2015

The Effect of Parental Size on Spermatophore Production, Egg Quality, Fertilization Success, and Larval Characteristics in the Caribbean Spiny Lobster, Panulirus argus

Mark J. Butler IV
Old Dominion University, mbutler@odu.edu

Alison Macdiarmid

Gaya Gnanalingam
Old Dominion University

Original Publication Citation

Butler, M. J., Macdiarmid, A., & Gnanalingam, G. (2015). The effect of parental size on spermatophore production, egg quality, fertilization success, and larval characteristics in the Caribbean spiny lobster, *Panulirus argus*. *ICES Journal of Marine Science, 72*((Supplement 1)), 115-123. doi:10.1093/icesjms/fsv015

ICES Journal of Marine Science

ICES Journal of Marine Science (2015), 72(Supplement 1), i115–i123. doi:10.1093/icesjms/fsv015

Contribution to the Supplement: '*Lobsters in a Changing Climate*'

Original Article

The effect of parental size on spermatophore production, egg quality, fertilization success, and larval characteristics in the Caribbean Spiny lobster, *Panulirus argus*

Mark J. Butler, IV[1]*, Alison Macdiarmid[2], and Gaya Gnanalingam[1]

[1]*Department of Biological Sciences, Old Dominion University, Norfolk, VA, USA*
[2]*National Institute for Water and Atmospheric Research, Wellington, New Zealand*

**Corresponding author: tel: +1 757 683 3609; fax: +1 757 683 5283; e-mail: mbutler@odu.edu*

Butler, M. J.IV, Macdiarmid, A., and Gnanalingam, G. The effect of parental size on spermatophore production, egg quality, fertilization success, and larval characteristics in the Caribbean Spiny lobster, *Panulirus argus*. – ICES Journal of Marine Science, 72: i115 – i123.

Received 24 August 2014; revised 7 January 2015; accepted 11 January 2015; advance access publication 17 February 2015.

The average size of spiny lobsters (Decapoda; Palinuridae) has decreased worldwide over the past few decades. Market forces coupled with minimum size limits compel fishers to target the largest individuals. Males are targeted disproportionately as a consequence of sexual dimorphism in spiny lobster size (i.e. males grow larger than females) and because of protections for ovigerous females. Therefore, overexploitation of males has led to sperm limitation in several decapod populations with serious repercussions for reproductive success. In the Caribbean spiny lobster, *Panulirus argus*, little is known about the effect of reduced male size on fertilization success or the role that individual size plays in gamete and larval quality. We conducted a series of laboratory experiments to test the relationship between male size and spermatophore production over multiple mating events and to determine whether spermatophore reduction and female size affected fertilization success or larval attributes in *P. argus* in the Florida Keys, FL (USA). We found that over consecutive matings, larger males consistently produced spermatophores of a greater weight and area than smaller males, although size-specific differences in sperm cell density were undetected and probably obscured by high variance in the data. Where spermatophores were experimentally reduced to mimic the decline in spermatophore size with declining male size, fertilization success (the number of fertilized eggs/total number of eggs extruded) declined, indicating that sperm availability is indeed limited. No maternal size effects on egg size or quality (C:N ratio) or larval quality (size, swimming speed, mortality) were observed. Our results demonstrate the importance of maintaining large males in populations of *P. argus* to ensure fertilization success and caution against their overexploitation through fishing, which may severely reduce reproductive success and thus population sustainability.

Keywords: egg quality, larvae, mating, *Panulirus argus*, reproduction, spermatophore.

Introduction

Fishing significantly alters the size structure of exploited populations with major consequences for mating systems, reproduction, and the sustainability of exploited populations (Rowe and Hutchings, 2003; Maxwell *et al.*, 2009; Butler *et al.*, 2011a; Garcia *et al.*, 2012; Kuparinen and Hutchings, 2012). Typically, the largest and oldest individuals are removed first, particularly where minimum size limits are enforced, which reduces the mean size of individuals in the population (Roberts and Polunin, 1991; Jennings and Lock, 1996; Heath and Speirs, 2012). Where there is sexual dimorphism in the size of adults and males grow larger than females, fishing can skew sex ratios in favour of females. Prohibitions on the removal of ovigerous females may further skew sex ratios. As highlighted by a number of decapod species, these fishing induced changes in size structure, abundance, and sex ratio can restructure mating systems and reduce the reproductive output of the population (Kendall *et al.*, 2002; Sato and Goshima, 2007; Sato *et al.*, 2010; Robertson and Butler, 2013). Such may be the case for the Caribbean spiny lobster, *Panulirus argus*.

Panulirus argus is ubiquitous throughout the Western Atlantic Ocean, Caribbean Sea, and Gulf of Mexico from NC, USA, to northeast Brazil (Briones-Fourzan *et al.*, 2008). Fisheries for *P. argus* are

some of the largest and most economically valuable in the Caribbean with an estimated annual regional value more than $450 million USD (CRFM, 2013). As a consequence of its high value and market demand, many regional populations of *P. argus* are currently fully capitalized or overfished (Chavez, 2009; Ehrhardt *et al.*, 2010). The species displays considerable sexual dimorphism; males grow more than three times larger (by mass) than females and are distinguishable from females by their broader sternum, elongated walking legs, and curved dactyls (Holthius, 1991). So although fishing reduces body size in both sexes, the decline is more pronounced in males (Bertelsen and Mathews, 2001; Cox and Hunt, 2005).

In unfished populations where large males are still present, lobsters demonstrate a lek-style mating system in which large males defend a den from other large males and females choose among males and their dens (Bertelsen and Cox, 2001). In heavily fished populations where large males have been removed this system breaks down, although competition among males still remains a major component of the mating system (Bertelsen and Mathews, 2001; Butler *et al.*, 2015). During mating, male and female *P. argus* couple and males deposit an external, tar-like spermatophore on the sternum of the female (Lipcius *et al.*, 1983; Figure 1). Females resist further mating attempts by males and scratch open the spermatophore 1–28 d after copulation to expose the non-motile sperm, which fertilize the eggs externally as they are extruded (Talbot and Summers, 1978; Butler *et al.*, 2011a). The fertilized eggs attach to the setose pleopods on the underside of the female's abdomen, and they are carried there for 3–4 weeks until they hatch (Saul, 2004). Throughout much of the Caribbean *P. argus* spawn year-round (Butler *et al.*, 2010), although spawning peaks in late spring and in subtropical areas, the breeding season is constrained to just spring–summer (Chubb, 2000). In Florida, for example, spawning generally commences in March and continues through August. However, the number of clutches and the duration of the spawning period vary with female size; larger females produce more clutches and become reproductively active earlier in the spawning period when compared with small, mature females (Bertelsen and Mathews, 2001). Mating of all palinurids occurs during the intermoult phase, and there is no clear link between molting and mating in *P. argus*, as there is in other spiny lobster species such as *Jasus edwardsii* (MacDiarmid and Butler, 1999).

