**Measuring *Daphnia* Life History in the Wild: the Efficacy of Individual Field Cages**

**Running Head:** O’Connor et al., - Effectiveness of field cages *in situ*

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Abstract

Life history studies are often conducted in a laboratory environment where it is easy to assay individual animals. However, factors such as temperature, photoperiod, and nutrition vary greatly between laboratory and field environments, making it difficult to compare results. Consequently, there is a need to study individual life histories in the field, but this is currently difficult in systems such as *Daphnia* where it is not possible to mark and track individual animals. Here, we present a proof of principle study showing that field cages are a reliable method for collecting individual-level life history data in *Daphnia magna*. As a first step, we compared the life history of paired animals reared outside and inside cages to test the hypothesis that cages allow free-flow of algal food resources. We then used a semi-natural mesocosm setting to compare the performance of individual field cages versus glass jars re-filled with mesocosm water each day. We found that cages did not inhibit food flow, and that differences in life histories between three clones detected in the jar assays were also detectable using the much less labour-intensive field cages. We conclude that field cages are a feasible approach for collecting individual-level life history data in systems such as *Daphnia* where individual animals cannot be marked and tracked.

Keywords: Field Experiment, *Daphnia*, multivariate phenotype, life history, field cage

**Mr Michael O’Connor, Mr Daniel Sadler, Dr Franziska Brunner, Mr Alan Reynolds, Dr Nicola White, Mr Stephen Price, and Dr Stewart Plaistow**

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Dear Editor,

We would like to thank you for the opportunity to revise our manuscript “Measuring *Daphnia* Life History in the Wild: the Efficacy of Individual Field Cages” (#ECE-2021-05-00782).

We have revised the manuscript to address the comments from the Associate Editor and reviewers and thank them for their help in improving this manuscript. In particular, we have clarified the preliminary nature of our study and the purpose of testing for clonal differences in the mesocosm setting. Our intention was to demonstrate that field cages are as useful at detecting clonal variation in the field as a more typical lab style assay, rather than trying to quantify clonal variation in life-histories *per* se. We have clarified this throughout the manuscript. Furthermore, we have included a discussion of the remaining differences between the field cages and an unrestricted, wild setting as requested. Please find our detailed responses to each of the comments in bold below.

Yours sincerely,

Mr Michael O’Connor, Mr Daniel Sadler, Dr Franziska Brunner, Mr Alan Reynolds, Dr Nicola White, Mr Stephen Price, and Dr Stewart Plaistow

Associate Editor Comments to Author:  
  
Associate Editor  
Comments to the Author:  
I've received two reviews on your manuscript and I'm afraid both are recommending rejection. The issues taken are similar - that the work is too preliminary and needs further work before presentation as a full paper. The experiments are unfortunately too small in scale. However, I think there's a useful concept here, so I'd like to see either an expanded paper incorporating the reviewers concerns or a reformulated manuscript that makes the preliminary aspects clearer and explains both the limitations and scope for further development. There is helpful feedback from the referees here, but I think it goes further than just revisions, so a "reject and resubmit" is my recommendation.

**Thank you for your careful consideration of our manuscript. We have chosen to reformulate the text to make the preliminary aspects of the study clearer (e.g. L 32, L 361) and incorporated a discussion of the limitation of field cages (L 546-557). Furthermore, we have reorganised some of our discussion to create a clearer outlook section explaining potential future uses of the cages (L 558-574).**

Reviewer: 1  
  
Comments to the Author  
In this study, the authors seek to compare a standard laboratory-based approach to studying Daphnia life history with an outdoor approach where enclosures are placed within mesocosms.  
  
The motivation for the study is compelling and very clearly described. A lot of what we know about Daphnia life history comes from tightly controlled lab experiments. These are obviously great in many ways, as we can carefully unpick chosen genetic or environmental contributions towards a particular phenotype. However, to what extent do these experiments capture real-world Daphnia biology? We don't really know.  
  
So, when it comes to the overarching aim of this study, I'm sold. I also think the experimental setup and description of the field cages and their comparators is good.

**Thank you very much for these positive comments.**  
  
However (I'm sorry, but there is a 'however'), I wonder if these experiments are really large enough to clearly show that clonal variation in life history traits uncovered the lab can be found in the field enclosures. There are just three genotypes (!) in the larger field experiment, and the study comprises of a pair of two quite modest experiments. Yes the three genotypes showed broadly the same LH across experiments, but one could have chosen a different three genotypes and found a completely different result. It just isn't comprehensive.

**We agree with the reviewer that three genotypes will not typically capture the full life history variation of a natural population. However, capturing the full extent of population level variation was not our intent; rather, we wanted to test whether low-effort field cages could capture genetic variation in life histories in the field as well as a more traditional high-effort laboratory style assay conducted in the field. Had we picked three clones that didn’t differ in their life-histories this would still have been fine as long as the field cage assay produced the same result as the jars. We have revised the text to make it clear that our mesocosm field cage experiment was a proof of principle study rather than an attempt to capture the full extent of life history variation possible (e.g. L 32, L 361).**

When I read the abstract, I was expecting a number of floating enclosures testing for field variation across many Daphnia genotypes. The mesocosms themselves are large (I couldn't find the size of them in the ms, but had a google), so there doesn't seem to be a reason why the authors couldn't have tested 10, 15 or even 20 genotypes across some replicate mesocosms. That way, one could comment on whether the genotypic variation uncovered in the lab is present in the field setup. Daphnia are the poster child for genetic variation - name a trait and lab studies tell us there is significant variation across clonal genotypes. Yeah, some genotypes will behave differently depending on lab or field setting, but one could get a handle on whether genotype is equally important in both settings. What we need to know if Daphnia genotypic variation is meaningful in ecologically complex environments.

