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Cover photograph –Barn Owl (*Tyto alba*) at Owl's Hole Pit Cave courtesy of Elyse Vogeli

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THE EFFECT OF HURRICANE ACTIVITY ON SEX DETERMINATION IN THE SCALY PEARL OYSTER: *PINCTADA LONGISQUAMOSA*.

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ABSTRACT

The scaly pearl oyster, *Pinctada longisquamosa*, is abundant in several anchialine ponds on San Salvador Island, Bahamas. In particular, we found established colonies in Mermaid Pond, Oyster Pond, Little Lake and Little Granny (Six-Pack) Pond. Following Hurricane Floyd (Sept. 14, 1999) and Hurricane Frances (Sept. 1, 2004), we noticed dramatic differences in the impact on oyster populations within these different ponds. The Oyster Pond and Mermaid Pond populations seemed relatively intact following each of these episodes. The Little Granny population, on the other hand, was profoundly decimated with less than 0.01 % survival. We used paraffin histology to follow gonad maturation and differentiation within these various populations. We learned that the *Pinctada* in Mermaid Pond exhibit protandrous hermaphroditism. Juveniles initially mature as males developing functional testes. Later, half the population undergoes a sex-change becoming female with testes redifferentiating as ovaries. This is a relatively common life-history strategy among sessile bivalves. In Little Granny Pond, (the population undergoing repetitive hurricane decimation), the life history trajectory is one of gonochorism. As these individuals mature, half develop into males and the other half develop directly into females bypassing the hermaphroditic transition. We offer several hypotheses to account for differences in these life history trajectories. Histology further revealed that the Oyster Pond population appears to be undergoing a dramatic senescence with concomitant loss of reproductive capacity.

INTRODUCTION

Sex determination is a developmental program engineered by natural selection to optimize the allocation of resources to each sex (Charnov & Bull 1977). In nature, some species exhibit strict genetic determination of sex, while others employ environmental cues to shift gender assignment in order to optimize their reproductive output (Bull 1980). Organisms that undergo sequential hermaphroditism begin life as one sex only to switch sex later in life in response to a specific environmental cues. In cases of protandry, (a male-first life history trajectory), female fitness is generally more strongly dependent on body size than is male fitness, and thus selection favors being male early in life when body size is small, and becoming female later in life, as body size increases (Warner, et al., 1975). Under stable environmental conditions, different individuals will be invested as males or females at any given time, and thus the strategy can be evolutionarily stable (St. Mary 1997). Conversely, if periodic environmental disturbances produce synchronous initiation of development of the entire population, an exclusively protandrous population will be characterized by a strongly male-biased sex ratio soon after achieving reproductive maturity. Because of frequency dependent selection for the rare sex (Fisher, 1930), we propose that such a population would be susceptible to invasion by an alternate sex determining strategy. We have uncovered an example in which environmental conditions may be acting across a very local landscape (different marine ponds on a single island in the Caribbean) to drive divergence from a pattern of protandrous hermaphroditism, to one of gonochoristic sex determination. We propose that a history of repetitive hurricane exposures has driven the scaly pearl oyster (*Pinctada longisquamosa*) from a typical protandrous hermaphroditism (in smaller mangrove-protected ponds) towards gonochoristic sex determination in ponds that are more exposed to episodic hurricane decimation. Confirmation of this association would provide a remarkable opportunity to examine the balance of factors contributing to the evolution of sex allocation.

METHODS

Histology

Oysters were collected from each study site and sorted by size (class "A" = 5.0 - 10.0 mm hinge length, class "B" = 10.0 – 15.0 mm hinge length, and on in 5mm increments). Hinge lengths were measured using hand-held calipers and measuring along the straight edge of the oyster's hinge. Ten specimens of each size class were kept. Their visceral masses were dissected and fixed in Bouin's fixative. Specimens were dehydrated and embedded in paraffin as described by Humason (1979). Eight-micron thick sections were made and slices were stained in Gomori's trichrome stain (Fisher Inc.). Slides were scored using an Olympus BH40 microscope with brightfield optics. Images were taken using a SPOT-RT digital camera.

