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Daniel A Sasson, Joseph F Ryan

Ctenophores (comb jellies) are emerging as important animals for investigating fundamental questions across numerous branches of biology (e.g., evodevo, neuroscience. and biogeography). Several ctenophore species including, most notably, *Mnemiopsis leidyi*, are known as invasive species, adding to the importance of studying the ecology of these animals. Despite the growing interest, relatively little is known about ctenophore reproduction. Like most ctenophores, *M. leidyi* is a simultaneous hermaphrodite capable of self-fertilization. In this study, we assess the influence of light on spawning, the effect of body size on spawning likelihood and reproductive output, and the cost of self-fertilization on egg viability in *M. leidyi*. Our results suggest that *M. leidyi* spawning is more strongly influenced by circadian rhythms than specific light clues, and that body size significantly impacts spawning and reproductive output. We also find a lower percentage of viable embryos from *M. leidyi* that were spawned alone versus those that were spawned in pairs, suggesting that self-fertilization may be costly in these animals. These results provide critical insight into the reproductive ecology of these ctenophores and provide a fundamental resource for researchers working with *M. leidyi* in the laboratory.

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Abstract

Ctenophores (comb jellies) are emerging as important animals for investigating fundamental questions across numerous branches of biology (e.g., evodevo, neuroscience. and biogeography). A few ctenophore species including, most notably, *Mnemiopsis leidyi*, are known as invasive species, adding to the importance of studying the ecology of these animals. Despite the growing interest, relatively little is known about ctenophore reproduction. Like most ctenophores, *M. leidyi* is a simultaneous hermaphrodite capable of self-fertilization. In this study, we assess the influence of light on spawning, the effect of body size on spawning likelihood and reproductive output, and the cost of self-fertilization on egg viability in *M. leidyi*. Our results suggest that *M. leidyi* spawning is more strongly influenced by circadian rhythms than specific light clues, and that body size significantly impacts spawning and reproductive output. We also find a lower percentage of viable embryos from *M. leidyi* that spawned alone versus those that spawned in pairs, suggesting that self-fertilization may be costly in these animals. These results provide insight into the reproductive ecology of these ctenophores and provide a fundamental resource for researchers working with *M. leidyi* in the laboratory.

Introduction

Ctenophores (comb jellies) are fascinating planktonic animals most easily recognized by eight rows of cilia that they use as their primary means of locomotion. Recent work suggests ctenophores are the sister group to the rest of all animals and therefore are especially informative as to the state of the most recent common ancestor of animals (Dunn et al., 2008; Hejnol et al., 2009; Ryan et al., 2013; Borowiec et al., 2015; Chang et al., 2015; Whelan et al., 2015) but see (Pisani et al., 2015). This phylogenetic position, the availability of nuclear and mitochondrial

genome sequences (Pett et al., 2011; Ryan et al., 2013), and the ease with which embryos can be collected and observed (Pang & Martindale, 2008b) has made the ctenophore *Mnemiopsis leidyi* an emerging model system in which to study animal evolution and development (Pang & Martindale, 2008a). In addition, since the invasion of *M. leidyi* into European waters from its native range on the Atlantic seaboard (Vinogradov et al., 1989; Reusch et al., 2010) has had profound impacts on European fisheries (Kideys, 2002; Oguz, Fach & Salihoglu, 2008; Finenko et al., 2013), interest in the biogeography and invasion dynamics of *M. leidyi* has continued to grow.

The reproductive biology and life-history of *M. leidyi* has likely played a major role in its ability to invade and establish populations in foreign waters. *M. leidyi*, like most ctenophores, are simultaneous hermaphrodites that have the ability to self-fertilize and have been observed to produce thousands of eggs a day (Baker & Reeve, 1974; Costello et al., 2006; Graham et al., 2009; Jaspers, Møller & Kiørboe, 2011; Lehtiniemi et al., 2012; Jaspers, Costello & Colin, 2014). Offspring may develop from egg to reproductive adult in as few as 13 days (Baker & Reeve, 1974; Costello et al., 2012). *M. leidyi* may even produce viable gametes as juveniles (Martindale, 1987).

A number of studies have described the spawning behavior of *M. leidyi* (Baker & Reeve, 1974; Pang & Martindale, 2008b). Early research suggested that *M. leidyi* spawns as a response to darkness (e.g., sunset) (Freeman & Reynolds, 1973), and a recent a study on the effects of starvation on egg production noted that most eggs were produced during the night (Jaspers, Møller & Kiørboe, 2015). However, current spawning protocols state that *M. leidyi* use light cues to trigger spawning, readily releasing gametes upon exposure to light after spending at least three to four hours in darkness (Pang & Martindale, 2008b).

Adult *M. leidyi* vary dramatically in body size and this variation can affect both the likelihood to spawn and the number of eggs produced (Baker & Reeve, 1974; Finenko et al., 2006; Graham et al., 2009; Jaspers, Møller & Kiørboe, 2011). Animals are more likely to spawn as they grow larger (Baker & Reeve, 1974) and larger animals generally produce more eggs per day (Baker & Reeve, 1974; Finenko et al., 2006; Jaspers, Møller & Kiørboe, 2011; Jaspers, Møller & Kiørboe, 2015). However, the threshold size before spawning begins has varied from between 10 and 15mm (Finenko et al., 2006; Jaspers, Møller & Kiørboe, 2011) to 32mm (Baker & Reeve, 1974) across studies. In general, *M. leidyi* in European waters where they are invasive tend to spawn at smaller sizes (Finenko et al., 2006; Jaspers, Møller & Kiørboe, 2011) than in their native range (Baker & Reeve, 1974; Graham et al., 2009). It is unclear what factors effect this wide variation in initial spawning size.