**We have revised the abstract to make a clearer distinction between the content of this study (a test of the reliability of the field cages) versus potential future uses of the field cages, to avoid creating a wrong impression of the size of the study at this point (L 32). We have also included a size description of the mesocosms in the methods now (L 424), thank you for pointing this out. A bigger experiment to test whether the extent and types of differences between a large number of clones would be comparable between the field and in the laboratory as suggested by the reviewer would indeed be interesting, however this was beyond the scope and manpower of the project in this case, and we have therefore included this idea in the final section discussing further development and uses of the field cages (L 559-562). Before such a study could be carried out it would be necessary to make sure that you have a reliable method for measuring individual life-history data in the field.**

I appreciate a lot of reviewers write something along the lines of "MOAR GENOTYPES" , and I like to think I'm not one of them, usually. If genotypic variation was not the overarching aim, then the authors could have done more to unpick the environmental effects by, for example, doing the field expt and measuring temp, food and whatever other ecological variables of interest across a couple of mesocosms, then replicating means of those values in the lab and looking at whether the rank order of the Daphnia genotypes is the same in lab and field across the traits of interest.

**Similarly to the suggestion above, we consider this a great idea for future uses of the field cages to address question of *Daphnia* evolutionary ecology. However, the purpose of this study was not to compare the lab environment and the field environment but to test a method (field cages) that will allow us to start collecting individual life-history data in the field. Something that hasn’t previously been published.**

When I look at the study presented, I'm afraid I just some see enough of a test of either environmental or genetic variation, despite being well written and addressing a question that is of interest in the field.  
It's just too preliminary.

**We certainly understand and share the wish to get into answering more advanced questions with the field cages right away. However, we feel that a proper testing stage of such field cages is often skipped or at least not published which is why we wanted to address this gap first. By demonstrating that field cages allow free flow of resources (experiment 1) and perform as well as a lab assay conducted in the field (experiment 2) we hope that we have robustly demonstrated the utility of a field cage approach for conducting the sort of studies proposed by the reviewer.**

Reviewer: 2  
  
Comments to the Author  
Review of the manuscript: “Measuring Daphnia life history in the wild: the efficacy of field cages” submitted by O´Connor et al.  
  
The authors investigated the use of net cages to study life history parameters of Daphnia magna. Net cages in the field should replace glass jars in which in situ field conditions are difficult to mimic and to maintain. Main result was that the net cages gave similar life history results compared to results obtained in glass jars.  
  
The interest to bring laboratory type of experiments into more complex field situation is increasing and technical solutions to do this are needed. The manuscript covers therefore an important aspect of modern ecological experimentation. The authors did detailed tests of basic life history parameters of Daphnia and a statistical comparison of cultivation in cages and glass jars. The manuscript is very well written and concise. However, I do not see an explicit ecological or evolutionary conceptual research question which was investigated with the technique such as related to for example to global change or other important topics pointed out in the introduction.

**Thank you for your positive comments on the interest of our study. We have revised our manuscript to clarify that we aimed to test the usefulness of the field cages to address questions of evolutionary ecology in the future, rather than performing a full-sized test of genetic or environmental variation at this point. We think that there is a lack of published robust tests of the reliability and usefulness of such field cages and therefore wanted to address this gap in a first instance.**  
  
-Abstract, line 36: The authors state that nutrient flow was not inhibited by cages; however I cannot find any nutrient measurements in the manuscript? Are this unpublished data?

**We have revised the sentence to clarify that the flow of algal food was not inhibited, which was what we meant to say (L 37). We apologies for the unclear terminology here. While we did not measure algal concentration as such, free algal flow is shown by the fact that *Daphnia* growth rates were greater in the part of the beakers outside of the cages even though algae were only added to the inside.**   
  
 -Net cages to cultivate Daphnia and which allow full access to food surrounding the cages   in laboratory and field environments are already described (see also literature cited, line 156 in introduction), one more example for laboratory investigations with "caged" Daphnia is Reichwaldt et al. , Oecologia 141:411-419(2004), showing “normal” grazing rates in cages and also tested effects of the cages on food by using empty control cages. For field experiments examples of large field net cages in enclosures (10m) are described do follow the effects of Daphnia population dynamics with and without diel vertical migration on surrounding phytoplankton (Haupt et al. Journal of Plankton Research 31: 515-524 (2009)).

**Thank you for pointing out these additional studies, we have included them in our discussion now (L 496, 497, 503-505). While these studies use cage designs, they differ clearly from our study in that they use population size cages holding at least 13 or in the other case hundreds of *Daphnia* while our cages are designed to study individual *Daphnia* to get more detailed individual level life history data. Furthermore, neither of these studies (nor most other studies we had already referenced in our manuscript) explicitly test for effects of the cages on *Daphnia* life histories since all of them are held within cages.**  
  
- Daphnia magna can have large number of eggs (up to 100), so field cages may suddenly include a large number of neonates. Is it more difficult to handle neonates in cages then in glass jars or do neonates escape the net cages and may affect outside dynamics?

**The mesh size of 170μm of the cages (now clarified on L 385 in the methods) does not allow even newborn *Daphnia* to escape the cages. We did not find the removal of neonates from the cages any more difficult than from the jars and specify this now in the methods as well (L 389-390). This possibility of a sudden change in the occupancy rate of the cages after reproductive events in fact demonstrates one of the advantages of the cages over the jars: while the jars limit the available food until the next water exchange, the cages allow for the constant influx of food particles, therefore limiting the confounding impact of reproductive events on the mother’s growth rate.**  
  
-The relevant technical point that ensures that the food is ~ the same inside and outside the cage is the size of the mesh covering the cage. For Daphnia, a usual size category for algae to be an edible food source is smaller 30 µm, very large mesh size above 250 µm may allow neonates to escape net cages. I may have overlooked it, but I did not find information about the important parameter mesh size of cages (Only 300 µm mesh size of some lids).

**We agree that this is an important point and have now specified the mesh size of the cages (L 385).**  
  
-The main argument of the manuscript is that such net cages as described will allow to measure "real field" individual life history variation in field experiments related for example to global change. Hence, Daphnia has also a complex behavior that will also influence life history, such as for example migration behavior to escape predation or UV radiation.  Exposing Daphnia in the described net cages will only allow them to live in the first few cm below the water surface. Daphnia is then not able to show their “real” field behavior or explore “real" field food conditions except within the very first few cm of the water column. This may even result in a disadvantage in very clear waters compared to glass jars (UV exposure without possibility to migrate deeper?)