Individual fecundity in female *P. argus* is size-dependent with larger individuals producing exponentially more eggs (Bertelsen and Mathews, 2001; Ehrhardt, 2005; MacDiarmid and Sainte-Marie, 2006). A reduction in female body size as a result of fishing, therefore, has clear implications for reproductive capacity in terms of egg production. What is less clear is the effect of reduced male size on reproductive dynamics. Decapods may experience sperm limitation if ejaculate size scales with male body size, mating history, expected female output, or future mating opportunities (reviewed by MacDiarmid and Sainte-Marie, 2006). The probability that populations are sperm limited is therefore closely related to population structure, mean male size, density, sex ratio, and the intensity of sexual competition (Sato *et al.*, 2010). Previous studies of *P. argus* have demonstrated a clear link between the availability of large males and female fecundity because spermatophore size, a function of male size, explains nearly half of the variance in clutch size (MacDiarmid and Butler, 1999) and because sperm:egg ratios—which are already low (~25:1)—are 40% lower in fished populations (Butler *et al.*, 2011a).

Building on the previous work of MacDiarmid and Butler (1999), we present the results of a series of laboratory experiments that describe the mating system of *P. argus* with special reference to the effect of male size on reproductive success. First, we experimentally manipulated the size of spermatophores on mated females to determine the potential effects of reduced male size on fertilization success and egg production by females. We then assessed the effects of male size on spermatophore production, depletion, and recovery over successive matings. Finally, using data from the spermatophore reduction and depletion/recharge experiments, we evaluated maternal effects—i.e. the effects of female size on egg and larval quality.

Methods

Spermatophore reduction experiment

To assess whether sperm availability can limit reproductive success in *P. argus*, we manipulated the size of spermatophores on mated females collected from the field and measured the resultant production of fertilized and unfertilized eggs. Female *P. argus* with spermatophores were collected by divers from the Florida Keys, FL, USA (a fished population), and the Dry Tortugas National Park, FL, USA (a no-take marine protected area; MPA) in February–March of 1998–2000. Lobsters were transported in aerated live wells to the Florida Fish and Wildlife Conservation Commission field laboratory in Marathon, FL, where the experiments took place.

Females were split into two size classes: small (carapace length 80–90 mm) and large (carapace length >90 mm) and kept in individual 75 l tanks with aeration and flow through seawater; lobsters were fed squid and shrimp *ad libitum* daily. These size classes of female lobsters represent those that are common in heavily fished vs. unfished populations of *P. argus* (respectively); thus, the results of this study provide specific insight into how current fishing practices potentially impact reproductive dynamics. Spermatophores on females were then manipulated with a scalpel and flat forceps such that lobsters belonged to one of three treatment groups: (i) a 50% reduction in spermatophore mass, (ii) a 75% reduction in spermatophore mass, and (iii) a no reduction control (Figure 1). Sperm cells are evenly distributed between the left and right halves (Butler *et al.*, 2011a) of the spermatophore, but are more concentrated in the posterior portion of each paired spermatophore. Therefore, the removal of one of the paired spermatophores is a proxy for a 50% reduction in spermatophore mass, which approximates the difference in spermatophore sizes transferred to females by large and small males (MacDiarmid and Butler, 1999); a 75% reduction in size was simply chosen as a third, extreme treatment. Although cutting the spermatophore transversely from anterior to posterior would have maintained the total area of the spermatophore, they were cut longitudinally to preserve as much as possible the tough outer layer of the spermatophore and prevent the exposure of the sperm before fertilization. The 50% reduction treatment would not have resulted in any inadvertent "leakage" of sperm from the remaining spermatophore, given that each spermatophore half is an independent unit produced by the paired testes and delivered via paired gonopores. Perhaps, sperm may have leaked from the remaining portion of the spermatophores in the 75% reduction treatment, but if so that procedural effect would be the same for both large and small lobsters.

Lobsters were then monitored daily for the presence of attached eggs, an indication of spawning and fertilization. After spawning, unfertilized eggs fail to attach to the female's pleopods, so we siphoned them from the tank bottom and counted them to estimate the abundance of unfertilized eggs (see below). When eyespots

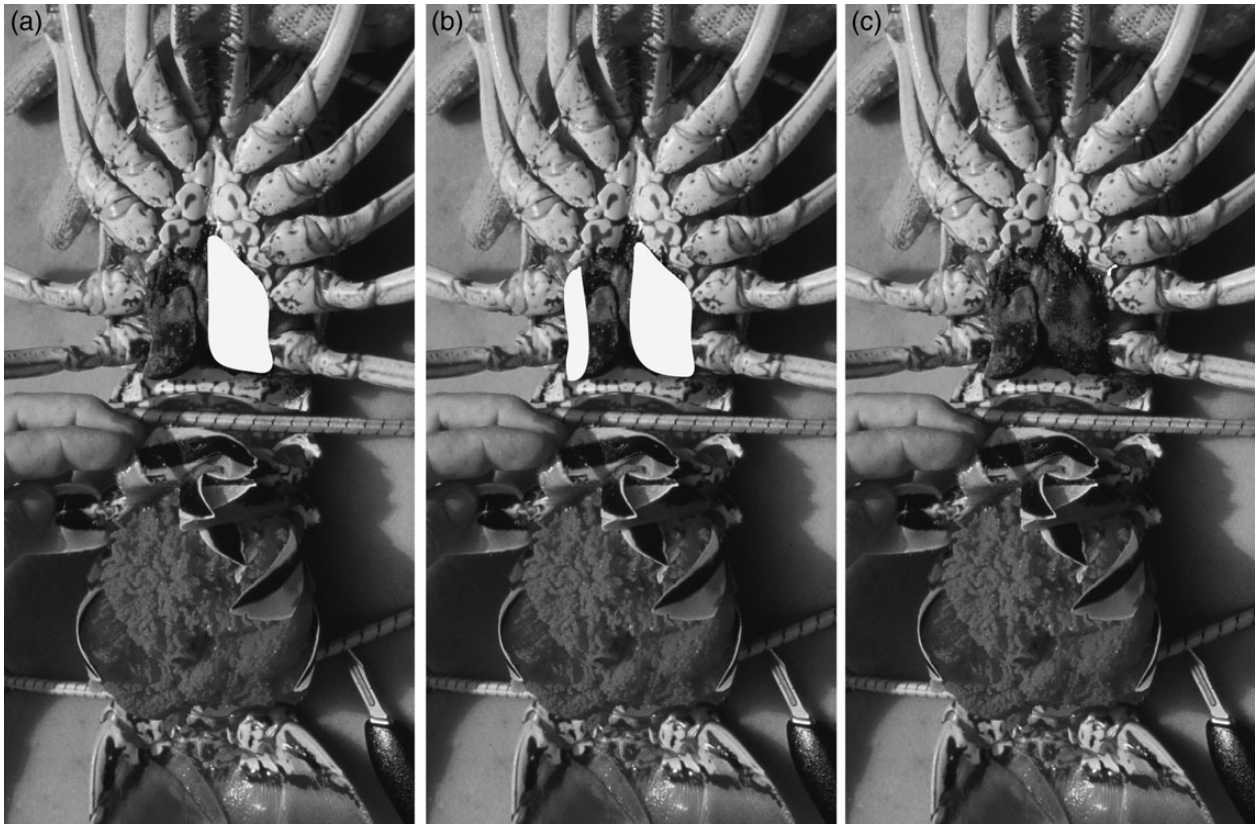

Figure 1. Visualization of the way in which spermatophore size was diminished in the spermatophore reduction experiment. In each frame is a ventral view of the same female *P. argus* with eggs from a previous clutch (visible under the abdomen) and a new spermatophore (black mass) that will be used to fertilize the next clutch. (a) 50% reduction treatment: white area depicts how half of the spermatophore was removed. (b) 75% reduction treatment: white areas depict how three-fourth of the spermatophore was removed. (c) Control: no manipulation of the spermatophore.

became visible in brooded eggs (~14 d after spawning), a scalpel was used to gently scrape off egg masses from the pleopods. Counts of unfertilized (siphoned from tank) and fertilized (scraped from female pleopods) eggs were made by taking three ~2 g subsamples (weighed to the nearest hundredth of a gramme) of each egg mass. Eggs were counted under a dissecting microscope to provide an estimate of individual female fecundity (number of fertilized eggs) and the total number of eggs extruded (number of fertilized + unfertilized eggs). Fertilization success is defined as the ratio of fertilized eggs retained on pleopods to the total number of eggs extruded. To evaluate differences in fertilization success as a function of the spermatophore reduction treatment, we used a two-factor ANOVA with female size class and treatment as factors.