While self-fertilization may provide the benefit of allowing *M. leidyi* to reproduce when conspecifics are not present, it may also come with the cost of inbreeding depression. Inbreeding depression has been shown to affect the viability of offspring in many systems (Charlesworth & Charlesworth, 1987; Crnokrak & Roff, 1999; Herlihy & Eckert, 2002). Rates of self-fertilization and inbreeding depression may be especially high in recently established populations where the population size and genetic diversity are low (Young, Boyle & Brown, 1996; Hedrick & Kalinowski, 2000). Thus, establishing the degree to which self-fertilization is costly in *M. leidyi* has particular significance for the management of areas where these ctenophores are invasive. However, to our knowledge, the costs associated with self-fertilization in *M. leidyi* have never been thoroughly investigated.

In this study, we aim to describe the reproductive cues, effect of body size on spawning, and potential costs of self-fertilization in *M. leidyi*. We first investigate spawning cues by placing

individuals under different light regiments. We then describe how body size influences spawning likelihood, egg production, and egg viability. Finally, we test whether self-fertilization in *M. leidyi* is costly by comparing the viability of eggs from ctenophores spawned individually to those spawned with a partner. If self-fertilization is costly, we predict that the offspring of *M. leidyi* spawning alone will have lower viability than those spawned in groups. Taken together, this study provides a detailed description of the reproductive ecology of *M. leidyi*, supplies revealing information for studying the invasive impact of these ctenophores, and therefore will become an important resource for establishing *M. leidyi* as a model system in the laboratory.

Materials & Methods

Collection

We carefully collected a total of 218 *M. leidyi* for the following experiments between June and October 2015 from the surface waters of Port Orange and St. Augustine, FL using a cteno-dipper (beaker on a stick) between the hours of 9:00 and 15:00. We generally collected animals on sunny days with low winds, but we did not keep a detailed record of the weather conditions for every collection day. We then transported them in buckets to the Whitney Laboratory for the Marine Biosciences in St. Augustine, FL. Upon arrival, the ctenophores were transferred first to a large beaker with filtered seawater. All seawater used in the experiments was pumped to the laboratory directly from the ocean and filtered with a 0.2 μm filter. The salinity of the seawater ranged between 35 – 36 ppt. We measured the polar length of every ctenophore along the oral/aboral axis to the nearest mm using calipers and placed them in individually marked 4” diameter glass dishes filled with 250mL of filtered seawater. Ctenophores were used in the experiments the same day of collection except for 39 individuals

that were kept overnight and used the following day (see below). Following the experiments, we released all ctenophores except for four individuals which were used for DNA and RNA extraction for another study. Due to this short time period in the lab, we did not feed any ctenophores. All experiments were conducted at room temperature (range 20 – 25°C).

Ctenophore distribution across experiments

The ctenophores we collected were often used in multiple analyses when appropriate. The 33 ctenophores used to test the Pang and Martindale protocol (2008b) were not used in any other analysis. All the remaining ctenophores we collected that were placed individually in bowls (N = 118) were used to measure the effect of body size on spawning likelihood. We did not refine our egg estimation protocol (see below) until partway through the experiment, so we measured the correlation between body size and egg production using 30 *M. leidyi*. Of these 30 ctenophores, we measured egg viability 24 hours later in all but one individual. Finally, we compared egg production and offspring viability between the aforementioned 30 ctenophores and 50 ctenophores that were placed in bowls in pairs (N = 25 bowls).

Light effects on spawning and egg production

We tested the protocol described in Pang and Martindale (2008b) using 33 *M. leidyi* that we collected between June 30, 2015 to August 12, 2016 (see supplemental data). These ctenophores ranged in size from 27 – 57mm (median: 43mm). These ctenophores were either placed in the experiment the day of collection (N = 25) or kept overnight in a large kreisel aquarium and placed in the experiment the day following collection (N = 39). We did not monitor animals for spawning while they were in the kreisel nor were animals fed. Between the hours of 10:00 and 18:00, we placed these animals in 4” dishes with 250ml of filtered seawater

126 in the dark for three to four hours. Upon exposure to light, bowls were monitored over the next
127 two hours for the presence of eggs.

128 We conducted a separate set of experiments to test the importance of light cues for
129 spawning on a subset of the *M. leidyi* that we collected from Port Orange (N=66, size range: 19 –
130 58mm, median: 41mm). On the day of collection, we separated each ctenophore into individual
131 4" diameter bowls filled with 250 mL of filtered seawater and haphazardly assigned individuals
132 to one of four treatments: A) constant light (N = 21), B) 11 hours of light and then four hours of
133 darkness (N = 15), C) seven hours of light and then eight hours of darkness (N = 12), or D)
134 constant darkness (N = 18). For the variable light treatments, 7w or 14w compact fluorescent
135 bulbs were attached to an automatic timer that turned off after the set amount of time. The
136 animals in the constant light treatment were placed under a lamp with a 15w compact fluorescent
137 bulb. All treatments began at 18:00 and ended at 9:00 the next day, at which point we exposed all
138 of the animals to light and immediately recorded whether eggs were present in each bowl. In this
139 experiment, we did not count the number of eggs spawned in each bowl, as we had not yet
140 developed our egg counting protocol (see below).

141 *Size effects on spawning, egg production, and egg viability*

142 In many systems, body size strongly influences reproductive output. We designed an
143 experiment to test the effect of body size on spawning likelihood, egg production, and offspring
144 viability. We tested the effect of size on spawning likelihood using the ctenophores already
145 spawned in the previous light cues experiment (N=66) and an additional 52 *M. leidyi* (total N =
146 118) that we collected. We measured the length of every ctenophore along the oral/aboral axis to
147 the nearest mm using calipers and then placed each in their own bowl with 250 mL of filtered

148 seawater. We left the additional 52 animals that had not already been spawned overnight in either
149 constant darkness (N = 26) or in a room with no artificial lights and an uncovered window to
150 experience natural changes in light (N = 26). We immediately recorded whether eggs were
151 present in each bowl on the following morning at 9:00. Since *M. leidyi* typically spawn hundreds
152 of eggs, we only considered bowls with at least 15 eggs as having a true spawn. We calculated
153 the effect of size on spawning likelihood using logistic regression and visualized the data with a
154 cubic spline.

