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THE EVOLUTION OF LIFE HISTORIES: NEW INSIGHTS ON DIFFERENTIAL SEX
DETERMINATION IN THE SCALY PEARL OYSTER *PINCTADA LONGISQUAMOSA*

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ABSTRACT

Pearl oysters (Pteriidae: Pinctada) are known sequential hermaphrodites, often switching from male to female over the course of a single lifetime. Sequential hermaphroditism arises out of differential fitness benefit for each sexual form. This study describes and compares the sex determination trajectories of five populations of the Scaly Pearl Oyster *Pinctada longisquamosa* from inland ponds on San Salvador Island, Bahamas as well as a marine population from Key Largo, Florida. Populations of *Pinctada* were found to differ in sexual allocation trajectory, suggesting possible adaptation to different selective environments. We propose that pond-specific differences in mortality associated with hurricane events may be driving the differential evolution of oyster life histories on San Salvador Island. Sex trajectories in a vulnerable population before and after the significant disturbance of a hurricane were found to be similar despite significant environmental changes, supporting a genetic and not environmental mechanism of sex determination. Evidence of sex-reversal capability was found in this same population. We also relate observed trends in oyster whole population sex ratio and maturation to larger theories on life history evolution.

INTRODUCTION

As sequential hermaphrodites, pearl oysters (Pteriidae: Pinctada) change gender over the course of a single lifetime. Many pearl oysters are protandrous, meaning they mature first as males and subsequently into females (Southgate and Lucas 2008). The evolution of sequential hermaphroditism has been explained in part by the size advantage hypothesis (Ghiselin 1969) which posits that size-specific fitness in each sexual form selects for individuals to change gender (in the case of protandry, from male to female) as they age. Under a different strategy of alternating sexuality, individuals exhibit greater sexual lability, or the ability to change gender multiple times according to environmental conditions such as food availability. The observation of both unidirectional protandry and alternating, reversible sexuality in phylum Mollusca raises questions as to the flexibility of sex determination within and between species in this group (Hoagland 1984).

The Scaly Pearl Oyster *Pinctada longisquamosa* is endemic to the West Atlantic, with populations found throughout the Florida Keys and Bahamas (Mikkelsen et al., 2004). The nature and plasticity of sex determination in this species of *Pinctada* are not well characterized. However, previous studies on *Pinctada longisquamosa* from inland ponds on San Salvador Island, Bahamas suggest that these oysters are protandrous hermaphrodites (Cole et al., 2007).

The six populations we studied fall on a conceptual gradient of vulnerability to physical disturbance and salinity variability as associated with hurricane events (Figure 1).

Populations subject to low salinity variability such as those in the Florida Keys and Oyster Pond are marine or near-marine due to the influence of ocean conduits, whereas environments of high salt variability such as those in Six Pack Pond do not have such conduits and have been hypersaline in recent years. Ponds of low hurricane vulnerability have small surface areas and substantial mangrove vegetation to serve as protective substrate during a storm; this may be contrasted with more open, less vegetated environments associated with high hurricane

vulnerability. This gradient in environments has been correlated with differences in oyster size distribution, a function of differing mortality regimes associated with hurricane disturbance. For instance, adult oysters in Six Pack are decimated whereas similar oysters in Oyster and Mermaid Ponds persist after hurricanes have hit San Salvador Island (Carlson et al., 2009).
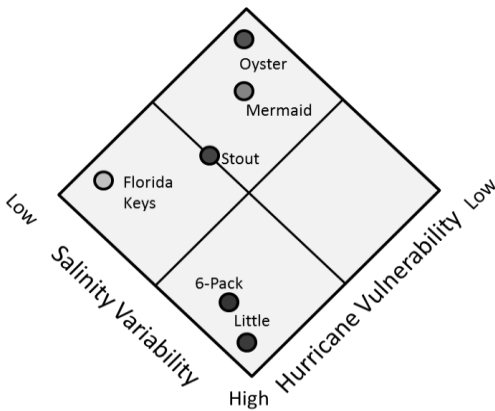

Figure 1. Conceptual gradient of hurricane-associated physical disturbance and salinity variability for six oyster populations.