Underwater Photography

Underwater photographs were taken using a Nikonos V underwater camera and natural lighting.

Suspended Carbon Analysis

At each study site, the richness of suspended organic material (a measure of potential nutrients for the oysters) was estimated by filtering one liter of pond water through a pre-weighed glass fiber filter. Filters were then dried and weighed. They were then "ashed" by placing in an oven for 4 hours at 500 degrees Fahrenheit. During this process all the organic content (principally carbon) is burned off, leaving behind the inorganic content of the sample. After allowing the filter to cool, the ash weight of the filters was recorded. We were then able to determine the "Ash-Free Dry Weight." The AFDW is the dry weight (inorganic + organic contents) minus the weight of the ash (inorganic contents only).

GEOGRAPHY OF OUR STUDY SITES

The geology of San Salvador Island can be attributed to forces acting during periods of interglacial inundation: deposition of biotic and abiotic calcium carbonate and erosion by wave action, and exposure during glacial episodes: wind-driven dune formation, rainwater dissolution and lithification of sand grains into various forms of limestone (Carew & Mylroie, 1994). The last interglacial period (the Sangamon) elevated sea level 6 meters above its current stand, whereas the last glacial episode (the Wisconsin) dropped sea level over 140 meters compared with today's shoreline. Currently, sea level appears to be rising at the rate of approximately 3mm per year, producing noticeable changes in coastal flora and fauna (Godfrey, 03). The island's current profile is a series of lithified dune ridges and intervening swales. The swales harbor numerous inland ponds or lakes ranging from fully marine to hypersaline conditions (See Dutke et al., 2008, this volume). These anchialine ponds have only become ponds within the last 6,000 years based on current altitude and published rates of sea level elevation. A typical pond on this island has undergone historic changes from freshwater (as groundwater lifts the freshwater lens breaching the overlying terrain), to brackish, marine and hypersaline as underlying seawater breaches the pond's profile and evaporation concentrates the salts. Ostracods from sediment samples have been used to document this trajectory within some of San Salvador's inland ponds (Sanger & Teeter 1982). Hence, organisms that have become established in the inland ponds have done so over a short period of time and any evolutionary changes that have come to distinguish these populations must be relatively recent

(See Holtmeier, 2001 for a similar study of "fast evolution" in the Bahamian pupfish).

ECOLOGY OF OUR STUDY SITES.

Four living populations of *Pinctada longisquamosa* (scaly pearl oysters) have been discovered on San Salvador island to date: those in Mermaid Pond (MM), Oyster Pond (OP), Little Granny Pond (LG) and Little Lake (LL) (See Figure 1).