**We agree that there are still important differences between the possible behaviour and environmental variation in our field cages compared to a *Daphnia* in the wild and have added a discussion section to point out those differences (L 546-557).**  
  
- Actually, the described mesh cages are then very similar to exposing Daphnia in glass jars (also no migration behavior possible, restricted food availability) and both systems do not allow a “real” test of field situations. Not surprising, both systems showed indeed very similar results. So is mainly an argument about less work effort in net cages compared to glass jars that is remaining as main advantage?  It would have been helpful to see how the described systems would have performed in experimental manipulated mesocosm systems with a clear ecological research question.

**Beyond the reduced workload of field cage tests compared to glass jars, we think there are some crucial differences in ecological realism. Most important is probably the flux of food which means that *Daphnia* in cages do not experience unnatural fluctuating food availability as food gets added at water exchanges and then depleted over time. Along with natural food availability, *Daphnia* in cages will experience any short term (e.g. diurnal) fluctuations in water temperature, water chemistry etc which will at least be distorted (e.g. for temperature changes) or completely prohibited (e.g. water chemistry) by the jars. This was even reflected in increased survival of *Daphnia* juveniles in the field cages compared to jars. We therefore politely disagree with the assessment of the two being very similar. There are of course also limitations of the field cages and differences to a fully wild situation which we now describe in more detail at the end of the discussion. This includes ways that diel migration might be incorporated into cage design.**

Introduction

Model organisms are an important part of experimental biology, especially in the field of evolutionary biology (Gasch, Payseur and Pool, 2016), where taxa such as *Drosophila* spp., *Caenorhabditis elegans* and *Daphnia* spp. allow the investigation and interpretation of some of evolution’s biggest questions (Kellogg and Shaffer, 1993). Traditionally, work on model organisms has often been restricted to the laboratory (Barata *et al.*, 2000). Laboratory experiments are useful for controlling natural environmental variation but also often result in individuals being studied in isolation (Kohler, 2002). This may exclude social aspects of the environment and other potentially important biological and environmental interactions (Morin, 1998). Since laboratory studies only capture a small part of the dynamic natural environment (Grodwohl, Porto and El-Hani, 2018), it can be difficult to know whether the results obtained in the laboratory are applicable to real-world field conditions (Ieromina *et al.*, 2014; Poorter *et al.*, 2016).

*Daphnia* are a well-established model organism used in a variety of studies across multiple fields including evolutionary biology, ecotoxicology, and genetics (Altshuler *et al.*, 2011; Miner *et al.*, 2012). *Daphnia*’sshort generation time, high fecundity, and clonality make it an ideal organism to carry out replicated experiments across multiple clonal lineages and environments (Ebert, 2005; Lampert, 2006). Furthermore, *Daphnia* are ecologically relevant; as a keystone species in many freshwater ecosystems, they act as both an algal grazer and as a prey species for a variety of aquatic predators (Hebert, 1978; Ebert, 2005; Lampert, 2006). Moreover, several *Daphnia* species have extensively mapped genomes accompanied by a plethora of genetic studies, allowing for understanding of life history and morphological responses and their evolution at a molecular level (Colbourne, Singan and Gilbert, 2005). However, much of our understanding of individual-level *Daphnia* biology is based on the results of laboratory studies (Barata *et al.*, 2000). Studies that take place in the field tend to be restricted to population level responses (e.g. (Zbinden, Haag and Ebert, 2008; Cabalzar *et al.*, 2019)). As a result, individual *Daphnia* life history responses in wild populations remain understudied (Burks, Jeppesen and Lodge, 2001; Bruijning, ten Berge and Jongejans, 2018).

A primary reason for this lack of individual based field studies, in both *Daphnia* and other aquatic model species, is the issue of replicated measurements of the same individuals. Difficulties associated with marking and tracking individuals for repeatable and reliable identification are magnified in the natural setting (Woodcock *et al.*, 2016). Combined with the increased financial and logistical constraints associated with field work (Morin, 1998), this has led to a reliance on laboratory experimentation for aquatic invertebrates. The laboratory is a poor substitute for the dynamic natural environment. Dynamic variables in the wild such as temperature (Lagerspetz, 2006), photoperiod (Korpelainen, 1986), and nutrition (Ieromina *et al.*, 2014) are often held constant in laboratory environments (Giebelhausen and Lampert, 2001). The lack of natural fluctuations affects the realism of laboratory studies, resulting in discrepancies; for example, in toxicity resistance between laboratory and field conditions in *Daphnia* (Hatch and Burton, 1999; Duchet *et al.*, 2010) and other model organisms such as *Hyalella azteca* (Clark *et al.*, 2015).

The need for individual-level field studies goes beyond the limitations of laboratory realism. For the model organism *Daphnia*, questions related to its evolutionary ecology, for example its ability to adapt to changing environments, can only be investigated under natural field conditions (Yurista, 2001; Duchet *et al.*, 2010). Like most organisms, *Daphnia* are extremely phenotypically plastic, meaning that they change their phenotype in response to the environment that they are exposed to (West-Eberhard, 1989; Pigliucci, 2001; Plaistow and Collin, 2014). Phenotypic plasticity is an integral part of responses to environmental change (Gienapp *et al.*, 2008; Fox *et al.*, 2019). It is therefore extremely important that experiments investigating the role of plasticity in evolution accurately reflect the natural environment that *Daphnia* are exposed to. Field cages represent a simple yet effective solution for studying phenotypic responses to changes in environmental conditions (O’Brien and Kettle, 1981; Bjergager *et al.*, 2012). Small containers with mesh sides allow the monitoring of individual organisms while exposing them to many of the natural fluctuations in parameters such as temperature and food as experienced by the rest of the population. In this sense, cages can act as a midway point between the laboratory and the field (Bjergager *et al.*, 2012). However, the design must ensure that the life history data collected is reflective of *Daphnia* living in a natural environment outside of the cage. Various field cage designs have been used in previous studies, both for cages holding individual *Daphnia* (Yurista, 2001; Bjergager *et al.*, 2012; Ieromina *et al.*, 2014; Bruijning, ten Berge and Jongejans, 2018) and for cages holding whole *Daphnia* populations (Reichwaldt, Wolf, and Stibor, 2004; Haupt *et al*., 2009), yet the assumption that being inside the cage reflects natural conditions has, to our knowledge, never been tested.