Sperm depletion and recovery

We determined if large and small adult males differ in their production of spermatophores and their ability to recharge sperm stores after mating, by repeatedly mating large males collected from an MPA, and small males from a fished population, with several females in a laboratory experiment. Divers collected males and females from the Florida Keys fished population and the Dry Tortugas National Park MPA during February and March of 1999–2001. The absence of large males in the heavily exploited Florida Keys population necessitated the collection of large individuals only from the Dry Tortugas. All males were collected before the onset of the breeding season and therefore had not mated since the previous year's mating season. A single large male (126–160 mm CL; $n = 22$) or small male (90–99 mm CL; $n = 19$) along with seven females ranging in size from 70–144 mm CL were placed into experimental tanks (1.75 m diameter; 1500 l) that received aerated filtered seawater from a flow-through system. Ambient seawater temperature and photoperiod were maintained and lobsters were fed shrimp and squid *ad libitum*. These experimental conditions (e.g. sizes and sex ratios) are typical of conditions in the wild during the reproductive season when females significantly outnumber males on coral reefs because large, agonistic males temporarily drive smaller males into alternative habitats (Bertelsen and Mathews, 2001; Butler *et al.*, 2015).

Females were checked for spermatophores daily. Flat forceps were used to remove intact spermatophores after they had hardened for 24 h. Before their removal, the spermatophore area was estimated. An outline of the mass was traced onto a sheet of a clear acetate paper, which was scanned and digitally measured in Image J. Once removed, spermatophores were weighed to the nearest hundredth of a gramme and refrigerated at 5°C in 10 ml of filtered seawater until sperm counts could be conducted. Females were returned to their original experimental tanks after the removal of the spermatophore so that they could remate. In the Florida Keys and Dry Tortugas, large *P. argus* females mate and produce up to three and perhaps four clutches within a given season, whereas

small females produce only a single clutch per year (Lyons *et al.*, 1981; Bertelsen and Mathews, 2001). As such, we could rely on females remaining receptive to courtship and remating during the course of the experiment. The male mating frequency in the wild is unknown, but the lek-style mating strategy of large males indicates that at least large males mate repeatedly during the breeding season.

To conduct sperm counts, spermatophores were laterally sliced into thin sections (~0.5 mm thick) with a scalpel into a Petri dish. The spermatophore sections were then washed from the Petri dish into a 25-ml test tube with 10–15 ml of sterile seawater; the test tube was capped and then mechanically shaken for 3 min to liberate sperm from the spermatophore matrix. A haemocytometer was then used to count the number of sperm cells in five separate 10 μl aliquots of each sample. The total sperm number and sperm density (total sperm number/spermatophore weight) was then calculated. This method for sperm counts has been used previously and it has been shown to consistently liberate >70% of the sperm from the spermatophore matrix (Butler *et al.*, 2011a).

Males repeatedly mated with the females in their experimental tanks and the order in which spermatophores were produced was recorded. On the cessation of mating (i.e. production of new spermatophores) in each experimental tank, typically 10 d to a month from first mating, males were removed and isolated in individual tanks for 1, 2, or 3 weeks to potentially "recharge" their sperm stores. After the recharge period, males were returned to their original experimental tank with the same set of females to resume mating.

Sperm depletion and recharge rates were assessed as a function of male size with individuals separated into large (>100 mm CL) and small (<100 mm CL) size classes. To test whether sperm attributes (i.e. spermatophore weight, spermatophore area, sperm density, and total number of sperm) varied relative to male size for the first deposited spermatophore, a one-factor MANOVA with canonical discriminant function analysis was performed. To determine whether male size affected sperm attributes over repeated matings (sperm depletion), we used a split plot MANOVA with male size as the whole plot factor, ejaculate number as the subplot factor, and individual as a randomized block. To assess whether male size affected sperm attributes after the set recharge period, we used a two-factor MANCOVA with the covariate "time between mating" and the independent variables male size and recharge period (i.e. 1, 2, or 3 weeks). Finally, paired *t*-tests were used to compare spermatophores produced initially and following the recharge period by the same individual.

Egg quality and larval characteristics

During sperm depletion/recharge experiments, the spermatophore on six mated females from every treatment group was left intact and the date of spermatophore deposition and the date of egg extrusion were recorded. This took anywhere between 0 and 19 d for different females with a mean time of 4 d. These females were removed from the experimental tanks and on the 10th day after egg extrusion random samples of the egg masses were removed, for the assessment of maternal effects on egg quality and to provide the estimates of fecundity. Females were then returned to holding tanks until their remaining clutch had hatched. Eggs and newly hatched larvae from these females in the sperm depletion/recharge experiment and from control females in the spermatophore reduction experiment were used to assess possible maternal effects on egg and larval quality.

For egg quality, the egg area was determined by measuring the diameter of 20 eggs per clutch with a dissecting microscope fitted with an ocular micrometre. The C:N content of eggs (a measure of egg lipid content and thus egg condition or quality; Giminez and Anger, 2001; Liddy *et al.*, 2003; Bas *et al.*, 2007) was also determined from the subsamples of each egg clutch. Subsamples were rinsed in sterile seawater, frozen, and stored at −20°C, then dried before C and N were measured with a Carlo–Erba elemental analyser and standard methods.

Larval condition was measured in three ways: larval size, survival, and swimming speed. The carapace length of 20 first stage phyllosome larvae per clutch was determined with a dissecting microscope fitted with an ocular micrometre. Larval survival was assessed in a starvation trial in which 20 first stage phyllosome larvae per clutch were individually housed in 15 ml Petri dishes filled with sterile seawater but no food at ambient light and temperature (~26°C). Larval mortality was assessed daily and sterile seawater was changed daily until all larvae had expired. The swimming speed of ten individual phyllosome larvae per clutch was also estimated in a seawater-filled, black swimming chamber (25 cm long × 10 cm wide × 5 cm deep) with a 1-cm grid scale on the bottom. Clear holes (0.5 cm dia) at either end of the plastic chamber permitted light (wavelength: 400–600 nm; intensity: 0.80–1.00 μmol m^{-2} s^{-1}) to enter either end of the chamber from a 300-W quartz-halogen filament lamp (Olympus model LGPS) projected from a fibre optic cable and through a filter. This wavelength and intensity is similar to that observed in the surface water (<25 m) of the open sea where first-stage larvae normally dwell (Butler *et al.*, 2011b). At the start of each trial, the room was darkened and a single larva was added to one end of the test chamber, while the chamber was illuminated from the opposite end. First-stage phyllosome larvae are positively phototactic, so they swam towards the light at the opposite side of the chamber. We measured the distance the larvae moved over a 5-s period and then let the larvae swim all the way to the lighted end of the chamber. Larvae were given a 1-min respite in total darkness before we illuminated the opposite end of the chamber and repeated the procedure. The swimming speed of each larva was tested in this manner ten times and the mean swimming speed determined for each larvae. The relationships between female size and egg characteristics (area, C:N ratio) and larval characteristics (larval carapace length, days until 100% mortality, and swimming speed) were analysed with linear regression.