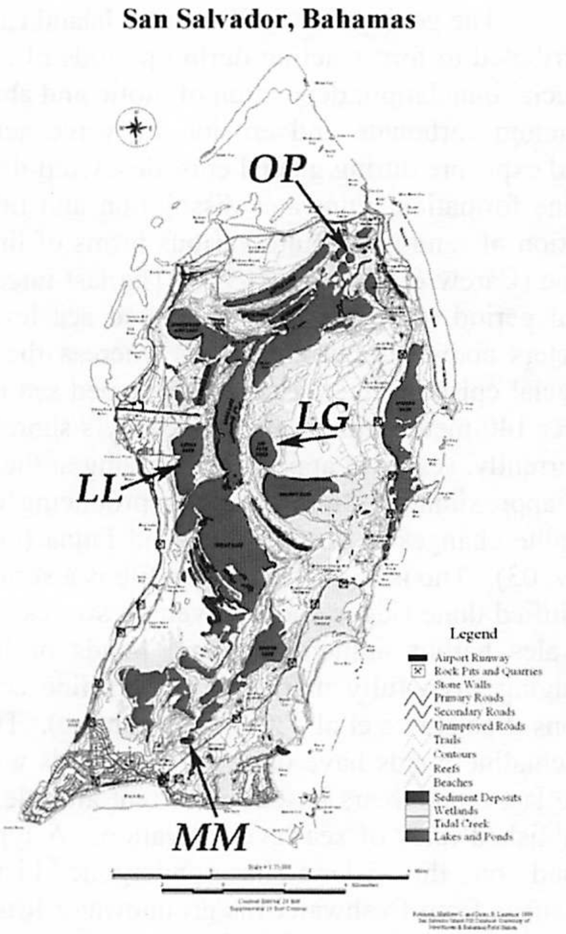

Figure 1. Map of San Salvador Island showing four ponds in which live populations of P. longisquamosa have been found. OP: Oyster Pond, LG: Little Granny Pond ("Six Pack"), LL: Little Lake, and MM: Mermaid Pond.

From: Robinson and Davis, 1999, San Salvador Island GIS Database. University of New Haven and Gerace Research Center (formally Bahamian Field Station).

The Mermaid Pond Community

Mermaid Pond represents a hurricane-sheltered habitat for pearl oysters. The pond is relatively small (low wave generation during storms), tidal, fully-marine (34 ppt) and supports a littoral red mangrove (*Rhizophora mangle*) prop-root community providing stable substrate for oyster colonization. The prop-root habitat is productive supporting all three of the pond's larger bivalves: the black mangrove oyster (*Isognomen alatus*), the burnt mussel (*Brachiodontes exustus*), and the Scaly Pearl Oyster (*Pinctada longisquamosa*), the subject of our study (Figure 2).

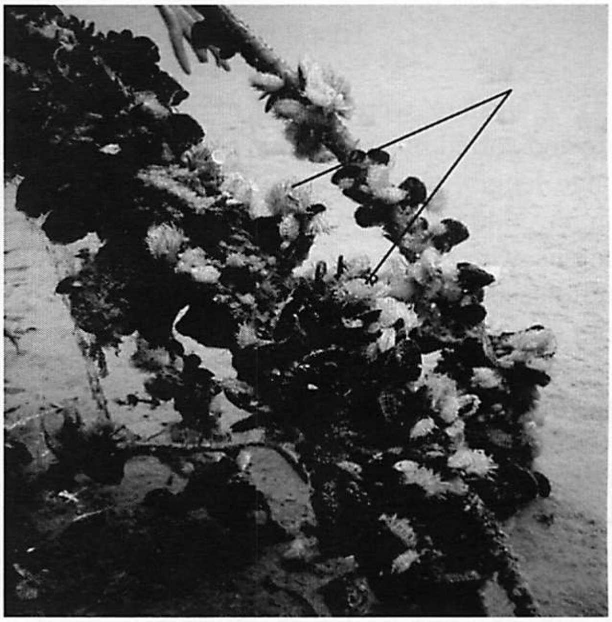

Figure 2. <u>Pinctada</u> (arrows) anchored to mangrove proproots along with <u>Isognomen</u>, (black mangrove or "tree" oyster).

Species diversity in this pond is relatively rich (Lanterman, et al., 2007). Mermaid pond is supported by at least two large underground conduits that conduct seawater over 2 km from the nearest ocean access with a 3 hour time lag (Button, et al., 2007). This tidal flux exchanges a considerable water volume twice daily with the open ocean. Water depth is typically 1-1.5 m except over the conduits. The surrounding topography indicates that hillside rainwater catchment for the pond is limited. This suggests that nutrient input from the surrounding landscape is also relatively limited.

Furthermore, the seawater with which this pond mixes during tidal exchange is oligotrophic. All in all, we suspect that, though relatively sheltered from storm activity, Mermaid Pond may be nutritionally impoverished in comparison with some of the more exposed ponds that constitute our study series.

The Little Granny Pond Community.

Little Granny Pond is severely exposed to hurricane impact in several ways. It has a large surface area making it vulnerable to wind-driven wave action, and it has no sheltering mangrove community. Its oysters must anchor to fragile *Batophora* algae fronds (Figure 3).