155 A number of the ctenophores produced thousands of eggs, making a direct count of all
156 eggs difficult. To address this challenge, we developed a protocol to allow us to estimate the
157 number of eggs in each 2" bowl. We drew a 2" diameter circle and placed a square within the
158 circle so that each point on the square touched the edge of the circle (Fig. 1). Finally, we divided
159 the square into eight equal sized triangles that we labeled 1 – 8. For each ctenophore, we counted
160 the number of eggs in two randomly selected triangles. Two triangles comprise 15.91% of the
161 total area of the circle, and so to estimate the total number of eggs in the dish we multiplied the
162 combined egg count by 6.285. We tested this protocol by comparing the estimated number of
163 eggs to the actual number of eggs produced by two ctenophores with lower egg counts. The
164 number of eggs estimated was close enough to the actual number of eggs (50 estimated vs. 42
165 actual and 31 estimated vs. 29 actual) that we feel that this measure provides us with at least a
166 way to compare relative egg production across individuals. To collect the eggs of the
167 ctenophores that spawned, we poured the water and eggs from each bowl through a 70-µm filter.
168 The eggs of each ctenophore were then pipetted into separate 2" diameter bowls filled with
169 filtered seawater. Eggs were allowed to settle in the bowl before we counted eggs. Estimated egg
170 production was log-transformed to increase normality. We then evaluated the correlation

between body size and estimated egg production using linear regression for the individuals that spawned (N = 30). Egg production from more *M. leidyi* was not included in this analysis because we developed this method of estimating egg production halfway through the study.

To determine egg viability, we recounted the number of eggs in each dish after 24 hours. *M. leidyi* typically develop into juvenile cydippids within 24 hours after fertilization at room temperature (Martindale & Henry, 1997; Martindale & Henry, 2015). Juveniles can easily be distinguished from undeveloped eggs due to ciliary movement, and since viable embryos can swim away from their original triangle into the water column, we counted the number of undeveloped eggs in the same triangles as in the egg production assay. We then estimated the number of undeveloped eggs in the entire dish using the method described above. Using this estimate we calculated the percent of undeveloped eggs (estimated undeveloped eggs / estimated total eggs) and subtracted that number from one to determine the percentage of viable eggs. We used linear regression to assess the effect of body size on egg viability (N = 29).

Costs of self-fertilization

If self-fertilization is costly, we would expect *M. leidyi* spawning alone to have reduced offspring viability compared to those spawning in pairs. To test for such a cost, 80 *M. leidyi* were randomly placed by themselves or with another individual in a 4” diameter bowl with 250 mL of filtered seawater. Individuals spawned overnight and the next morning we estimated the number of eggs present in each bowl and the percent of viable offspring 24 hours later (see above). We compared estimated egg production and egg viability from ctenophores spawned alone (N = 30 for egg production, N = 29 for egg viability as we accidently did not count one bowl for viability) to ctenophores spawned in pairs (N = 25) using Student’s t-test.

193 All statistical analyses were run in JMP 11.0 (SAS Institute, Cary, NC).

194 **Results**

195 *Spawning cues*

196 Following the recent spawning protocol, only three of 33 (9.1%) animals had produced
197 any eggs. Furthermore, the few ctenophores that did spawn often released only a few eggs
198 (median = 19 eggs, range 18 – 177 eggs).

199 When placed in bowls overnight, we found no difference in spawning likelihood between
200 ctenophores kept in constant light (20/21 [95%] spawned), four hours of darkness (15/15 [100%]
201 spawned), eight hours of darkness (12/12 [100%] spawned), or constant darkness (17/18 [94%]
202 spawned).

203 *Size effects on spawning and egg viability*

204 The ctenophores in this experiment varied in size from 12 – 70mm (median = 38mm). As
205 *M. leidyi* grow larger, the likelihood of spawning significantly increases (Fig. 2, Logistic
206 regression, N = 118, $\chi^2 = 62.0$, $p < 0.0001$). All but three ctenophores larger than 30mm spawned
207 overnight, while only one ctenophore smaller than 26mm produced eggs.

208 We saw large variation in the number of estimated eggs spawned (range = 25-3934 eggs,
209 median = 484 eggs). Larger individuals generally produced more eggs (Fig. 3, N = 30, $r^2 = .38$,
210 $p < 0.001$). The light conditions the ctenophores experienced overnight did not affect egg
211 production (ANOVA, $F_{5,28} = 1.45$, $p = 0.24$). We also found a weak but insignificant positive
212 correlation between body size and egg viability (Fig. 4, N = 29, $r^2 = 0.12$, $p = 0.07$).

213 *Costs of self-fertilization*

We compared the egg production between *M. leidyi* that spawned alone (N = 30) with *M. leidyi* that spawned in pairs (N = 25). We found no difference between treatments in the estimated number of eggs produced (Fig. 5, Student's t-test, t-ratio = 0.005, p = 1.0). However, we did find that a higher percentage of offspring from individuals that spawned in pairs (N = 25) had developed after 24 hours when compared with individuals that spawned by themselves (N = 29, Fig. 6, Student's t-test, t-ratio = 2.3, df = 52, p = 0.025).