Natural breeding populations of protandrous species are consistently male-biased (Saucedo and Monteforte 1997, Sewell 1994, Charnov 1982). Charnov (1982) speculates that sequential hermaphrodites should have gender compositions dominated by the first-maturing sex. Among protandrous species, this may be caused by an inherent attribute: female fecundity increases with size at a greater rate than male fecundity. The breeding sex ratio under protandry will be male-biased because larger females are more productive than their male counterparts, and therefore a greater proportion of individuals in the population will be selected to remain male to balance out greater female output per capita. In line with this theory, we expected oyster populations with larger size distributions, such as Oyster and Mermaid, to be more significantly male-biased than populations with small sizes, such as Florida Keys and Six Pack.

Among other animal systems, background mortality has been demonstrated to in-fluence the nature of fundamental life history trade-offs between reproduction and survival. For instance, evolving under heightened mortality, individuals may trade-off early reproduction at the cost of late survival (Kirkwood and Rose 1991); this has been observed among fruit fly strains subject to persistently high adult mortality (Gasser et al., 2007). These ideas imply that sex determination patterns, which reflect trends in reproductive investment, may be strongly affected by background adult mortality.

Accumulating evidence on the life histories of *Pinctada longisquamosa* suggest that localized selection pressures may be driving population-level differences in oyster sex determination on San Salvador Island (Cole et al., 2007, Carlson et al., 2009).

This study seeks to augment past descriptions of the sex determination trajectories of six populations of the Scaly Pearl Oyster, particularly in relation to the hurricane disturbance gradient. Additionally, we assess the sex reversal capabilities of the hurricane-vulnerable Six Pack Pond population, examine oyster sex allocation before and after the major hurricane disturbance of Hurricane Noel, which hit San Salvador Island in November 2007, and survey oyster maturity and whole-population sex ratio in the context of larger theories in life history evolution.

METHODS

Between January 2006 and June 2011, 2,495 oysters were collected from six populations of *Pinctada longisquamosa* in Key Largo, Florida and on San Salvador Island, Bahamas. Upon collection, these oysters were sorted into size classes based on hinge length as measured using handheld calipers, with each size class increasing in five mm hinge length increments. Specimens were dissected, preserved in Bouin's Fixative, and then dehydrated and embedded in paraffin wax per Humason (1979). 7-9 micron thick sections were placed on egg albumen-subbed slides and in turn stained using Gomori Trichrome stain (Fisher Inc.). Slides were examined under an Olympus microscope and corre-

sponding oysters assigned male, female, hermaphrodite, or indeterminate identification based upon the absence or presence of sperm and eggs (Figure 2). This data was used to create plots of percent females and percent mature oysters at each hinge size class, a function of oyster age (Figure 3).

To survey sex reversal, 40 live oysters from Six Pack Pond were confirmed female in January 2011 by the presence of eggs using a field needle biopsy procedure (Saha and Layzer 2008) and 10% methylene blue dye. These oysters were placed in two cork-plugged iron mesh minnow traps which were subsequently zip tied to shallow underwater rebar posts anchored in the pond. In June 2011, these oysters were recollected and fixed in Bouin's Fixative for histological sex scoring and assessment of potential sex reversal.
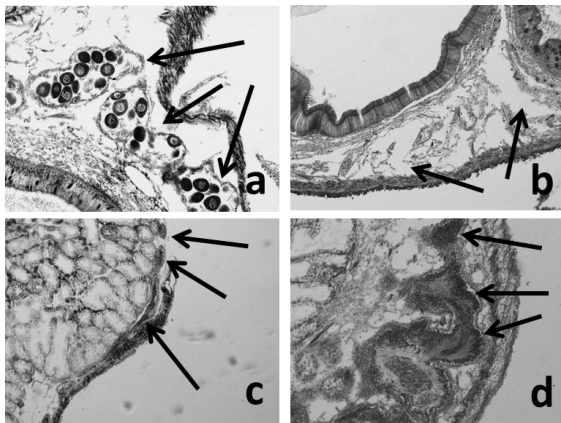

Figure 2. Histological cross-sections of Six Pack Pond caged oysters. (a) A female with maturing oocytes; (b) an indeterminate oyster with empty gonads; (c) a male undergoing early spermatogenesis, and (d) a male with mature spermatozoa.