Results

Spermatophore reduction and sperm limitation

When spermatophores were experimentally reduced in area, the total number of eggs that were released did not differ significantly among treatments (Figure 2). However, fertilization success (number of fertilized eggs/total eggs extruded) was lower for females whose spermatophores had been reduced (Figure 2; ANOVA: $F = 13.59$, $p < 0.001$, $d.f. = 1$). *Post hoc* tests indicated that differences lay between the control and 50% reduction treatments (Tukey HSD, $p = 0.030$) and the control and 75% reduction treatments (Tukey HSD, $p = 0.003$) with no detectable difference between the reduction treatments. When fertilization success was assessed for large and small females separately, differences among treatments were only significant for small females (Figure 2; ANOVA: $F = 13.78$, $p = 0.001$, $d.f. = 1$); the results were similar but not significant for large females (ANOVA: $F = 1.34$, $p = 0.27$, $d.f. = 1$), although the results for large females are tentative given the small sample sizes, hence lower power of the tests when the data were divided into two lobster size groups.

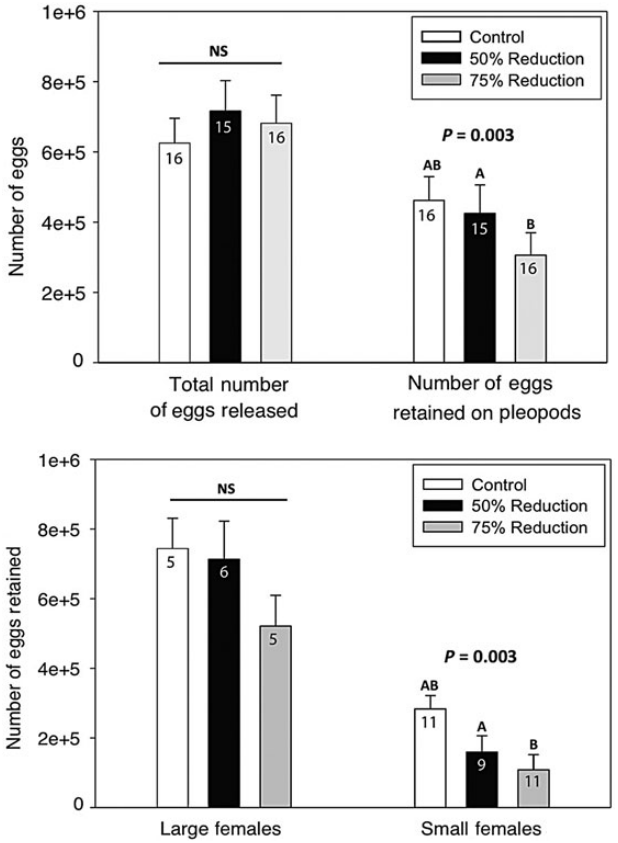

Figure 2. Results of the spermatophore reduction experiment; bars are the means with standard errors (top panel). The total number of eggs released (at left) by female *P. argus* in the three treatments and the total number of fertilized eggs retained by female lobsters (at right). (bottom panel) The number of fertilized eggs produced by large (at left) and small (at right) female lobsters in the three treatment groups. Note that the number of eggs retained by female lobsters reflects fertilization success because unfertilized eggs do not attach to the females pleopods. NS results and significant results (with *P*-values) among treatment groups are shown in each panel; treatments sharing a letter are not significantly different from each other.

Spermatophores depletion and recharge over multiple matings

Males took 1–22 d between consecutive matings with a mean time of 2.5 d between mating events. Attributes of initial spermatophores varied significantly between large and small males (Wilks' lambda, $p < 0.0001$), with large males typically producing spermatophores that were heavier and of a larger surface area (Figure 3; spermatophore weight: $F = 13.41$, $p = 0.001$, *d.f.* $= 1$; spermatophore area: $F = 14.07$, $p = 0.001$, *d.f.* $= 1$). However, the number of sperm cells and sperm density for initial spermatophores did not differ significantly between large and small males (number of cells: $F = 3.41$, $p = 0.72$, *d.f.* $= 1$; density: $F = 0.01$, $p = 0.943$, *d.f.* $= 1$), although the values for large males are consistently greater than those for small males and the lack of a difference may very well be due to the high variance in these data.

Males fertilized between 2 and 27 females consecutively; larger males (>100 mm) mated with 15 females on average and small males (<100 mm) mated with 11 females on average over a period of up to 1 month. This difference was not statistically significant (*t*-test: $t = 0.25$, $p = 0.80$, *d.f.* $= 28$). For males of all sizes