Discussion

Previous work has suggested that *M. leidyi* uses light cues to induce spawning (Freeman & Reynolds, 1973; Pang & Martindale, 2008b; Martindale & Henry, 2015); however, our attempts at replicating this spawning cue failed. Instead, we found that almost every *M. leidyi* over a certain size spawned overnight regardless of the light/dark cycle and despite slight differences in power levels of the light bulbs used in these experiments; even those individuals that were placed under constant light consistently spawned. This result suggests that *M. leidyi* spawns using a circadian rhythm rather than specific light cues, at least when initially brought into the lab. The results of BLAST searches suggest that the *M. leidyi* genome contains a number of orthologs involved in animal circadian rhythm including *Clock* and ARNTL (data not shown) These and other circadian rhythm genes have been associated with reproduction and reproductive timing in a number of systems (Boden & Kennaway, 2006; Leder, Danzmann & Ferguson, 2006; Liedvogel et al., 2009). Functional genomic analyses into how these circadian-rhythm genes affect spawning could potentially provide solid evidence linking circadian rhythms and *M. leidyi* spawning. Given the phylogenetic position of ctenophores as the sister lineage to the rest of animals (Dunn et al., 2008; Ryan et al., 2013; Borowiec et al., 2015; Chang et al., 2015; Whelan et al., 2015); but see (Pisani et al., 2015), such a study would also address to what extent the

237 genetic circuitry underlying animal circadian rhythm was present in the last common animal
238 ancestor.

239 Previous spawning protocols were described for *M. leidyi* populations near Woods Hole,
240 Massachusetts (Pang & Martindale, 2008b). To our knowledge, spawning protocols have not
241 previously been described for *M. leidyi* in the Atlantic waters of northern Florida. While these
242 two *Mnemiopsis* populations had previously been classified as a separate species (Massachusetts
243 = *Mnemiopsis leidyi*, Agassiz 1865, northern Florida = *Mnemiopsis mccradyi* Mayer, 1900), they
244 are now generally considered to be separate populations of the same species (Pang & Martindale,
245 2008a; Bayha et al., 2015), although this has yet to be extensively tested genetically. Populations
246 within species may differ in their reproductive timing or cues (e.g. Partecke, Van't Hof &
247 Gwinner, 2004; Moore, Bonier & Wingfield, 2005; Jaspers, Møller & Kiørboe, 2011) and so it
248 could be that the spawning behavior we observed is unique to the northern Florida population of
249 *M. leidyi*. Alternatively, spawning behavior could change across seasons with changes to day
250 length or water temperature (e.g. Sastry, 1963; Fell, 1976; Taranger et al., 1998).

251 Body size plays an essential role in ctenophore reproduction. Spawning occurs almost
252 exclusively in larger *M. leidyi* (>30mm), although a few individuals smaller than 30mm spawned
253 and a few animals larger than 40mm did not spawn (Fig. 3). Interestingly, this result differs from
254 *M. leidyi* reproduction in the Caspian and Baltic Seas where individuals commonly spawn when
255 over 10 mm and the most common size of spawning individuals is between 20 and 30 mm
256 (Finenko et al., 2006; Jaspers, Møller & Kiørboe, 2011). Why these populations differ in size of
257 reproduction is unclear, but they may be influenced by water temperature, resource abundance,
258 or low salinity (Finenko et al., 2006; Jaspers, Møller & Kiørboe, 2011; Jaspers, Møller &
259 Kiørboe, 2015). The differences in the non-native *M. leidyi* might also be a result of selection for

body size or age of reproductive maturity due to selective pressures imposed by ship-ballast transport.

Not surprisingly, larger individuals in our study produced more eggs than smaller individuals (Fig. 4). Body size may correspond to nutritional status rather than age (Reeve, Syms & Kremer, 1989) and so larger ctenophores may simply be those well fed enough to produce gametes. The production of gametes is costly (Hayward & Gillooly, 2011) and smaller ctenophores preferentially allocate resources to somatic growth rather than gamete production (Reeve, Syms & Kremer, 1989). Since larger individuals consume more prey (Bishop, 1967; Finenko et al., 2006) they likely have more resources available to produce eggs than smaller individuals.

Body size may also affect offspring viability. We found that the percentage of developed eggs after 24 hours increased as individuals grew larger (Fig. 5), although this result was marginally not significant. If body size truly does affect offspring viability it may be due to sperm volume. If sperm are limited, especially in small individuals, larger animals may simply have more sperm available to fertilize eggs. Alternatively, larger animals may provision more resources to their eggs than smaller animals, which may increase egg viability or development speed. This possibility could be tested by comparing the size of eggs across body sizes.

We also found that *M. leidyi* individuals spawning alone had a lower percentage of developed offspring after 24 hours than ctenophores that spawned in pairs (Fig. 6). What contributes to this apparent cost to self-fertilization is unclear. It could be that spawning pairs simply fertilize more eggs than individuals spawning alone, which could occur if sperm are limited. Another possibility could be that the percentage of eggs fertilized did not differ between

treatments but that fewer fertilized eggs developed for individuals spawning alone. Although we did not differentiate between unfertilized eggs and non-developing embryos in this study, we did commonly observe embryos that appeared to have arrested development after only a few stages of cell division in both treatments. These results are consistent with a reduction in offspring viability due to inbreeding depression.

Interestingly, ctenophores in pairs did not produce more eggs than those spawning alone (Fig. 5). The average size of the ctenophores did not differ between treatments, suggesting that, when paired, ctenophores either reduce the number of eggs spawned or only one of the two ctenophores spawned eggs. This latter option, referred to as egg-trading, may indicate the intriguing possibility that ctenophores alternate between releasing sperm and eggs when in pairs or groups. Egg-trading has been reported in other simultaneously hermaphroditic systems including sea slugs, tobacco fish, and polychaetes (Leonard & Lukowiak, 1984; Sella, 1985; Petersen, 1995). This behavior could be used to reduce the chance of self-fertilization in *M. leidyi*. However, the underlying assumption of egg-trading is that individuals spawn with the same partners multiple times; we would not expect this to be the case in *M. leidyi* under natural circumstances since movement is largely governed by water flow.