For comparison of sex determination trajectories before and after the disturbance of Hurricane Noel, oyster sex data from Six Pack, Mermaid, and Oyster ponds were pooled into 'before Noel' and 'after Noel' groups and directly compared. The algal diets of oysters from these populations in November 2006 and January 2009 were compared by recording abundances of algal morphotypes in select oyster gut

sections from three size classes per pond, and calculating diversity using the Shannon Index (Shannon 1948).

Whole population sex-ratios were determined by using known gender compositions for each class at each time of sampling and then incorporating randomly gathered whole-population distributions of oyster hinge lengths to estimate the percentage of mature male and female oysters in the population, as well as a male:female ratios among breeding oysters. Oyster maturation curves for the six populations were produced by totaling the number of oysters with mature gametes (male, female, or hermaphrodite) divided by the total number of oysters sampled from each size class.

RESULTS

Sex Determination in *Pinctada longisquamosa*

Sex allocation trajectories for the oysters, plotted as percent females in each size class for each population, suggest a large range of sex determination patterns in *Pinctada longisquamosa*. The Florida Keys, Oyster Pond, and Mermaid Pond oysters have steadily greater percentages of females with each size class. This may be contrasted with the Stout Lake and Six-Pack Pond oysters in which populations became mature with a significant (greater than 30%) female population, and among which feminization was not linearly related to size. Little Lake showed a curious depression in female-bias in the oldest oysters. Rates of feminization, (trajectory slopes), were also highly variable with the Florida Keys population feminizing at a faster rate, the Oyster Pond and Mermaid Pond populations at an intermediate rate, and the Six Pack Pond population with a minimal slope (Figure 2).

Of the 40 Six Pack Pond female oysters placed in cages in January 2011, 37 were still living in June 2011 and were subsequently scored for gender. 25 of these oysters were still female, three were indeterminate, and nine displayed evidence of spermatogenesis (Figure 2), meaning these oysters had undergone sex rever-

sal from female to male within a 5 month interval. These population-level differences highlight plasticity in oyster sex determination, possibly via phenotypic adaptation according to environmental conditions or genetic adaptation through the mechanism of natural selection.
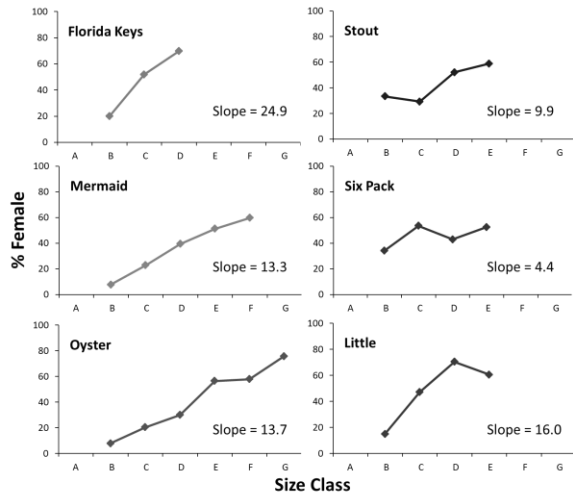

Figure 3. Sex determination trajectories for six oyster populations, with size class a function of hinge length to indicate oyster age.

A Natural Experiment to Assess Environmental or Genetic Sex Determination

A comparison of Oyster, Mermaid, and Six Pack Pond oyster sex determination trajectories before and after the disturbance of Hurricane Noel in November 2007 reveals little deviation of oyster life histories in any of the three populations (Figure 4). As we shall see, the life-history trajectory for each population remain unchanged despite dramatic changes in environmental conditions within each pond including salinity and algal diversity.