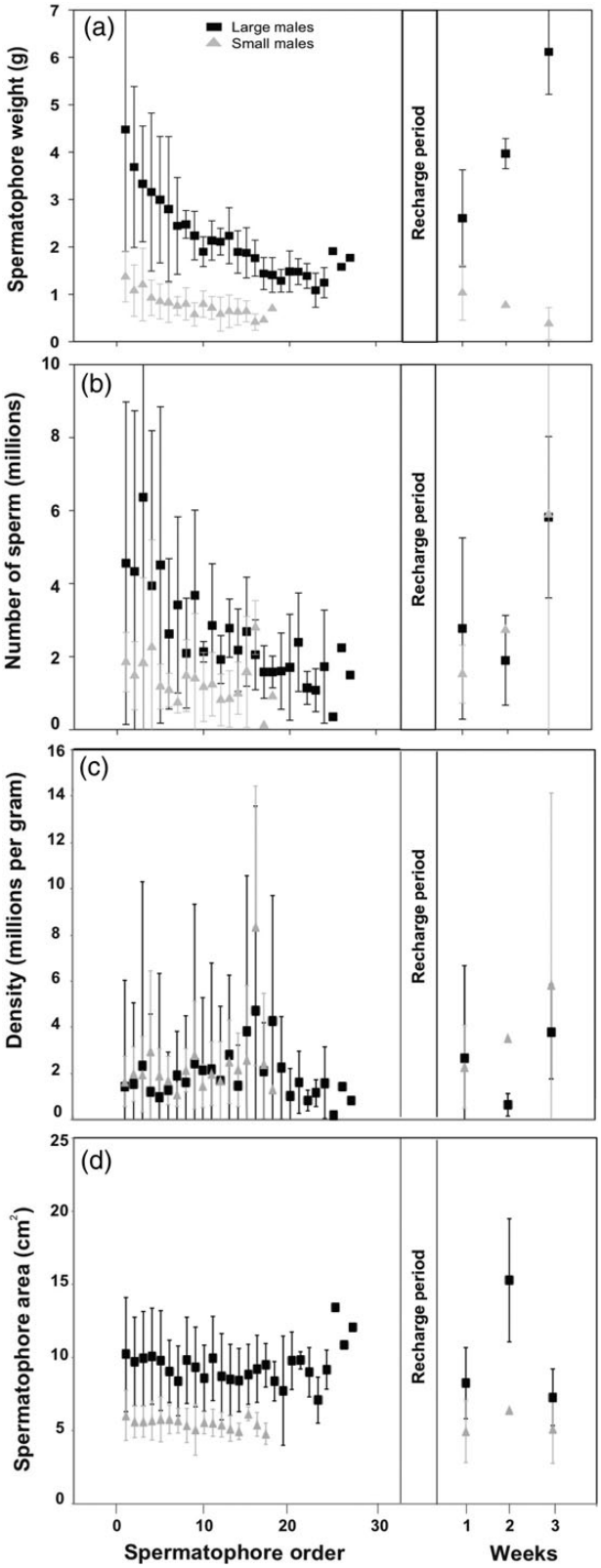

Figure 3. Results of the sperm depletion experiment for large (dark squares; $n = 30$) and small (grey triangles; $n = 11$) male *P. argus*; shown are the means with 1 s.d. Spermatophore weight (top panel), number of sperm per spermatophore (second panel), sperm density (third panel), and spermatophore area (bottom panel) are shown for consecutive matings and after a non-mating recharge period of 1, 2, or 3 weeks.

combined, the spermatophore weight and area decreased markedly as the number of spermatophores per individual increased (Figure 3; spermatophore weight: $F = 6.26$, $p < 0.001$, $d.f. = 41$; spermatophore area: $F = 2.82$, $p = 0.003$, $d.f. = 41$).

For all males combined, no significant differences in the total number of sperm cells and sperm density were observed over repeated matings. But there is a large amount of variance in the data, particularly for the smaller males, which likely obscures a clear result when all the data are combined. However, when analysed by size class, spermatophore weight, spermatophore area, and the total number of sperm cells declined as the number of spermatophores extruded per individual increased (Figure 3; spermatophore weight: $F = 75.18$, $p < 0.001$, $d.f. = 1$; spermatophore area: $F = 101.70$, $p < 0.001$, $d.f. =$; total number of cells: $F = 15.55$, $p < 0.001$, $d.f. = 1$).

After a recharge period of 1, 2, or 3 weeks, larger males again had heavier spermatophores that were larger in area (Figure 3; spermatophore weight: $F = 6.73$, $p = 0.018$, $d.f. = 1$; spermatophore area: $F = 4.65$, $p = 0.044$, $d.f. = 1$), but there were no differences between large and small males in the total number of sperm cells or sperm density (Figure 3; number of cells: $F = 1.82$, $p = 0.19$, $d.f. = 1$; sperm density: $F = 0$, $p = 0.99$, $d.f. = 1$). This same result was observed regardless of the length of the recharge period (Figure 3). There were also no differences in sperm attributes between initial spermatophores and spermatophores produced following the recharge period (paired t-test, $p > 0.05$), even when size classes were analysed separately, with the sole exception of spermatophore weight for the small males ($t = 3.8$, $p < 0.01$, $d.f. = 7$).

Egg quality and larval characteristics

Maternal size did not influence the measured egg characteristics (i.e. area and C:N ratio), larval carapace length, or larval survival (Figure 4). The slope of the regression of larval swimming speed with female carapace length differed significantly from zero and was unexpectedly negative, but that result was of borderline significance ($F = 5.51$, $p = 0.02$, $d.f. = 43$) and the relationship explained very little variance in the data, as shown by the low r^2 value ($r^2 = 0.09$; Figure 4).

Discussion

The rationale for this study was to obtain a greater understanding of the effects of male and female *P. argus* size on gamete production and quality, fertilization success, and larval characteristics associated with survival. Given the dramatic reduction in lobster size in exploited populations, correlations between offspring quality or quantity with paternal or maternal size could adversely affect the reproductive potential of those populations. Our results indicate that smaller males produce lighter and smaller spermatophores over consecutive mating events. Increased female body size, on the other hand, had no discernible effect on egg or larval characteristics beyond the well-known exponential relationship between maternal size and egg production.

Our finding that reduced spermatophore size did not alter the number of eggs extruded by females, but instead reduced fertilization success, indicates that smaller clutches produced by females mated by small males are not a result of reduced egg output by the female but instead reflect the sperm limitation of fertilization success. Spermatophore reduction also appeared to have a greater impact on the egg retention and hence fertilization success of smaller (<90 mm CL) females, as has been similarly noted for the Western rock lobster *P. cygnus* (Kuris, 1991). Large *P. argus* males actually allocate larger spermatophores to large females (MacDiarmid and Butler, 1999) and larger spermatophores are associated with positive residual clutch weights (MacDiarmid and Sainte-Marie 2006), so perhaps this results in an over-apportionment of a viable sperm available for fertilization, which would explain why our experimental reductions in spermatophore size were less effective on large females than smaller ones. Conversely, if males over-apportion sperm to larger females but are less generous to smaller females, as is true in the American lobster, *Homarus americanus*, then smaller females may receive under-apportioned spermatophores relative to large females (Gosselin *et al.*, 2003). Females across a range of taxa including fish (Skinner and Watt, 2007), insects, birds, and amphibians differentially allocate reproductive resources according to the attractiveness of their mate (see review by Sheldon, 2000). In decapods, evidence of differential allocation of eggs based on mate quality is limited to freshwater crayfish (Galeotti *et al.*, 2006).

For *P. argus*, the apparent inability to mediate egg output suggests that females should select larger males as mates so as to maximize fertilization success. Indeed, large females of all palinurid species thus far tested (*P. argus*, *P. guttatus*, *Jasus edwardsii*) experience lower fertilization success when mated with smaller males (MacDiarmid and Butler, 1999; Robertson and Butler, 2013; Butler *et al.*, 2015), implying that all experience strong selection for larger mates. Although true for some spiny lobsters (e.g. *P. guttatus*; Robertson and Butler, 2013; *J. edwardsii*; Butler *et al.*, 2015), it has not proven true in experiments with *P. argus* (Butler *et al.*, 2015). This discrepancy among species in female selectivity of males by size is difficult to reconcile and bears further study.

Spermatophores produced by large males were significantly heavier and larger initially and following multiple matings. This is a finding in accord with previous studies of sperm limitation in *P. argus* (MacDiarmid and Butler, 1999; Butler *et al.*, 2011a). The number of sperm cells in the spermatophore and sperm density, however, was not different between the size classes (the exception being the number of sperm cells within spermatophores following multiple matings). This despite indications that the mean number of sperm for large males was almost always higher than for small males (Figure 3). So perhaps the high variance in these results obscures a size-specific difference in sperm abundance in addition to the clear difference in spermatophore size. We also believe that our data on the size-specific male recovery of sperm stores after depletion are probably inconclusive. Although there was no statistical difference between spermatophore attributes after recharge periods of 1–3 weeks, the sample sizes were small, variances high, and means inconsistent.