Our results also suggest that individuals may be more efficient when spawning alone than with others. Because paired *M. leidyi* did not spawn more eggs than individuals that spawned alone, more total viable offspring were produced per individual when spawned alone than when paired despite their reduced percentage of offspring that developed. However, we only spawned each ctenophore once. Gamete production is costly (e.g. Hayward & Gillooly, 2011), and if individuals spawning alone require a longer refractory period for gametogenesis before spawning again than paired individuals, this increase in viable offspring may only be temporary.

Comparing the reproductive output and viability between paired and single individuals over multiple days could provide more resolution on the costs associated with self-fertilization.

The ability to self-fertilize almost certainly enhances the capability of ctenophores to spread when invading new areas. However, the costs to self-fertilization that we've demonstrated may at least slow down their invasive capabilities. These costs may be especially high at the initial stages of an invasion when population numbers and genetic diversity are low. Our self-fertilization experiment only examined one stage of development (i.e., 24 hours after spawning) in one generation and yet we still found evidence that self-fertilization is costly. Additional costs likely do not appear until later in life or after multiple generations of self-fertilized offspring. An experiment investigating the multi-generational effects of self-fertilization may provide a clearer picture of the hurdles, or lack of hurdles, *Mnemiopsis* faces when initially invading a new area.

Conclusions

Due to their evolutionary position as sister taxa to all other animals (Ryan et al. 2013), ctenophores in general, and *M. leidyi* in particular, are quickly emerging as new model systems from which to understand evolution, development, regeneration, and even human disease (Pang & Martindale, 2008a; Maxwell et al., 2014). Understanding the reproductive ecology of ctenophores is a necessary step in establishing these animals as tractable models for these areas of research. This study has reinforced the importance of body size in *M. leidyi* reproduction and has provided the first suggestions that self-fertilization may be costly in ctenophores. However, ctenophore reproduction in natural systems is still very much a mystery. For example, little is known about how common it is for *M. leidyi* to self-fertilize in the wild. We have shown that spawning likely follows a circadian rhythm, which may be a mechanism to increase the odds of

out-crossing if all animals spawn simultaneously. If self-fertilization is indeed costly, additional adaptions to increase the chance of out-crossing are likely. This work provides a fundamental resource for researchers working with *M. leidyi* in their laboratory, as well as, a jumping-off point from which future studies of *M. leidyi* reproductive biology can be launched.

Acknowledgements

We acknowledge Leslie Babonis, Kira Carreira, Marta Chiodin, Bailey Steinworth, and Allison Zwarycz for help with collecting *Mnemiopsis leidyi*. We thank Mark Martindale and David Simmons for advice on ctenophore husbandry and spawning. Finally, we would like to thank two reviewers for their helpful comments and suggestions.

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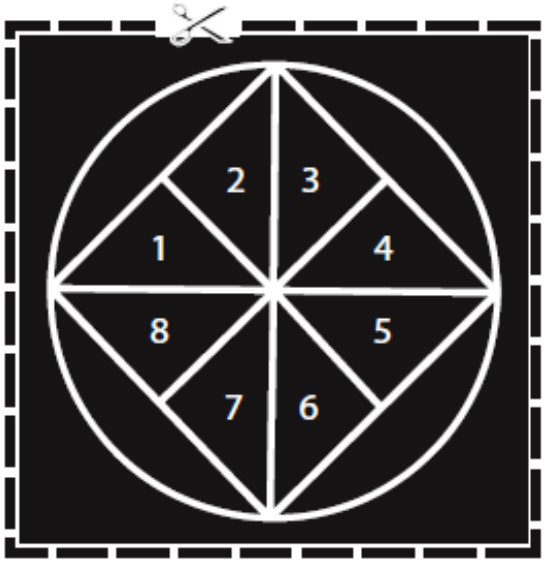

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Fig. 1 The diagram used to estimate egg numbers. Each triangle (labeled 1 – 8) represents 7.96% of the total area of the circle. We counted the eggs in two triangles and then multiplied the total by 6.285 to estimate the total number of eggs in the dish. Scaled to actual size used for 2” bowls.