In the present study we wanted to address this lack of field cage validation and designed a field cage that allowed individual *D. magna* life history data to be collected in the context of a natural environment. First, in a laboratory experiment, we tested the hypothesis that cages allow free-flow of algal food resources by comparing the life-history of paired animals reared inside and outside cages. We then conducted a second experiment in a semi-natural mesocosm setting where we compared the performance of individual field cages to the performance of assays conducted in glass jars that were re-filled with mesocosm water each day, using three clones collected from the same population. We hypothesised that if cages performed adequately, clonal difference in life-histories detected in jars would also be detected in cages.

Methods

All *Daphnia magna* used were taken from laboratory lines isolated from Brown Moss Nature Reserve (52°57'01.2"N 2°39'05.6"W). *D. magna* were kept under standard conditions at 21°C and a 14:10 light:dark photoperiod for two generations in the laboratory before the experiment to reduce any potential maternal effects (see Plaistow & Collin, 2014; Plaistow *et al*., 2015). The offspring from the second F3 clutch was then used for experimentation. Individuals in the laboratory experiment were fed ad libitum once daily on a high concentration food diet of 200 cells per µL of the algae *Chlorella vulgaris*.

*Experiment 1: Comparing the life-histories of animals inside and outside field cages*

The first experiment took place in the laboratory, under controlled conditions of 21 and a 14:10 light:dark photoperiod using a single *D.* *magna* clone from Brown Moss. 43 paired replicates were set up in caged and uncaged treatments, split over two time blocks, for a total of 86 individuals. We used Finum® permanent filters (model Brewing Basket M, Finum, UK) as individual field cages. Each cage consisted of a plastic cup-shaped frame (length 7.3cm, diameter 6cm) with sides and base composed of a 170μm stainless steel mesh (Fig. 1). 43 cages were placed inside 300mL glass jars filled with 260mL of artificial pondwater and enriched with 1.2mL algal extract (Baird *et al.*, 1989). A single *D. magna* neonate was placed inside the mesh cage with another neonate placed outside the cage such that each had access to roughly equivalent volumes of media in the jar (see Fig. 1). The fine mesh size prevented neonate *Daphnia* from passing through, hand removing neonates from the inside of the cage with a pipette was similar to removing them from a glass jar. Each jar was filled with algal food (*Chlorella vulgaris*, 200 cells/ µL) pipetted inside the cage and swirled gently to allow homogenisation of the medium. Experimental containers were exchanged every other day to prevent build-up of algae along the bases of the cage and beaker. Life history data was collected until individuals had dropped their second clutch as described in Plaistow and Collin (2014). Each animal was checked daily and photographed within 24h after being born, upon reaching sexual maturity (assessed as the first time eggs appeared in the brood pouch), and after dropping their second clutch using a Canon EOS 350D digital camera connected to a Leica MZ6 dissecting microscope at 2.5x magnification. All images were then measured using the software ImageJ (Schneider, Rasband and Eliceiri, 2012). This methodology allowed us to collect data on the following life-history traits: length at maturity (mm), length at second clutch (mm), age at maturity (days), age at second clutch (days), mean fecundity (mean number of neonates produced in clutches one and two), average offspring size (mean length across five neonates from clutch one and five neonates from clutch two of each individual *D. magna*), juvenile growth rate ((length at maturity - length at neonate)/age at maturity), and adult growth rate ((length at second clutch - length at maturity)/(age at second clutch - age at maturity)).

The data were analysed using R version 4.0.2 (R Core Team (2020), 2020). Any differences in the multivariate phenotype of caged and uncaged individuals were tested for using a permutational multivariate analysis of variance (perMANOVA). We calculated pairwise Gower distances using vegdist {vegan} to account for differences in scales between the life history variables (Gower, 1971). The calculated distance matrices were then used in perMANOVAs run for 9999 permutations using the adonis function {vegan} (Oksanen *et al.*, 2013). Principal component analysis (PCA) was used to visualise the multivariate phenotypes using the prcomp function. Ellipses within the PCA plots show 95% percent confidence intervals around the centroids of the treatment groups. Life history traits were subsequently investigated individually using linear mixed models (LMMs), including the experimental block as a random factor (*Life history trait~Cage treatment+(1|block)*, to evaluate whether potential differences between caged and uncaged *D. magna* were caused by strong effects in a few traits or weak effects in many. Residual distributions were evaluated and where the Shapiro-Wilks test showed non-normal distributions, or the Levene’s test indicated heteroscedasticity, boxcox transformations were performed on the data using powerTransform {car} (Fox and Weisberg, 2011). A Chi-squared test was used to test for differences in mortality between caged and uncaged treatments.

*Experiment 2: Comparing the performance of cages and jar assays in the field*

The second experiment took place in a circular mesocosm of 2m diameter and 1m water depth at Ness Botanic Gardens (53°16'19.56" N, 3°2'44.16" W) in March 2019, during which time water temperature fluctuated around 2.5 and photoperiod increased from 11.5h to 12.8h. We compared the life-histories of three *D. magna* clones (BMH175, BMH47 & BMH 30) using two different methods: our individual field cages and a normal glass jar assay similar to that used in the laboratory, where the water in each jar was replaced on a daily basis. To set up the experiment, 10 replicates of each clone were added to individual jars and cages resulting in 60 individuals in total (see Fig. 1). Each cage was attached to a polystyrene ring, allowing the cages to float at the surface of the mesocosm (see Fig. 1b). Cages were attached in groups of three, with one replicate from each clone forming part of the trio. Each trio of floats was then weighted using a falcon tube filled with gravel, to prevent strong winds from capsizing the experimental containers (Fig. 1c). Each jar was filled with 150mL of mesocosm water and placed on a submerged plastic bench such that jars sat at roughly the same height in the water column as the field cages (Fig. 1b). This ensured that temperature was consistent across both treatments. The jars were filled daily using water from the mesocosm (filtered through the mesh lid to prevent predators from entering). All experimental containers were covered with a fine mesh (300µm) held by a durable elastic band to prevent escape should the cage/jar capsize. These mesh lids also served to prevent airborne predators or resting eggs from entering the containers. Growth data for both jar and caged *D. magna* was recorded until they reached maturity, collected by photographing each *D. magna* daily using a GXM-HD51 digital microscope at 2.5x magnification and measuring the images on ImageJ (Schneider, Rasband and Eliceiri, 2012). As a result, we compared the juvenile growth rate, size at maturity and age at maturity of the three different clones when reared in field cages, or in submerged glass jars filled with fresh media each day.