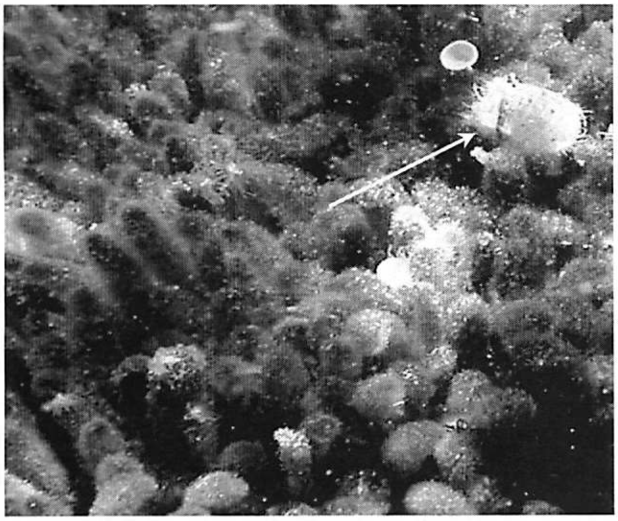

Figure 3. Pinctada (arrow) anchored to Batophora "finger" algae in Little Granny Pond.

During reconnaissance trips made in January 2001 (16 months following Hurricane Floyd) and January 2005 (four months following Hurricane Frances), we found that the Little Granny oyster population was decimated, their shells driven into deep piles along the shoreline. Oysters from the more protected ponds seemed relatively undamaged. Little Granny is also somewhat hypersaline (45 ppt) and marked by a relatively impoverished species diversity. The interior is deep (3 - 4.5 meters) and covered in uniform, deep flocculent sediment. As one approaches shore, the "floc"-bottom rises to a depth of 1-3 meters. Finally, in the shallows, (one meter or less) soft sediment gives way to a hard carbonate, shelly-sand bottom that is covered in a dense monoculture of "finger"-algae (*Batophora oerstedi*), the principle (fragile) substrate for *Pinctada longisquamosa*. Closer to shore, and clustered around the freshwater seeps, we found both black mangrove oysters and burnt mussels anchored to the carbonate substrate. Overall in comparison with Mermaid Pond, Little Granny Pond can be characterized as species-poor, but population dense. With an absence of tidal turnover, and a huge drainage area covering the surrounding scrub forest, this pond may be more nutrient-rich than the less saline Mermaid Pond. Preliminary data suggest that suspended organic matter is more abundant in Little Granny Pond than in Mermaid, and a casual examination of oyster gut contents in paraffin sections suggest that the oysters of Little Granny Pond are feeding better than those in Mermaid Pond (more abundant algae packed in the gut).

Oyster Pond and Little Lake.

Two other ponds on the island harbor *P. longisquamosa*; Oyster Pond, and Little Lake. Oyster Pond resembles Mermaid Pond in being small, protected, tidal and supportive of a relatively rich biodiversity (Cole, et al., 2007). Its shoreline supports a dense and productive mangrove prop-root community. Little Lake, on the other hand, is the largest of the lot, with little hard substrate, and low seawater turnover. It most closely resembles Little Granny in its hurricane exposure. We view these ponds as potential replicates of the natural experiment that is underway in Mermaid and Little Granny Pond.

NATURAL HISTORY OF THE PEARL OYSTER

No work has been published on the life history of *P. longisquamosa*, so its life-history characteristics cannot be assumed. Nevertheless, it is worthwhile noting the characteristics of its commercially important sibling species, to provide a context for what we have been finding.