It is also possible that the larger seminal fluid component of the spermatophore in large males when compared with small males is of consequence to sperm viability and hence fertilization success. Similar results have been found for the blue crab *Callinectes sapidus* (Kendall *et al.*, 2002). As with blue crabs, however, the consequences of reduced seminal fluid in terms of female reproductive success are unknown. Accessory fluid has several functions that include acting as an antibacterial agent or nutritive substance for sperm, as an aid for storage and retention of sperm (Tram and Wolfner, 1999), or as a sperm plug (Carver *et al.*, 2005). New genetic evidence based on paternity analysis (S. Johnson, Univ. Otago, pers. comm.) suggests that female *J. edwardsii* may engage in multiple copulations to fertilize a single egg clutch. This phenomenon appears unlikely in *P. argus* where more detailed examinations

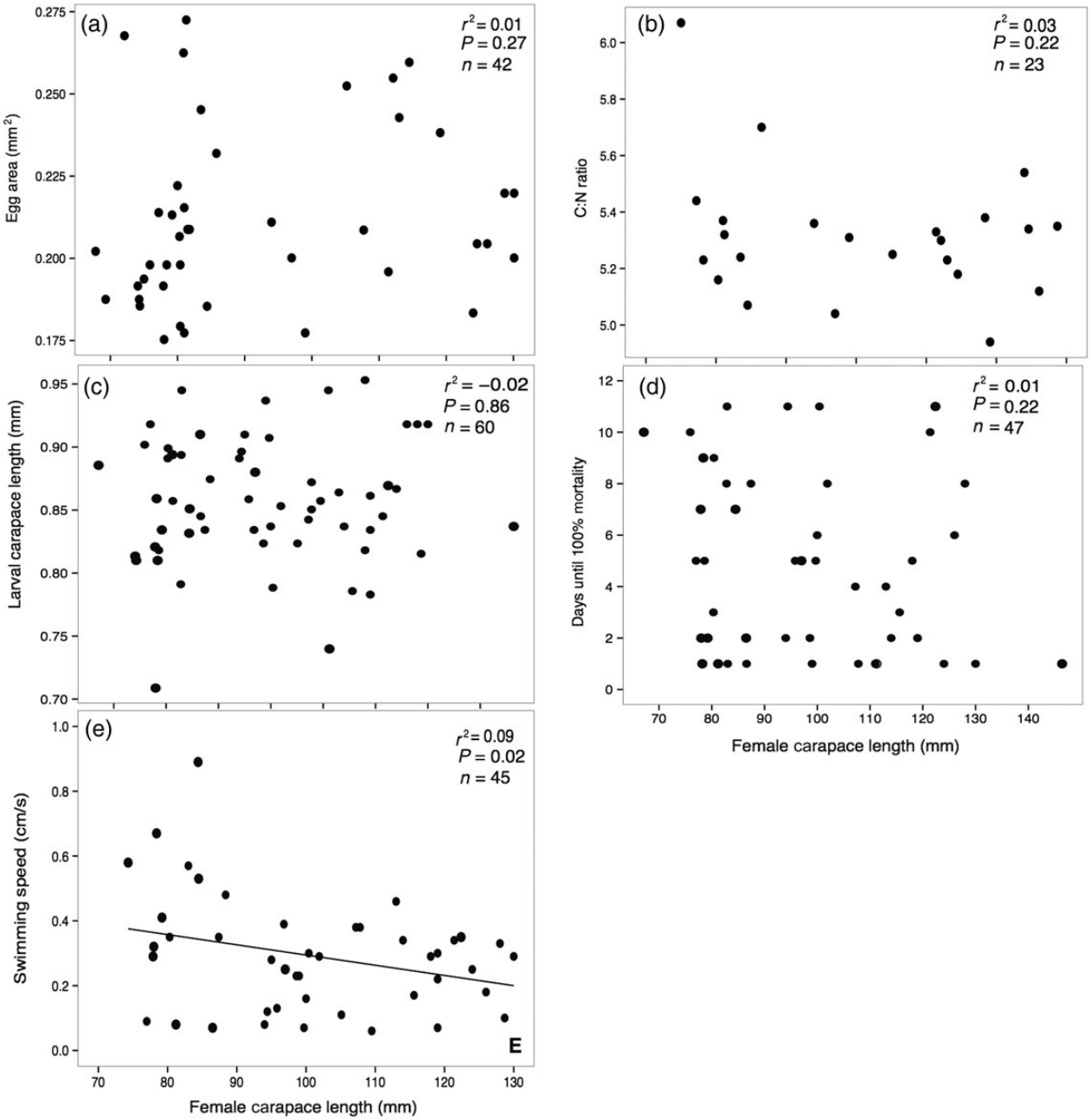

Figure 4. The relationships between the size of *P. argus* females and several egg and larval quality attributes, including: (a) egg area, (b) C:N ratio of eggs, (c) larval carapace length, (d) larval mortality, and (e) larval swimming speed. Regression summary statistics are shown in each panel (r^2 = variance explained, ANOVA *p*-value, *n* = sample size), although a regression line is only plotted for the one instance where there was a significant regression.

of spermatophore structure (Butler *et al.*, 2011a) indicates that reports of layered spermatophores (Mota-Alves and Pavia, 1976) are most likely due to new, unused spermatophores laid upon previously exhausted spermatophores used to fertilize a prior clutch. Thus, to avert sperm competition, large males may use accessory fluid to enhance the size of their perceived investment or to act as a barrier to secondary matings. Indeed, previous experiments with *P. argus* demonstrate that spermatophores provide both physical and chemical cues perceptible by females that inhibit them from further mating before utilization of the existing spermatophore (Butler *et al.*, 2011a).

As first articulated by Dewsbury (1982), the cost to males of producing ejaculates is physiologically non-trivial and all the more so for multiple ejaculates (Wedell *et al.*, 2002). Evidence of depletion over the production of successive spermatophores in our study clearly demonstrates this. Large male *P. argus* produced more than 25 ejaculates during a mating cycle and small males around 15, but the size of the ejaculates declined precipitously after just a few repeated matings. Similarly, small male king crabs *Paralithodes camtschaticus* experience reduced reproductive success after seven matings, whereas large crabs can mate up to nine times before experiencing a reduction in fertilization success (Powell *et al.*, 1974).

Large *P. argus* also require a week or so hiatus from mating to restore spermatophore size; small males appear to need even longer. This is similar to observations of blue crabs *Callinectes sapidus* that require between 9 and 20 d to fully recover sperm stores (Kendall *et al.*, 2001). Other crab species, such as the coconut crab *Birgus latro* and stone crab *Haplogaster dentata*, are not thought to recover sperm stores until the next reproductive season (Sato, 2012).

Maternal effects on offspring fitness have garnered considerable attention, particularly in relation to exploited marine species (Berkeley *et al.*, 2004a; Green, 2008; Venturelli *et al.*, 2010). In addition to evidence for exponential increases in fecundity relative to size or age, positive correlations between maternal size or age and larval characteristics provide further support for the protection of mature size and age structure in exploited animal populations (Berkeley *et al.*, 2004b; Birkeland and Dayton, 2005). In lobsters, the relationship between maternal size and fecundity is clearly established (Saul, 2004; MacDiarmid and Sainte-Marie, 2006). In comparison, knowledge of maternal influences on egg and larval characteristics is limited, which is disconcerting given the increasing truncation of the size range of exploited lobster populations. Maternal size influences on gamete and/or offspring quality have been observed in the European clawed lobster *Homarus gammarus* (Moland *et al.*, 2010), some populations of the American clawed lobster *Homarus americanus* (Attard and Hudon, 1987), and in the temperate spiny lobster *J. edwardsii* (A. MacDiarmid, unpub. data). However, maternal size resulted in no detectable difference on egg or larval characteristics in *P. argus* with the possible exception of larval swimming speed, the result of which we question given its low r^2 value. According to Marshall *et al.* (2010), there are few evolutionary arguments for a maternal size–offspring quality relationship unless the physical environment is the primary driver selecting for maternal effects.