All statistics were performed in R version 4.0.2 (R Core Team (2020), 2020). We fitted linear models (LMs) for each life history trait as a response variable and treatment (Cage, jar) and clone (BMH175, BMH47 & BMH 30) as fixed factors (*Life history trait~Cage treatment\*Clone*). As above, any data observed to be non-normal or heteroscedastic was boxcox transformed using powerTransform {car} (Fox and Weisberg, 2011). Mortality differences between experimental treatments was again tested for using a Chi-square test.

Results

*Experiment 1: Comparing the life-histories of animals inside and outside field cages*

There was a marginally significant difference in the multivariate phenotype of *Daphnia* *magna* individuals reared inside and outside of the cages (perMANOVA: F1, 58 = 1.746, P=0.05, Fig. 2b). The accompanying biplot revealed that *D. magna* within the cages tended to mature at a smaller size but grow more as an adult. This observation was confirmed by the univariate analysis which demonstrated that individuals reared inside the cages had a slower juvenile growth rate (LMM: F1, 29 = 8.468, P=0.007), smaller size at maturity (LMM: F1, 29 = 13.91, P<0.001) and produced slightly smaller offspring (LMM: F1, 29 = 6.794, P=0.014). All other traits did not differ between treatments (LMM: P > 0.05, Table 1). There was no difference in mortality between caged and uncaged treatments (Chi-square: X2 = 0, df = 1, P=1).

*Experiment 2: Comparing the performance of cage and jar assays in the field*

Being reared in a field cage or in a jar had no effect on the juvenile growth, size at maturity and age at maturity across the three tested clones (LMs, all P>0.3, Table 2). However, we did detect differences in the life-histories of the three different clones which were independent of the cage treatment, specifically in juvenile growth rates (LM, Clone effect, F2,36 =3.335, P=0.047). There was also a significant difference in *Daphnia* mortality between cage and jar treatments, with significantly more of the jar-reared *Daphnia* dying before reaching maturity (Chi-square: X = 3.889, df = 1, P=0.048).

Discussion

*Daphnia* is an ideal model organism for investigating if and how shallow freshwater organisms can adapt to environmental change (Altshuler *et al.*, 2011; Miner *et al.*, 2012). However, attempts to study adaptation in *Daphnia* in the wild are hampered by the fact that it is not possible to mark and track individual animals. Field cages are a simple solution that may allow us to study individual-level phenotypic response to environmental conditions *in situ* (O’Brien and Kettle, 1981; Haupt *et al*., 2009; Bjergager *et al.*, 2012). Testing the assumption that animals inside field cages experience the environment in the same way as animals on the outside of the cages is an essential first step which is not addressed by most field cage using studies. In this study we used a simple laboratory experiment to demonstrate that cages do not limit access to algal resources. Then, in a second experiment conducted in a mesocosm under semi-natural conditions we demonstrated that clonal variation in life histories was as detectable in field cages as it was in a much more labour-intensive laboratory style assay conducted in the field. Juvenile survival was also improved in the field cages.

Model organisms have been incredibly useful for understanding many aspects of biology from gene functions to eco-evolutionary dynamics. However, their value for understanding aspects of global change biology is limited if we cannot study them in the wild and quantify individual-level responses to the real dynamic multifaceted cues of a natural environment. In some cases, we can’t study them in the wild because we know very little about their ecology (Parichy, 2015). But in other cases, such as *Daphnia*, the problem is simply that we cannot mark and track individuals. Field cages have previously been utilised as a way of getting around this problem (O’Brien and Kettle, 1981; Yurista, 2001; Bjergager *et al.*, 2012; Ieromina *et al.*, 2014; Bruijning, ten Berge and Jongejans, 2018). They have successfully been used to record life history data of population embedded individuals and small groups in the laboratory (Reichwaldt, Wolf and Stibor, 2004; Bruijning, ten Berge and Jongejans, 2018), semi-natural (Bjergager *et al.*, 2012), and natural environments (Yurista, 2001; Haupt *et al*., 2009; Ieromina *et al.*, 2014), and to develop models explaining population level changes from individual life history parameters (Bruijning, ten Berge and Jongejans, 2018). O’Brien and Kettle (1981) used a dye experiment to demonstrate that media in and out of cages is quickly mixed, and reported that the growth rate of populations kept in cages in the field were comparable to those of populations kept in the laboratory with excess food, although no data was presented. Furthermore, Reichwaldt, Wolf and Stibor (2004) observed that algal growth rates were unchanged by daily addition and removal of fine mesh cages in their jars, but did not include *Daphnia* populations in and out of the cage treatment for comparison. None of the previous studies explicitly tested the assumption that animals reared inside field cages experience the environment in the same way as animals on the outside of the cages. And importantly, no study has ever previously tested the assumption that the cage mesh is fully permeable to food, which is key to individuals within cages experiencing the environmental conditions and provides individuals reared inside and outside cages with the same resource availability. Testing this assumption is critical if field cages are going to be a useful tool for understanding how individual level responses to environmental change scale up to the population, community and ecosystem-level (O’Brien and Kettle, 1981; Bruijning, ten Berge and Jongejans, 2018).