Analysis of oyster gut contents before and after Hurricane Noel in 2007 illustrates substantial shifts in oyster diet particularly in Six Pack Pond (Figure 5). Oyster diets in Six Pack Pond were less evenly distributed and less species-rich after the hurricane than before the hurricane as compared with Mermaid or Oyster Ponds. This is evidenced by a drop in Shannon Diversity Index H from 2.1 to 1.26 between sample points in Six Pack Pond; the other two ponds increased in algal diversity (1.95 - 2.18
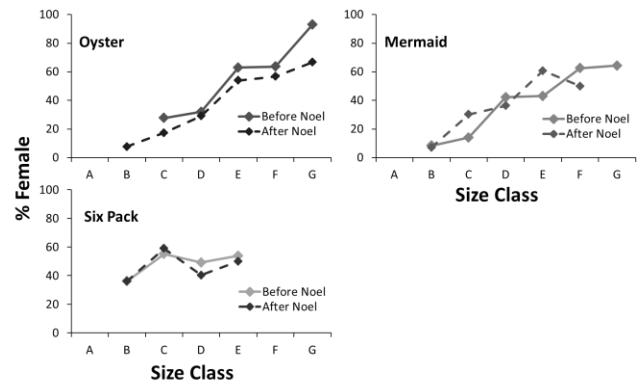

Figure 4. Oyster sex determination trajectories for three populations before and after the disturbance of Hurricane Noel in November 2007.
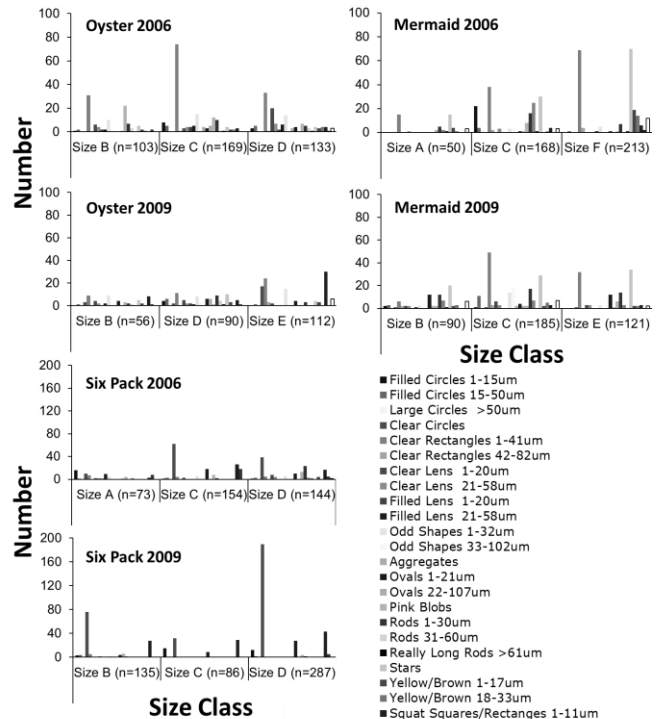

Figure 5. Gut contents for Oyster, Mermaid, and Six Pack pond oysters at 3 size classes before and after Hurricane Noel in 2007. Each bar represents abundance of a specific algal morphotype.

for Mermaid; 2.16 - 2.34 in Oyster Pond), after the hurricane (Figure 5). Despite significant changes in salinity and diet, oysters in Six Pack Pond did not alter their sex determination trajectory. This supports a theory suggesting that the unique life-history trajectory of oysters in Six

Pack Pond is genetically determined rather than environmentally driven.

Pinctada Breeding Population Sex Ratios

We also examined the overall sex ratio of each population, pooling all individuals and using demographic profiles to weight different size classes. A pooled analysis of gender composition for each of the oyster populations reveals a male bias in all populations with the exception of Little Lake and Six Pack Pond (Table 1).

Pond	% Male	% Female	Male:Female
Florida Keys	49.1 ± 2.7	45.3 ± 2.7	1.08
Oyster	32.7 ± 8.7	26.3 ± 7.1	1.25
Mermaid	51.6 ± 3.2	41.8 ± 3.6	1.24
Stout	31.4 ± 15.3	25.5 ± 21.6	1.23
Six Pack	27.8 ± 4.9	28.1 ± 8.6	0.99
Little	33.7 ± 3.9	54.9 ± 6.3	0.61

Table 1. Average estimated whole-population gender compositions for six oyster populations with standard errors. Data averaged for all time points available.

It is interesting to note the great variability in percent mature males and females in each population. This distinction between populations such as Mermaid and Oyster Pond is a function of consistent differences in oyster maturity, but may not relate to breeding oyster male:female ratio, as both populations are similarly male-biased.