Although studies on the effects of fishing on female size and reproductive patterns are known for many species of spiny lobster (reviewed in MacDiarmid and Sainte-Marie, 2006), detailed mating and fertilization dynamics are poorly documented. Our study reveals that the mating system of *P. argus* is considerably more complex than previously believed. Years of continuous fishing and the consequent human manipulation of lobster demographic structure has added to this complexity. For heavily exploited populations like those in the Florida Keys, where the largest lobsters that normally dominate matings are noticeably absent (Bertelsen and Mathews, 2001; Butler *et al.*, 2015), successful reproduction now depends on smaller individuals. Smaller males that in healthy unfished populations would typically be outcompeted for access to females are afforded the chance to mate repeatedly with available females in overfished populations. Given both the value of, and the reliance on, *P. argus* fisheries in the Western Atlantic, it is imperative that we incorporate knowledge of mating systems in population assessments (Rowe and Hutchings, 2003) and undertake research to better understand the implications of this. This study, like others before it, clearly demonstrates the importance of maintaining large individuals in exploited populations if we wish to maintain healthy breeding populations of *P. argus* into the future.

Acknowledgements

We are grateful for help with our laboratory experiments by a dedicated group of individuals, including J. Heisig-Mitchell, D. Behringer, D. Hansen-Behringer, J. Schratwieser, S. Donahue, and D. Robertson. The use of laboratory space at the Florida Fish and Wildlife Conservation Commission, Marathon Field Laboratory, is greatly appreciated. This research was supported by an award to M. Butler from the National Science Foundation (OCE-9730195).

References

Attard, J., and Hudon, C. 1987. Embryonic development and energetic investment in egg production in relation to size of female lobster (*Homarus americanus*). Canadian Journal of Fisheries and Aquatic Sciences, 44: 1157–1164.

Bas, C. C., Spivak, E. D., and Anger, K. 2007. Seasonal and interpopulational variability in fecundity, egg size, and elemental composition (CHN) of eggs and larvae in a grapsoid crab, *Chasmagnathus granulatus*. Helgoland Marine Research, 61: 225–237.

Berkeley, S. A., Chapman, C., and Sogard, S. M. 2004a. Maternal age as a determinant of larval growth and survival in a marine fish, *Sebastes melanops*. Ecology, 85: 1258–1264.

Berkeley, S. A., Hixon, M. A., Larson, R. J., and Love, M. S. 2004b. Fisheries sustainability via protection of age structure and spatial distribution of fish populations. Fisheries, 29: 23–32.

Bertelsen, R. D., and Cox, C. 2001. Sanctuary roles in populations and reproductive dynamics of spiny lobster. *In* Spatial Processes and Management of Marine Populations, Alaska Sea Grant AK-SE-01-02, pp. 591–605. Ed. by G. H. Kruse, N. Bez, A. Booth, M. W. Dorn, S. Hills, R. N. Lipcius, and D. Pelletier *et al.* University of Alaska Sea Grant, Fairbanks, AK, USA.

Bertelsen, R. D., and Mathews, T. R. 2001. Fecundity dynamics of female spiny lobster (*Panulirus argus*) in a south Florida fishery and Dry Tortugas National Park lobster sanctuary. Marine and Freshwater Research, 52: 1559–1565.

Birkeland, C., and Dayton, P. K. 2005. The importance in fishery management of leaving the big ones. Trends in Ecology and Evolution, 20: 356–358.

Briones-Fourzan, P., Candela, J., and Lozano-Alvarez, E. 2008. Postlarval settlement of the spiny lobster *Panulirus argus* along the Caribbean coast of Mexico: Patterns, influence of physical factors, and possible sources of origin. Limnology and Oceanography, 53: 970–985.

Butler, M. J., IV, Bertelsen, R., and MacDiarmid, A. 2015. Mate choice in temperate and tropical spiny lobsters with contrasting reproductive systems. ICES Journal of Marine Science, 72(Suppl. 1): i101–i114.

Butler, M. J., IV, Heisig-Mitchell, J. S., MacDiarmid, A. B., and Swanson, R. J. 2011a. The effect of male size and spermatophore characteristics on reproduction in the Caribbean spiny lobster, *Panulirus argus*. New Frontiers in Crustacean Biology, 15: 69–84.

Butler, M. J., IV, Mojica, A. M., Sosa-Cordero, E., Millet, M., Sanchez-Navarro, P., Maldonado, M. A., and Posada, J.*et al.* 2010. Patterns of spiny lobster (*Panulirus argus*) postlarval recruitment in the Caribbean: a CRTR Project. Proceedings of the 62nd Gulf and Caribbean Fisheries Institute, 62: 361–369.

Butler, M. J., IV, Paris, C. B., Goldstein, J. S., Matsuda, H., and Cowen, R. K. 2011b. Behaviour constrains the dispersal of long-lived spiny lobster larvae. Marine Ecology Progress Series, 422: 223–237.

Carver, A. M., Wolcott, T. G., Wolcott, D. L., and Hines, A. H. 2005. Unnatural selection: effects of a male-focused size-selective fishery on reproductive potential of a blue crab population. Journal of Experimental Marine Biology and Ecology, 319: 29–41.

Chavez, E. A. 2009. Potential production of the Caribbean spiny lobster (Decapoda, Palinura) fisheries. Crustaceana, 82: 1393–1412.

Chubb, C. F. 2000. Reproductive biology: issues for management. *In* Spiny Lobsters: Fisheries and Culture, 2nd edn, pp. 245–276. Ed. by B. F. Phillips, and J. Kittaka. Blackwell Scientific Press, Oxford.

Cox, C., and Hunt, J. H. 2005. Change in size and abundance of Caribbean spiny lobsters *Panulirus argus* in a marine reserve in the Florida Keys National Marine Sanctuary, USA. Marine Ecology Progress Series, 294: 227–239.

CRFM. 2013. CFRM Annual Report April 1 2012-March 31 2013. CRFM Administrative Report. 43pp.

Dewsbury, D. A. 1982. Ejaculate cost and male choice. The American Naturalist, 119: 601–610.

Ehrhardt, N. M. 2005. Population dynamic characteristics and sustainability mechanisms in key Western Central Atlantic spiny lobsters, *Panulirus argus*, fisheries. Bulletin of Marine Science, 76: 501–525.

Ehrhardt, N. M., Puga, R., and Butler, M. J., IV. 2010. Large ecosystem dynamics and fishery management concepts: the Caribbean spiny lobster, *Panulirus argus*, fisheries. *In* Towards Marine Ecosystem-Based Management in the Wider Caribbean. Ed. by L. Fanning, R. Mahon, and P. McConney. Amsterdam University Press, Amsterdam.

Galeotti, P., Rubolini, D., Fea, G., Ghia, D., Nardi, P. A., Gheradi, F., and Fasola, M. 2006. Female freshwater crayfish adjust egg and clutch size in relation to multiple male traits. Proceedings of the Royal Society of London, Series B, 273: 1105–1110.

Garcia, H. N., Kolding, J., Rice, J., Rochet, M. J., Zhou, S., Arimoto, T., and Beyer, J. T.,*et al.* 2012. Reconsidering the Consequences of Selective Fisheries. Science, 335: 1045–1047.