In our first experiment we reared paired individuals inside and outside of field cages and fed algae on the inside of the field cage each day to test the hypothesis that animals reared outside the cage do not do significantly worse than animals on the inside where the food was placed each day. In fact, we found that individuals reared on the outside of cages actually did slightly better by growing faster, maturing at larger sizes and producing slightly larger offspring. Although we cannot fully explain why animals on the outside did better, we suspect it could be because the volume of media on the outside of the cage (approx. 145ml) was actually slightly greater than the volume of media inside the cage (approx. 115ml), a result of the tapered shape of the cage. Irrespective of what caused the difference in the life histories of animals reared inside and outside cages, the fact that animals on the outside do not do worse than animals on the inside of cages where the food was put each day strongly supports the hypothesis that cages allow the free flow of algae. As a result, our findings support the idea that field cages could be used to quantify individual life histories in wild environments.

In order to test the hypothesis that field cages are useful for quantifying individual *Daphnia* life-histories in the wild, we conducted a second experiment where we compared the life history of three *D. magna* clones from the same population using field cages and a typical laboratory style assay conducted in a semi-natural mesocosm. Both approaches allowed us to detect differences in the life-histories of the three clones that were the same irrespective of the method used. Moreover, there were no differences in the estimates of mean life history traits for each clone measured in jars and in field cages. Therefore, we can conclude that both methods were comparable in their ability to quantify clonal variation in individual level life-histories. However, there are a number of reasons why the field cages are preferable to the jar approach. First, there is a significant difference in workload and required visits to the experimental site because jars must be changed every day and cannot be left in the wild for long periods of time. Second, changing jars every day increases disturbance and the requirement to handle and conceivably stress the *Daphnia*. This can also increase the risk of mortality; we observed that 13 *Daphnia* died in the jars but only 5 died in the cages. Third, *Daphnia* kept in jars might experience the same temperature variation and photoperiod, but the individuals being measured are still to some extent isolated from their environment and from dynamic changes occurring throughout the day in factors such as density cues, kairomones, and oxygen. For a jar assay the dynamism of such cues is constrained by the frequency of the jar changes. While the lack of constant food flux through the day did not affect growth rates at early spring temperatures, nutrient limitation in closed containers is likely to have a greater impact in the summer when temperature dependent growth rates reach their maximum.

There are of course still important differences between our field cages and a truly wild setting. The main differences lie in the cages’ restriction of movement and associated behaviours such as diel vertical migration. While the cages can be left free to float across the entire surface of a mesocosm or pond, the *Daphnia* do not get to choose where they graze, nor do they get to move vertically in the ponds. Diel vertical migration could however be easily mimicked by moving the cages (even automatically) between depths in the morning and evening as done by Haupt *et al.* (2009) with larger population level cages. Or alternatively by building cages as columns that allow free vertical movement. Furthermore, animals in cages are not directly exposed to predation which is often considered to be the strongest selection pressure operating in *Daphnia* populations (Lass and Spaak, 2003). However, individuals inside cages are exposed to predator cues and the large effect these have on individual life-histories (Hammill, Rogers and Beckerman, 2008), allowing researchers to separate the threat of predation from actual predation effects on populations in wild or semi-wild conditions.

Demonstrating that field cages can be a useful and reliable way to measure individual *Daphnia* life-histories in the field opens up a number of future possibilities. First of all, this opens up the possibility to study the differences between the laboratory and the field explicitly, for example by comparing life histories of a large number of clones between the two settings and testing whether the extent of genotypic variation is comparable. Second, the cages will allow studying *Daphnia*’s role in food webs and the wider community and its impact on ecosystem function in a more realistic way. Field cages will conceivably allow us to generate accurate individual level data required to parameterize models such as integral projection models (IPMs) that are used to predict population level responses to real environmental change from natural environments. Bruijning et al (2018) have recently used such an approach to parameterise IPMs for laboratory populations of *Daphnia*. But no study has yet used such an approach in wild or semi-wild populations. Finally, *Daphnia magna* is one of the most important ecotoxicology organisms. Although ecotoxicology is useful for defining acceptable doses of chemicals that can be released into the environment, this doesn’t necessarily help us to understand the long-term impact that exposures to novel anthropogenic stressors have in natural environments where populations are genetically variable and environments are dynamic. Field cages used in combination with replicated mesocosm studies could be one way forward (O’Brien and Kettle, 1981; Bjergager *et al.*, 2012; Ieromina *et al.*, 2014).

Conclusion

In summary, our ability to understand aspects of global change biology in model organism such as *Daphnia* is limited if we cannot study them in the wild and quantify individual-level responses to natural environments. By demonstrating that individual-level field cages don’t limit access to resources, and that cages are as capable of detecting clonal variation in life-history traits as more labour-intensive jar assays, our results demonstrate that field cages are a feasible approach for collecting individual life-history data in natural environments. Having the capacity to measure genetic variation in responses to environmental cues in natural populations will, we hope, enhance the value of *Daphnia* studies aimed at predicting population-level responses to environmental change.

References

Altshuler, I. *et al.* (2011) “An integrated multi-disciplinary approach for studying multiple stressors in freshwater ecosystems: Daphnia as a model organism,” in *Integrative and Comparative Biology*. doi: 10.1093/icb/icr103.

Baird, D. *et al.* (1989) “The Long‐Term Maintenance of Daphnia magna Straus for Use in Ecotoxicity Tests: Problems and Prospects,” in *Proceedings of the First European Conference on Ecotoxicology*, pp. 144–148.

Barata, C. *et al.* (2000) “Comparing population response to contaminators between laboratory and field: An approach using Daphnia magna ephippial egg banks,” *Functional Ecology*. doi: 10.1046/j.1365-2435.2000.00445.x.

Bjergager, M. B. A. *et al.* (2012) “Synergy between prochloraz and esfenvalerate in Daphnia magna from acute and subchronic exposures in the laboratory and microcosms,” *Aquatic Toxicology*. doi: 10.1016/j.aquatox.2011.12.001.

Bruijning, M., ten Berge, A. C. M. and Jongejans, E. (2018) “Population-level responses to temperature, density and clonal differences in Daphnia magna as revealed by integral projection modelling,” *Functional Ecology*. doi: 10.1111/1365-2435.13192.