Maturation in *Pinctada longisquamosa*

Maturation curves for *Pinctada longisquamosa* indicate great variability between populations. In most populations, older oysters are more likely to possess mature gametes and therefore actively participate in reproduction than younger oysters. The Oyster Pond and Stout Lake populations are consistently less mature than others, with no oyster size class greater than 68% and 50% mature, respectively. With 87% of its class B oysters possessing evident gametes, the Florida Keys population dis-

plays maturation at a substantially smaller size than all others (Figure 6). These trends indicate substantial differences in how oysters trade-off survival and reproduction, a context which could relate directly to differential evolution of sex determination in *Pinctada longisquamosa*.
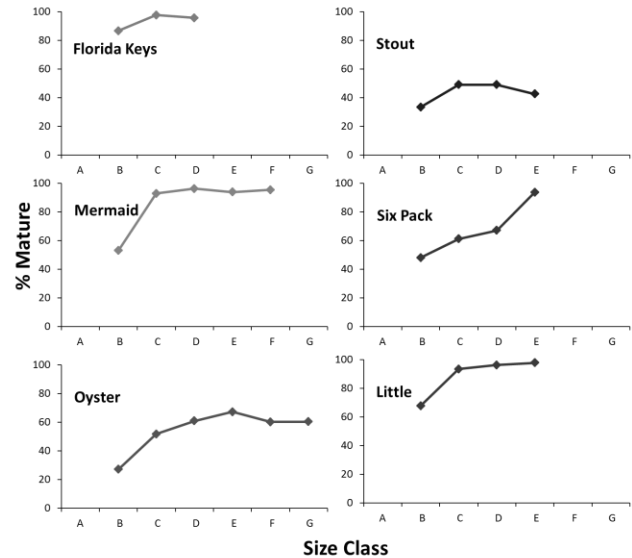

Figure 6. Percent mature oysters at each size class for six oyster populations. Oysters possessing evident eggs, sperm, or both were considered mature.

DISCUSSION

Protandrous sequential hermaphroditism is a reasonably common life history trait in the animal kingdom, documented in the Apodid Sea Cucumber *Leptosynapta clarki* (Sewell 1994), the shrimp *Rhynchocinetes uritai* (Bauer and Thiel 2011), and the Pacific Oyster *Crassostrea gigas* (Guo et al, 1998), among many others. Evidence presented here indicates that populations of *Pinctada longisquamosa* in Mermaid Pond, Oyster Pond, and the Florida Keys are also protandrous, with oysters becoming feminized as they grow larger. In these populations, feminization rate expressed as a function of oyster size appears to be dependent on oyster size distribution. With larger mean oyster sizes, the Mermaid and Oyster Pond populations feminize at a slower pace than the Florida Keys oysters. Large females are expected to have a distinct

fitness advantage over small females according to the size advantage hypothesis proposed by Ghiselin (1969), but the size advantage of any given oyster will be relative to other oysters in its population. If oysters in the Florida Keys do not grow as large as in Oyster or Mermaid, they will be selected to mature as females at smaller sizes.

Deviation from protandry is also apparent in certain populations of *Pinctada longisquamosa*, namely that of Six Pack Pond. Among these oysters, gender is not directly correlated with oyster size. Sexual lability, or the ability to change gender according to environmental influences, is hypothesized to evolve among environments in which individuals have minimal control over the environment in which they will grow and mature (Charnov and Bull 1977). As a highly variable environment of great vulnerability to hurricane disturbance, Six Pack Pond represents a characteristic environment for oysters to evolve sexual lability. This possibility is further supported by evidence that up to 24% of oysters in Six Pack are capable of a switch from oogenesis to spermatogenesis in as little as five months. This gender switching in Six Pack is not consistent with the definition of unidirectional protandry, and may be a signature of environmentally-driven alternating sexuality (Hoagland 1984).