Giminez, L., and Anger, K. 2001. Relationships among salinity, egg size, embryonic development, and larval biomass in the estuarine crab *Chasmagnathus granulata* Dana, 1851. Journal of Experimental Marine Biology and Ecology, 260: 241–247.

Gosselin, T., Sainte-Marie, B., and Bernatchez, L. 2003. Patterns of sexual cohabitation and female ejaculate storage in the American lobster (*Homarus Americanus*). Behavioral Ecology and Sociobiology, 55: 151–160.

Green, B. 2008. Chapter 1 Maternal effects in fish populations. Advances in Marine Biology, 54: 1–105.

Heath, M. R., and Speirs, D. C. 2012. Changes in species diversity and size composition in the Firth of Clyde demersal fish community (1927–2009). Proceedings of the Royal Society, Series B, 279: 543–552.

Holthius, L. B. 1991. FAO Species Catalogue. Marine Lobsters of the World. An annotated and illustrated catalogue of species of interest to fisheries known to date. FAO Fish Synopsis, 125.

Jennings, S., and Lock, J. M. 1996. Population and ecosystem effects of reef fishing. *In* Reef Fisheries, pp. 193–218. Fish and Fisheries Series, 20. Chapman & Hall, New York.

Kendall, M. S., Wolcott, D. L., Wolcott, T. G., and Hines, A. H. 2001. Reproductive potential of individual male blue crabs, *Callinectes sapidus*, in a fished population: depletion and recovery of sperm number and seminal fluid. Canadian Journal of Fisheries and Aquatic Sciences, 58: 1168–1177.

Kendall, M. S., Wolcott, D. L., Wolcott, T. G., and Hines, A. H. 2002. Influence of male size and mating history on sperm content of ejaculates of the blue crab *Callinectes sapidus*. Marine Ecology Progress Series, 230: 235–240.

Kuparinen, A., and Hutchings, J. A. 2012. Consequences of fisheries-induced evolution for population productivity and recovery potential. Proceedings of the Royal Society of London, Series B, 279: 2571–2579.

Kuris, A. M. 1991. A review of patterns and causes of crustacean brood mortality. *In* Crustacean Egg Production, pp 117–143. Ed. by A. Wenner, and A. M. Kuris. CRC Press, Boca Raton, FL.

Liddy, G. C., Phillips, B. F., and Maguire, G. B. 2003. Survival and growth of instar 1 phyllosoma of the western rock lobster, *Panulirus cygnus*, starved before or after periods of feeding. Aquaculture International, 11: 53–67.

Lipcius, R. N., Edwards, M. L., Hernkind, W. F., and Waterman, S. A. 1983. In situ mating behaviour of the spiny lobster *Panulirus argus*. Journal of Crustacean Biology, 3: 217–222.

Lyons, W. G., Barber, D. G., Foster, S. M., Kennedy, F. S., and Milano, G. R. 1981. The spiny lobster of the middle and upper Florida Keys: population structure, seasonal dynamics and reproduction. Florida Marine Research Publications, 38: 1–45.

MacDiarmid, A. B., and Butler, M. J., IV. 1999. Sperm economy and limitation in spiny lobsters. Behavioural and Ecological Sociobiology, 46: 14–24.

MacDiarmid, A. B., and Sainte-Marie, B. 2006. Reproduction. *In* Lobsters: Biology, Management, Aquaculture and Fisheries, pp 45–77. Ed. by B. Phillips. Blackwell Publishing, Oxford, UK.

Marshall, D. J., Heppell, S. S., Munch, S. B., and Warner, R. R. 2010. The relationship between maternal phenotype and offspring quality: do older mothers really produce the best offspring? Ecology, 91: 2862–2873.

Maxwell, K. E., Matthews, T. R., Bertelsen, R. D., and Derby, C. D. 2009. Using age to evaluate reproduction in the Caribbean spiny lobster, *Panulirus argus* in the Florida Keys and Dry Tortugas, United States. New Zealand Journal of Marine and Freshwater Research, 43: 139–149.

Moland, E., Olsen, E. M., and Stenseth, N. C. 2010. Maternal influences on offspring size variation and variability in wild European lobster *Homarus gammarus*. Marine Ecology Progress Series, 400: 165–173.

Mota-Alves, M. I., and Pavia, M. P. 1976. Frequencia de acasalamentos en langostas do genero Panulirus White (Decapoda, Palinuridae). Arquiveria Ciênces Marina, 16: 61–63.

Powell, G. C., James, K. E., and Hurd, C. L. 1974. Ability of male king crab, *Paralithodes camtschatica* to mate repeatedly, Kodiak, Alaska, 1973. Fishery Bulletin US, 72: 171–179.

Roberts, C. M., and Polunin, N. V. C. 1991. Are marine reserves effective in management of reef fisheries? Review in Fish Biology and Fisheries, 1: 65–91.

Robertson, D., and Butler, M. J., IV. 2013. Mate choice and sperm limitation in the spotted spiny lobster *Panulirus guttatus*. Marine Biology Research, 9: 69–76.

Rowe, S., and Hutchings, J. A. 2003. Mating systems and the conservation of commercially exploited marine fish. Trends in Ecology and Evolution, 18: 567–572.

Sato, T. 2012. Impacts of large male selective harvesting on reproduction: illustration with large decapod crustacean resources. Aqua-BioScience Monographs, 5: 67–102.

Sato, T., and Goshima, S. 2007. Effects of risk of sperm competition, female size, and male size, on the number of ejaculated sperm in the stone crab *Hapalogaster dentata.* Journal of Crustacean Biology, 27: 570–575.

Sato, T., Yoseda, K., Okuzawa, K., and Suzuki, N. 2010. Sperm limitation: possible impacts of large male selective harvesting on reproduction of the coconut crab *Birgus latro*. Aquatic Biology, 10: 23–32.

Saul, S. 2004. A review of the literature and life history study of the Caribbean spiny lobster, Panulirus argus. Caribbean Southeast Data Assessment Review Workshop Report, SEDAR-DW-05. Sustainable Fisheries Division Contribution No. SFD- 2004-048.

Sheldon, B. C. 2000. Differential allocation: tests, mechanisms and implications. Trends in Ecology and Evolution, 15: 397–402.

Skinner, A. M. J., and Watt, P. J. 2007. Strategic egg allocation in the zebra fish, *Danio rerio*. Behavioural Ecology, 18: 905–909.

Talbot, P., and Summers, R. G. 1978. The structure of sperm from *Panulirus*, the spiny lobster, with special regard to the acrosome. Journal of Ultrastructural Research, 64: 341–351.

Tram, U., and Wolfner, M. F. 1999. Male seminal fluid proteins are essential for sperm storage in *Drosophila melanogaster*. Genetics, 153: 837–844.

Venturelli, P. A., Murphy, C. A., Shuter, B. J., Johnston, T. A., Van Coeverden de Groot, P. J., Boag, P. T., and Casselman, J. M.*et al.* 2010. Maternal influences on population dynamics: evidence from an exploited freshwater fish. Ecology, 91: 2003–2012.

Wedell, N., Gage, M. J. G., and Parker, G. A. 2002. Sperm competition, male prudence and sperm-limited females. Trends in Ecology and Evolution, 17: 313–320.

Handling editor: Stéphane Plourde