Burks, R. L., Jeppesen, E. and Lodge, D. M. (2001) “Pelagic prey and benthic predators: Impact of odonate predation on Daphnia,” *Journal of the North American Benthological Society*. doi: 10.2307/1468092.

Cabalzar, A. P. *et al.* (2019) “Parasite-mediated selection in a natural metapopulation of Daphnia magna,” *Molecular Ecology*. doi: 10.1111/mec.15260.

Clark, S. L. *et al.* (2015) “Comparative sensitivity of field and laboratory populations of Hyalella azteca to the pyrethroid insecticides bifenthrin and cypermethrin,” *Environmental Toxicology and Chemistry*. doi: 10.1002/etc.2907.

Colbourne, J. K., Singan, V. R. and Gilbert, D. G. (2005) “wFleaBase: The Daphnia genome database,” *BMC Bioinformatics*. doi: 10.1186/1471-2105-6-45.

Duchet, C. *et al.* (2010) “Population-level effects of spinosad and Bacillus thuringiensis israelensis in Daphnia pulex and Daphnia magna: Comparison of laboratory and field microcosm exposure conditions,” *Ecotoxicology*. doi: 10.1007/s10646-010-0507-y.

Ebert, D. (2005) *Ecology, Epidemiology and Evolution of Parasitism in*, *Evolution*. doi: 10.1108/02634501111102760.

Fox, J. and Weisberg, S. (2011) *An {R} Companion to Applied Regression,*. 2nd ed. Sage: Thousand Oaks (CA).

Fox, R. J. *et al.* (2019) “Beyond buying time: The role of plasticity in phenotypic adaptation to rapid environmental change,” *Philosophical Transactions of the Royal Society B: Biological Sciences*. doi: 10.1098/rstb.2018.0174.

Gasch, A. P., Payseur, B. A. and Pool, J. E. (2016) “The Power of Natural Variation for Model Organism Biology,” *Trends in Genetics*. doi: 10.1016/j.tig.2015.12.003.

Giebelhausen, B. and Lampert, W. (2001) “Temperature reaction norms of Daphnia magna: The effect of food concentration,” *Freshwater Biology*. doi: 10.1046/j.1365-2427.2001.00630.x.

Gienapp, P. *et al.* (2008) “Climate change and evolution: Disentangling environmental and genetic responses,” *Molecular Ecology*. doi: 10.1111/j.1365-294X.2007.03413.x.

Gower, J. C. (1971) “A General Coefficient of Similarity and Some of Its Properties,” *Biometrics*. doi: 10.2307/2528823.

Grodwohl, J. B., Porto, F. and El-Hani, C. N. (2018) “The instability of field experiments: building an experimental research tradition on the rocky seashores (1950–1985),” *History and Philosophy of the Life Sciences*. doi: 10.1007/s40656-018-0209-y.

Hammill, E., Rogers, A. and Beckerman, A. (2008) “Costs, benefits and the evolution of inducible defences: A case study with Daphnia pulex,” Journal of Evolutionary Biology. 10.1111/j.1420-9101.2008.01520.x

Hatch, A. C. and Burton, G. A. (1999) “Sediment toxicity and stormwater runoff in a contaminated receiving system: Consideration of different bioassays in the laboratory and field,” *Chemosphere*. doi: 10.1016/S0045-6535(99)00023-5.

Haupt, F. *et al*. (2009) “Daphnia diel vertical migration: Implications beyond zooplankton,” *Journal of Plankton Research*. 10.1093/plankt/fbp003

Hebert, P. D. N. (1978) “The Population Biology of Daphnia (Crustacea, Daphnidae),” *Biological Reviews*. doi: 10.1111/j.1469-185x.1978.tb00860.x.

Ieromina, O. *et al.* (2014) “Population responses of Daphnia magna, Chydorus sphaericus and Asellus aquaticus in pesticide contaminated ditches around bulb fields,” *Environmental Pollution*. doi: 10.1016/j.envpol.2014.05.020.

Kellogg, E. A. and Shaffer, H. B. (1993) “Model organisms in evolutionary studies,” *Systematic Biology*. doi: 10.1093/sysbio/42.4.409.

Kohler, R. (2002) *Landscapes and Labscapes; Exploring the lab-field border in biology*. London: University of Chicago press.

Korpelainen, H. (1986) “The effects of temperature and photoperiod on life history parameters of Daphnia magna (Crustacea: Cladocera),” *Freshwater Biology*, (16), pp. 615–620.

Lagerspetz, K. Y. H. (2006) “What is thermal acclimation?,” *Journal of Thermal Biology*. doi: 10.1016/j.jtherbio.2006.01.003.

Lampert, W. (2006) “Daphnia: Model herbivore, predator and prey,” *Polish Journal of Ecology*.

Lass, S. and Spaak, P. (2003) “Chemically induced anti-predator defences in plankton: A review,” in *Hydrobiologia*. doi: 10.1023/A:1024487804497.

Miner, B. E. *et al.* (2012) “Linking genes to communities and ecosystems: Daphnia as an ecogenomic model,” *Proceedings of the Royal Society B: Biological Sciences*. doi: 10.1098/rspb.2011.2404.

Morin, J. P. (1998) *Experimental Ecology: Issues and Perspectives.* 1st Editio. Oxford: Oxford University Press.

O’Brien, W. J. and Kettle, D. (1981) “A zooplankton bioassay chamber for lab and field use,” *Journal of Plankton Research*. doi: 10.1093/plankt/3.4.561.

Oksanen, J. *et al.* (2013) *Package vegan: Community Ecology Package*, *R package version 2.3-1*. doi: 10.4135/9781412971874.n145.

Parichy, D. M. (2015) “The natural history of model organisms: Advancing biology through a deeper understanding of zebrafish ecology and evolution,” *eLife*. doi: 10.7554/eLife.05635.001.

Pigliucci, M. (2001) *Phenotipic plasticity: beyond nature and nurture*, *Heredity*.

Plaistow, S. J. *et al.* (2015) “Offspring provisioning explains clone-specific maternal age effects on life history and life span in the water flea, daphnia pulex,” *American Naturalist*. doi: 10.1086/682277.

