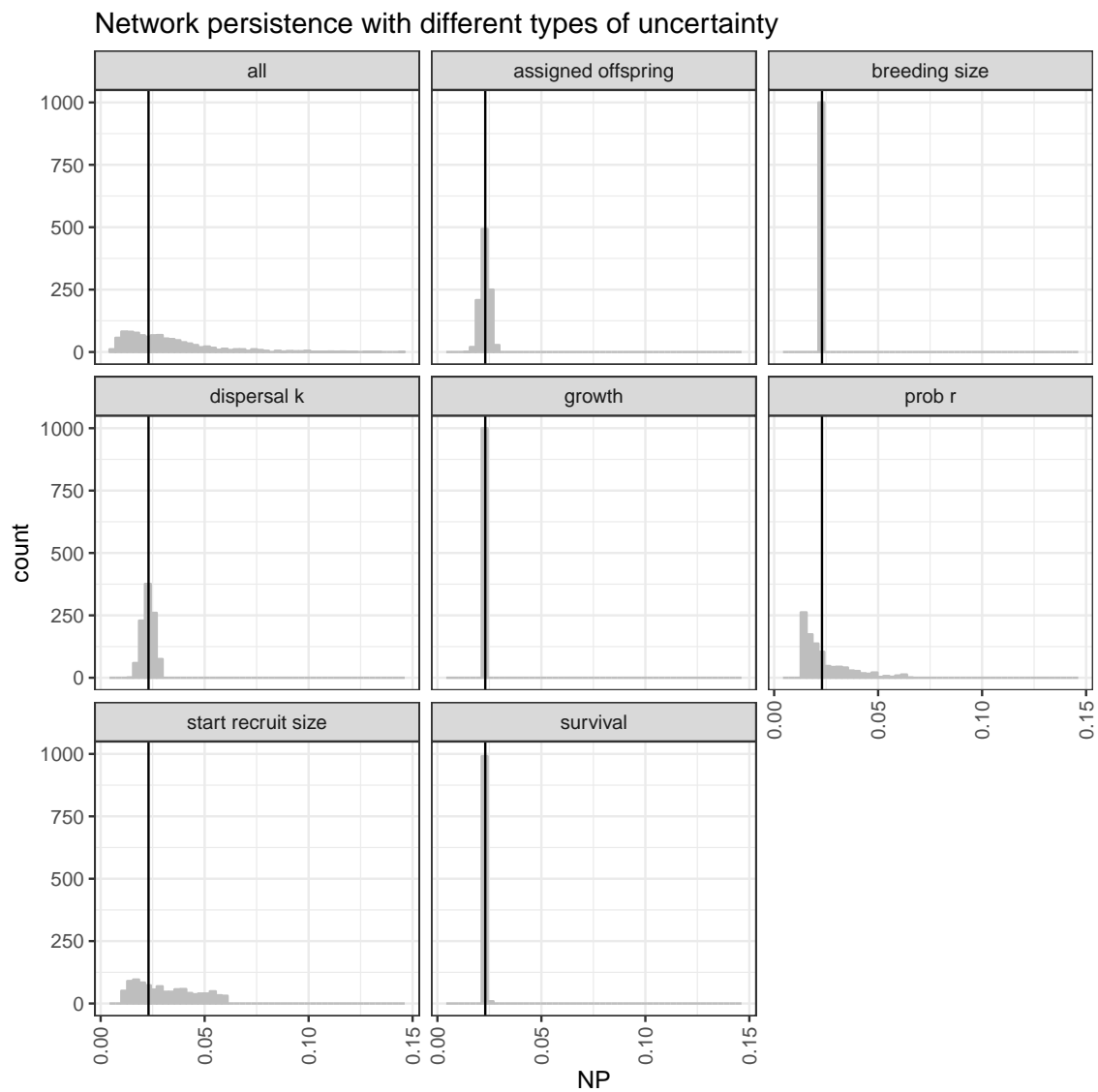


Figure B.5: WRITE A CAPTION!

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