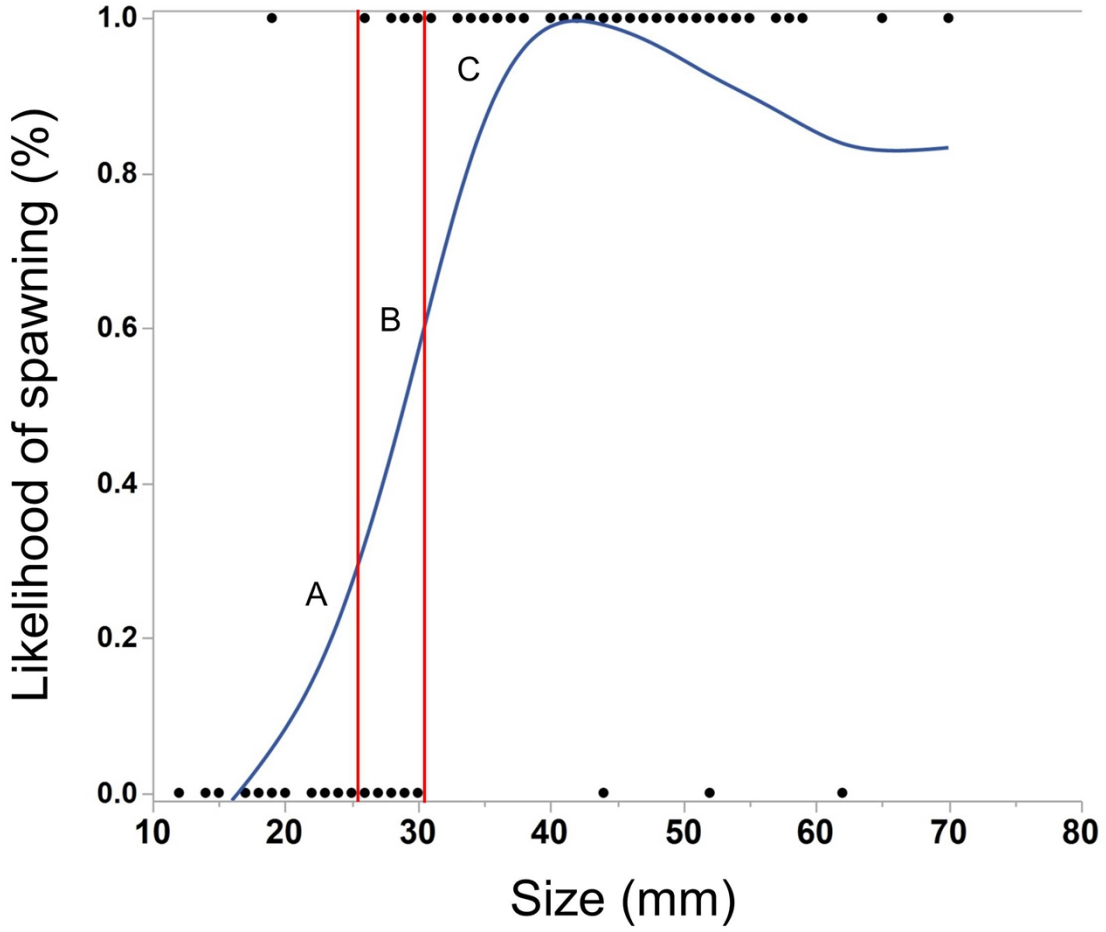

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Fig. 2 Cubic spline showing the effect of body size on the likelihood to spawn. Points along the lower x-axis indicate individuals that did not spawn, while point on the upper x-axis indicate individuals that did spawn. Multiple individuals may be represented by a single point. Ctenophores smaller than 26mm (section A) rarely spawned (1/22 = 5%) while those larger than 30mm (section C) almost always spawned (77/80 = 96%). Nearly half of the individuals between 26 and 30mm spawned (section B, 6/16 = 38%). Lambda value of cubic spline set to 1.

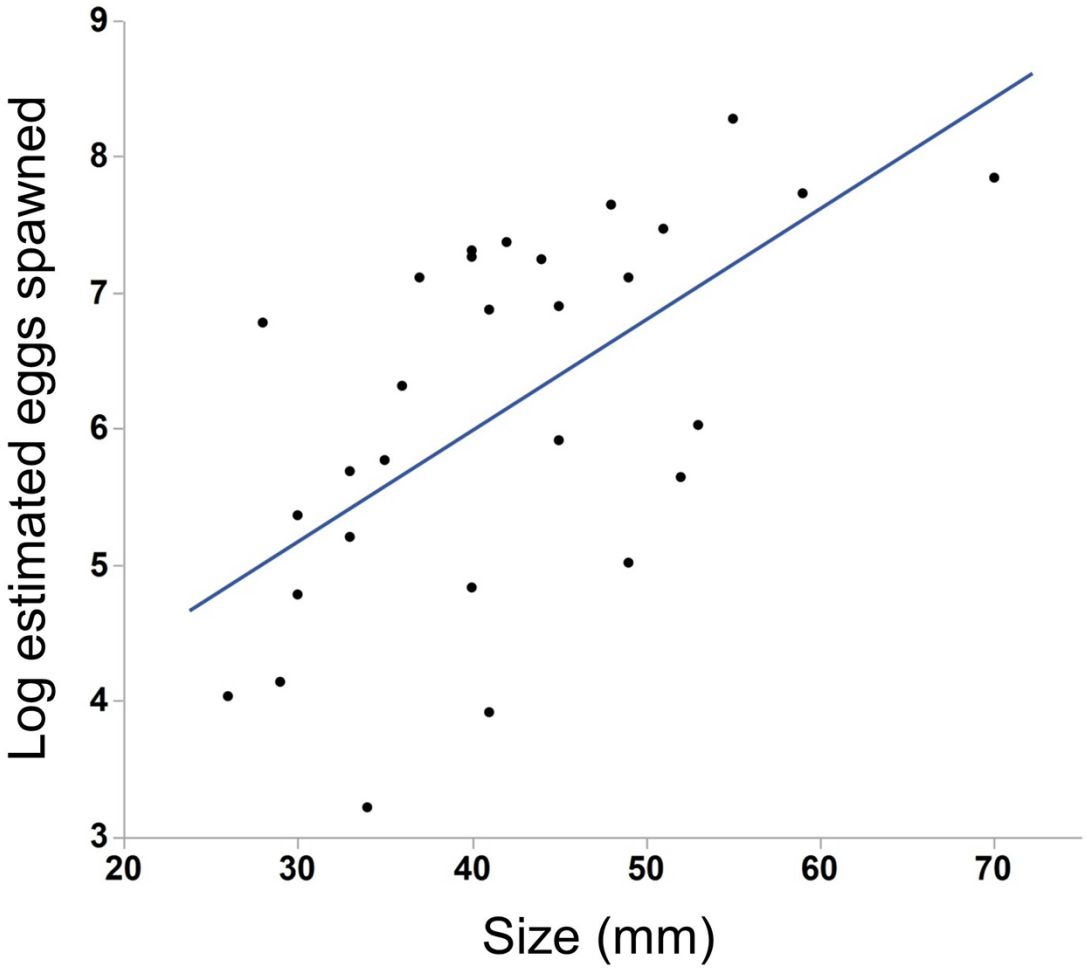

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Fig. 3 The effect of body size on egg production. Larger individuals generally produced more eggs than smaller individuals (N = 30, $r^2 = .38$, $p < 0.001$). Only those animals that spawned 25 or more eggs are included in the analysis and figure.