Plaistow, S. J. and Collin, H. (2014) “Phenotypic integration plasticity in Daphnia magna: an integral facet of G × E interactions - Plaistow - 2014 - Journal of Evolutionary Biology - Wiley Online Library,” *Journal of Evolutionary biology*. doi: 10.1111/jeb.12443.

Poorter, H. *et al.* (2016) “Pampered inside, pestered outside? Differences and similarities between plants growing in controlled conditions and in the field,” *New Phytologist*. doi: 10.1111/nph.14243.

R Core Team (2020) (2020) “R: A language and environment for statistical computing.,” *R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria*.

Reichwaldt, E. S., Wolf, I. D. and Stibor, H. (2004) “The effect of different zooplankton grazing patterns resulting from diel vertical migration on phytoplankton growth and composition: A laboratory experiment,” *Oecologia*. 10.1007/s00442-004-1645-9

Schneider, C. A., Rasband, W. S. and Eliceiri, K. W. (2012) “NIH Image to ImageJ: 25 years of image analysis,” *Nature Methods*. doi: 10.1038/nmeth.2089.

West-Eberhard, M. J. (1989) “Phenotypic plasticity and the origins of diversity,” *Annual review of ecology and systematics. Vol. 20*. doi: 10.1146/annurev.es.20.110189.001341.

Woodcock, B. A. *et al.* (2016) “Replication, effect sizes and identifying the biological impacts of pesticides on bees under field conditions,” *Journal of Applied Ecology*. doi: 10.1111/1365-2664.12676.

Yurista, P. M. (2001) “Growth, Survivorship and Reproduction of Daphnia middendorffiana in Several Arctic Lakes and Ponds,” *Journal of Plankton Research*. doi: 10.1093/plankt/23.7.733.

Zbinden, M., Haag, C. R. and Ebert, D. (2008) “Experimental evolution of field populations of Daphnia magna in response to parasite treatment,” *Journal of Evolutionary Biology*. doi: 10.1111/j.1420-9101.2008.01541.x.

Tables

**Table 1.** Summary of individual life history trait analyses by ANOVAs for the laboratory experiment. To meet the assumptions of the ANOVA, boxcox transformations were performed where indicated by Shapiro Wilks or Levene's test. Lambda values are listed for transformed variables.

|  |  |  |  |
| --- | --- | --- | --- |
| *LH trait* | *Df* | *F value* | *P value* |
| Age at maturity  *Lambda=0.9290* | 1  29 | 1.8448 | 0.1849 |
| Size at maturity | 1  29 | 13.907 | **0.0008 \*\*\*** |
| Juvenile growth rate | 1  29 | 8.4675 | **0.0068 \*\*** |
| Adult growth rate | 1  29 | 0.0778 | 0.7823 |
| Average clutch number | 1  29 | 0.4291 | 0.5128 |
| Average offspring size | 1  29 | 6.7941 | **0.0143 \*** |

**Table 2:** Summary of individual life history trait analyses by ANOVAs for the field experiment. To meet the assumptions of the ANOVA, boxcox transformations were performed where indicated by Shapiro Wilks or Levene's test. Lambda values are listed for transformed variables.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| LH trait | Treatment | Df | F value | P value |
| Age at maturity  Lambda=-0.2369 | Cage type  Clone  Cage type: Clone  Residuals | 1  2  2  36 | 0.7829  2.6932  0.0866 | 0.3821  0.0813  0.9172 |
| Size at maturity | Cage type  Clone  Cage type: Clone  Residuals | 1  2  2  36 | 0.0471  0.2798  0.0344 | 0.8294  0.7576  0.9663 |
| Juvenile growth rate | Cage type  Clone  Cage type: Clone  Residuals | 1  2  2  36 | 1.0798  3.3349  0.3612 | 0.3057  **0.0469 \***  0.6993 |

Figure Legends

**Figure 1**. a) The field cage design used in the initial laboratory experiment consisting of a Finum Brewing Basket M coffee filter within a glass beaker, with one *Daphnia* within the cage and one outside. b) The jars used in the field experiment, with a secured mesh gauze on top, as displayed under the jar. c) A set of three cages were tied together for the field experiment, with each containing one *Daphnia*, so each set of three contained a replicate from each clone. The field cages were attached to a falcon tube containing gravel as a weight. Each cage was secured with a mesh lid surrounded by polystyrene floats.

**Figure 2**. Principal components analysis of life history parameters across clones for the laboratory experiment. Contributions to principal component space is shown in the biplot (a) PC1 (41.77% of variation) vs PC2 (27.03%). The life history parameters measured are: adult growth rate (grad), juvenile growth rate (grjuv), average clutch number (avgclNo), length at second clutch (L2cl), length at maturity (LMat), age at maturity (agemat), average offspring size (aveoffsize), and age at second clutch (age2cl). (b) 95% confidence intervals of group means are plotted for “Outside Cage” and “Inside Cage” *Daphnia.* Lines indicate distance of each individual from respective group centroids.

**Figure 3**. Life history traits; a) age at maturity, b) size at maturity, c) juvenile growth rate, in *D. magna* of three clones (30, 47, 175) in two different containers; caged (C) and jar (J). Edges of the box represent the median, and the 25th and 75th percentiles, and the whiskers cover the 95th percentiles. Filled circles represent potential outliers.

Data Accessibility Statement

Daphnia life history data for both lab and mesocosm experiments are publicly available on the Dryad Digital Repository doi:10.5061/dryad.zgmsbcccq

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Author Contribution

**Michael O’Connor**: formal analysis (equal); investigation (lead); methodology (equal); project administration; visualization (equal); original draft preparation (lead); review and editing. **Daniel Sadler**: formal analysis (equal); investigation; visualization (equal); review and editing. **Franziska Brunner**: formal analysis (equal); validation (equal); review and editing. **Alan Reynolds**: formal analysis (supporting); investigation (supporting). **Nicola White**: investigation (supporting). **Stephen Price**: investigation (supporting); methodology (equal). **Stewart Plaistow**: conceptualization (lead); methodology (equal); supervision; validation (equal); review and editing.

Conflict of Interest

The authors declare that they have no conflict of interest.

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