Given this, it may be surprising that the sex determination trajectory of Six Pack Pond oysters did not differ drastically before and after Hurricane Noel hit the island in 2007. The hurricane brought substantial precipitation to San Salvador. Without tidal flushing from marine conduits, Six Pack saw a drop in salinity from a hypersaline 43.4 g/L Total Dissolved Solutes in June 2007 to a below-marine 31.7 g/L TDS in March 2008. The hurricane also decimated the adult population of Six Pack Pond, but not of the more sheltered, marine-flushed Mermaid or Oyster ponds (Carlson et al., 2009). While both the salinity and algal diet of Six Pack's oysters of 2009 were different from 2006, the two distinct populations had very similar life history trajectories. This suggests that the unique life history pattern of alternating sexuality observed in Six Pack Pond is genetically determined and not a physiological adaptation to differences in environment.

We suggest that the marine *Pinctada longisquamosa* population from Key Largo, Florida be considered representative of both the hypothetical parent population and a genetic out-group of the oysters that first settled and persist within the inland ponds of San Salvador Island. As such, differences in sex determination between the Florida Keys population and San Salvador oyster populations represent recent adaptations to local selection pressures.

Ponds spared the physical upheaval of tropical storms, salinity fluctuations, and most natural enemies such as Mermaid and Oyster may allow oysters to grow substantially larger than in the Florida Keys and yet retain protandry, albeit at a diminished rate of feminization. This may be due to the continual persistence of adult female oysters even after storm events: in these populations, young oysters maturing after a hurricane will have a selective advantage to mature as males first instead of competing for egg production with larger females. This selective environment will maintain the gender-specific size advantage that conserves protandry.

Populations in open environments not flushed by marine conduits, such as large Six Pack Pond, are especially subject to the physical disturbance of hurricane wave action as well as large salinity fluctuations. In these ponds, large proportions of adults die off during hurricane events every 5-10 years (Carlson et al., 2009). Under resulting low population densities and potentially skewed sex ratios, surviving adults able to change gender should have enhanced fitness. Moreover, subsequent generations of oysters would not have to compete with a large cohort of adult females and may therefore feminize earlier for better fitness. This selective environment will decouple the gender-specific size advantage and thereby weaken protandry.

Four of the populations of *Pinctada longisquamosa* have male-biased sex ratios. This phenomenon is expected among protandrous hermaphrodites. For instance, the

protandrous pearl oyster *Pinctada mazatlanica* has been reported to have as low as 11% females in natural populations (Saucedo and Monteforte 1997, Arnaud-Haond et al., 2003). Another protandrous sequential hermaphrodite, *Leptosynapta clarki*, has similarly been reported to reach breeding population female compositions of a maximum 29% (Sewell 1994). Interestingly, breeding sex ratio was not directly related to oyster size distribution. The case of a lower female composition among Oyster pond's large-bodied oysters is consistent with Charnov's expectation (1982) that enhanced female fitness among the largest individuals could skew a population toward unusual male-bias as compared with populations of smaller demography such as Six Pack and Little.

Inhabiting an environment of relatively low mortality compared particularly to Six Pack Pond and Little Lake, but also to the Florida Keys, oysters in Oyster Pond exhibit post-reproductive degradation, or the phenomenon of senescence. In Oyster Pond, the oldest oysters of size classes E, F, and G do not participate in reproduction as they do in other populations. We hypothesize that this may be due to the occurrence of 'antagonistic pleiotropy' as proposed by Kirkwood and Rose (1991). In their higher-mortality evolutionary past, Oyster pond oysters traded off early reproduction at the cost of accumulating late-life deleterious mutations. Now, under reduced mortality, older oysters persist deep into adulthood and experience reproductive degradation. In this low-mortality environment, oysters able to avoid senescence will have a selective advantage, resulting in a gradual shift of resources toward physiological maintenance instead of early reproduction (therefore resulting in reduced maturity in early size classes B and C).

Life history trade-offs of the opposite direction are favorable in higher-mortality environments such as Six Pack Pond and the Florida Keys, where oysters able to reproduce earlier have an acute selective advantage and few oysters persist to grow past class E. For this reason, enhanced maturity in size class B should evolve among these populations. As a side note, it is interesting that these high-mortality environments also display earlier feminization than low-mortality Mermaid and Oyster. We suggest that egg production at smaller sizes is more favorable in these populations in which older, more productive females do not survive to have a distinct size fitness advantage.

ACKNOWLEDGMENTS

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