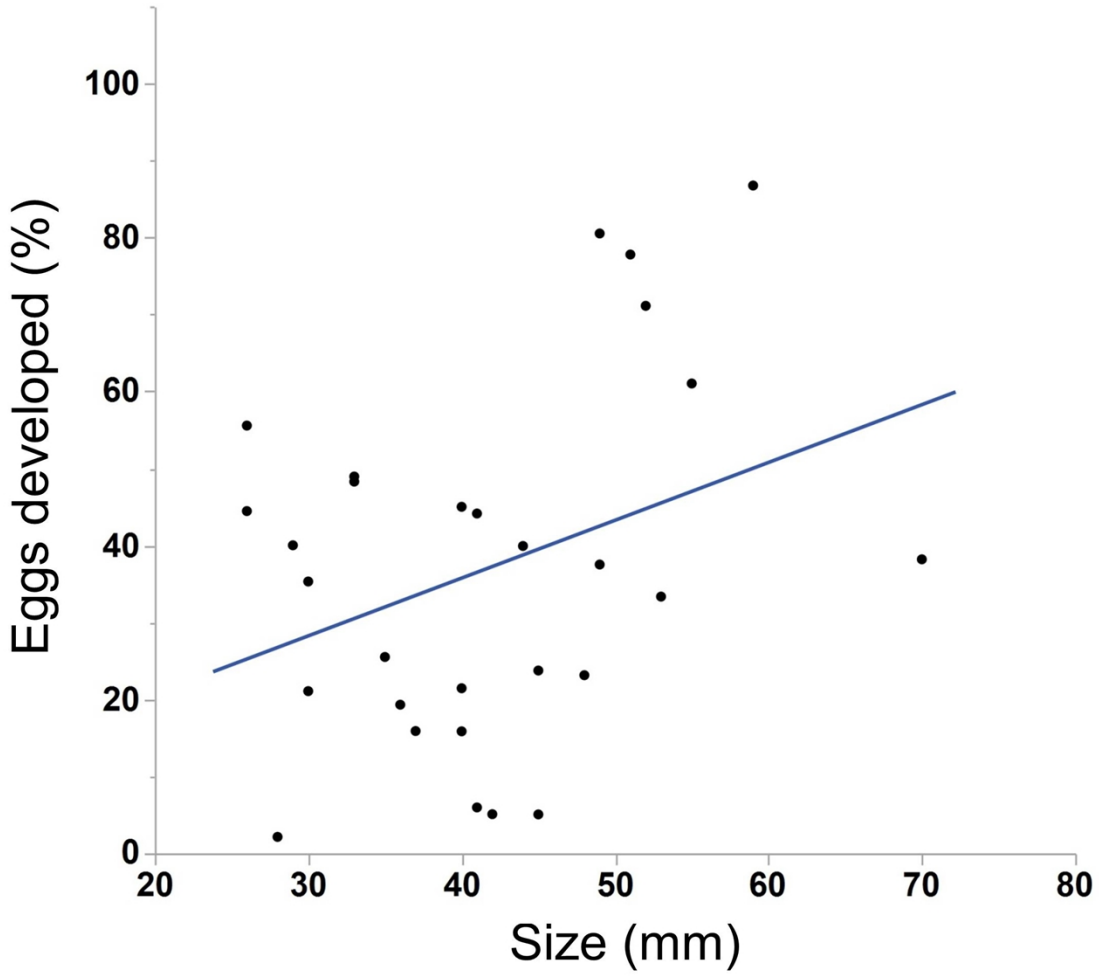

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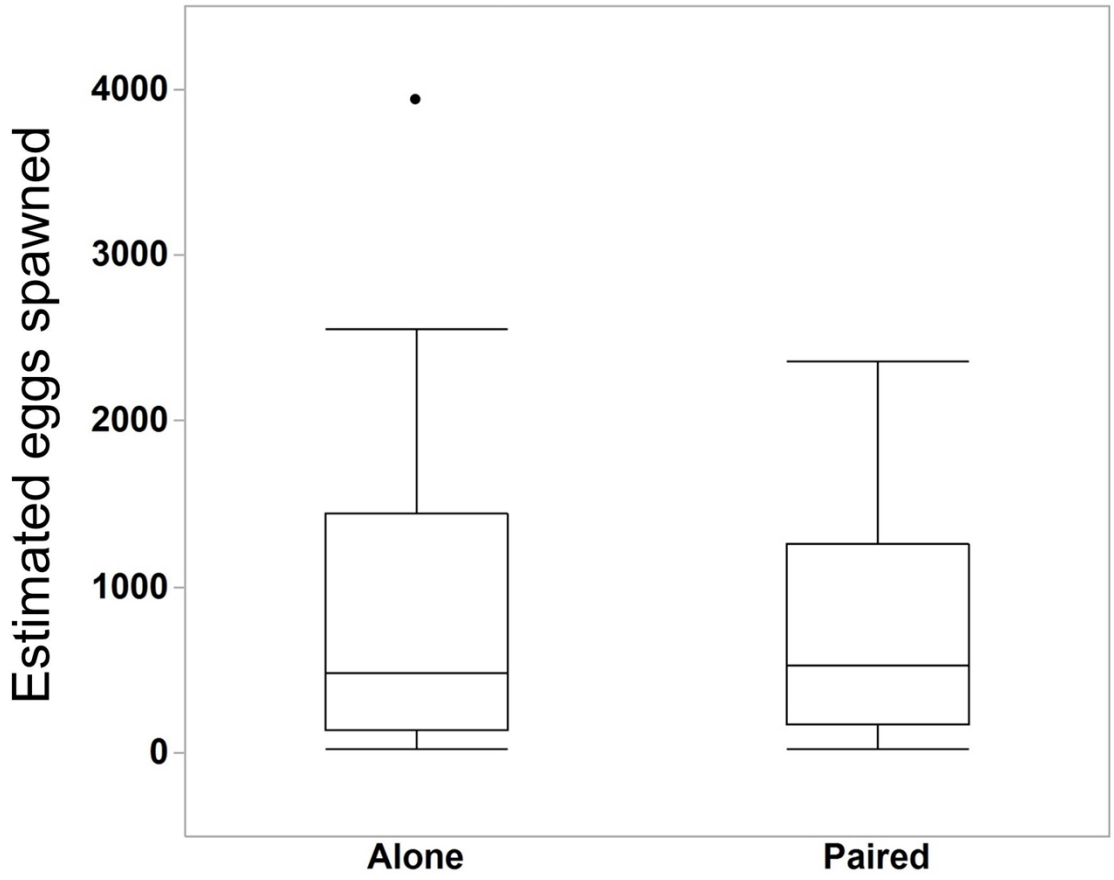
Fig. 4 Correlation between body size and egg viability. Body size positively correlated the percentage of eggs that developed after 24 hours, although the result was marginally not significant ($N = 29$, $r^2 = 0.12$, $p = 0.07$).



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483 Fig. 5 The estimated number of eggs in bowls of individuals that spawned alone (N = 29) and in pairs (N = 25). Surprisingly, two *M. leidyi* spawning together did not produce more eggs than individuals spawning alone (Student's t-test, t-ratio = 0.005, p = 1.0). The data point above the Alone box plot indicates an individual that spawned an estimated 3,934 eggs. Removing that data point does not change the overall findings of the analysis.

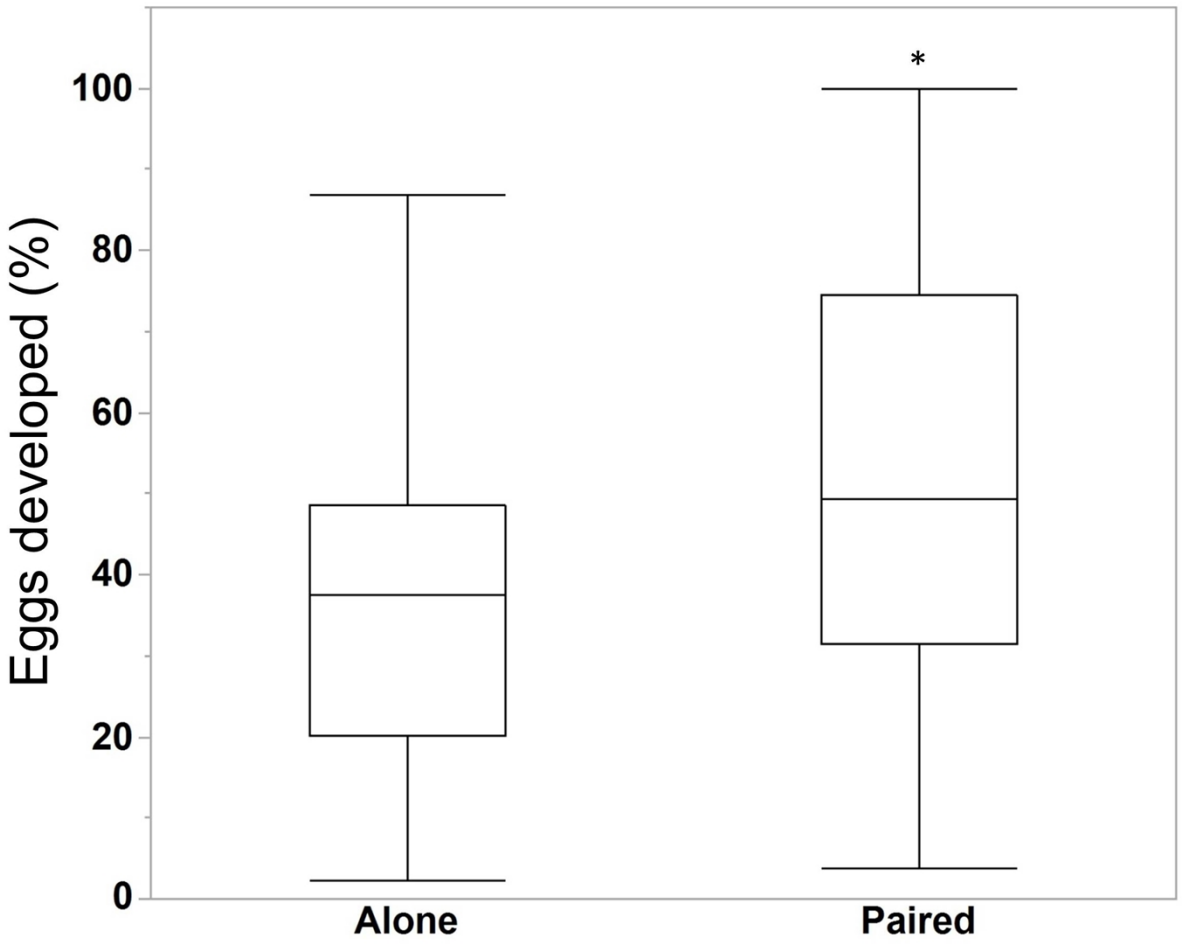

Fig. 6 The percentage of eggs developed after 24 hours for individuals spawning alone (N = 29) and in pairs (N = 25). A higher percentage of eggs developed for *M. leidyi* in pairs, possibility suggesting a cost to self-fertilization (Student's t-test, t-ratio = 2.3, df = 52, p = 0.025). Asterisk indicates significant difference across treatments.