Pearl oysters are sessile bivalves (family Pterriidae), more closely related to mussels than edible oysters of the family Ostreidae (Temkin, 2006, Mikkelson, et al., 2004). In coastal environments, *Pinctada* colonies attach to sea grass, especially *Thalassia testudinum*. Adults attach themselves to substrate using byssal threads rather than cement. Pearl oysters filter feed on algal phytoplankton. In studies on coastal populations of *P. imbricata*, (the Atlantic Pearl Oyster), breeding has been shown to occur throughout the year though peak breeding appears to occur during the colder parts of the year (Urban, 2000a). Arntz & Farnbach, (1991), and Urban & Tarazona, (1996) suggest that cold-water upwelling causes an increase in nutrient availability that may support gonad development, and this subsequently drives reproductive conditioning to peak during the colder months. Coastal populations of *P. imbricata* have a broad period of reproductive readiness, with two annual peaks in spawning as evidenced by individuals with "spent" gonads. These open ocean oysters grow rapidly, following a modified von Bertalanffy growth curve (Urban, 2002). In these conditions (temperature 23-27°C), oysters grow to 50 mm shell diameter in less than a year (approximately 5mm/month).

Oysters breed by releasing gametes through their excurrent siphons. Fertilization occurs in the water column, and embryos develop into veliger larvae commonly known as "spat". After approximately one month as free-swimming members of the plankton community, veliger larvae settle, attach to an appropriate substrate, and metamorphose into 0.2 mm juveniles (Ruffini, 1984). During their first year of growth, juveniles typically attain sexual maturity as males. Following further growth and development, (two years in *P. imbricata*), hermaphroditic females that began life as males; undergo a sexual transformation into females. In stable populations, this results in a gradual shift towards a 50:50 sex ratio. The sex ratio of one Pacific population of *P. imbricata* typically favors males 8:1 (Saucedo & Monteforte, 1997). This is likely due to age-dependent mortality favoring smaller, younger oysters that are more likely to be phenotypic males, whereas older, larger oysters are more likely to be females. (This may represent a less severe case of the scenario we shall be tracing in our inland ponds).

Oysters From San Salvador's Inland Ponds.

<u>Origins.</u> Ponds supported by conduits have an obvious and direct avenue for colonization in that they are connected by subterranean waterways to the sea. This may account for oysters found in both Mermaid and Oyster Pond. (It is curious that coastal populations of *Pinctada longisquamosa* have not yet been discovered on San Salvador Island, despite an abundance of suitable habitat and numerous reconnaissance trips.) The more interior ponds lacking conspicuous conduits (Little Granny Pond for example) represent a different puzzle. Their colonization may have lead through less conspicuous subterranean waterways, or, more likely, through air-born routes as waterfowl move between ponds and serve as vectors of larval dispersal. It seems possible that gene flow is still a factor in shaping these inland populations.

<u>Demography</u>. We have begun to generate a picture of the life history of oysters from the inland marine ponds on San Salvador Island (Cole, et al., 2007). Growth rates are slow compared to other *Pinctada* species. For Little Granny Pond, shell diameters follow a von Bertalanffy growth curve (Swanson, et al., 2008, this volume), comparable to that described for *P. imbricata*, (Urban, 2000b). During the first year of growth, hinge-length increases about 1 mm/month. During the second year, growth slows to approximately 0.25 mm/month. The largest adults were rarely over 24 mm and had slowed to approximately 0.1 mm/yr. Overall, *P. longisquamosa* grows about 1/5 as fast as *P. imbricata* and achieves a maximum only one third the size. In Mermaid and Oyster Pond, larger individuals (>30 mm hinge length) are relatively common, occasionally exceeding 35 mm hinge length. We have not yet determined growth dynamics for these populations. We suspect that the prevalence of larger oysters in Oyster and Mermaid Ponds is not due to differences in growth dynamics, but to protection from hurricane impact, so that these populations

contain both older individuals as well as juveniles whereas Little Granny Pond appears to be constituted by only very young individuals due to Hurricane decimation in both 1999 and 2004.

Nutrient availability. Demographics and sex determination in mollusks can be dramatically affected by nutrient availability. To get a snapshot of nutrient conditions within our various ponds, we filtered water samples and measured suspended carbon in the form of ash-free dry weight. Figure 4 displays our findings for the four ponds that currently support live oyster populations. It is noteworthy that the two ponds that exhibit the most dramatic turnover through marine conduits also exhibit the lowest levels of suspended nutrients. The two larger lakes that possess limited turnover and large rainfall catchments also exhibit the highest levels of suspended nutrients.

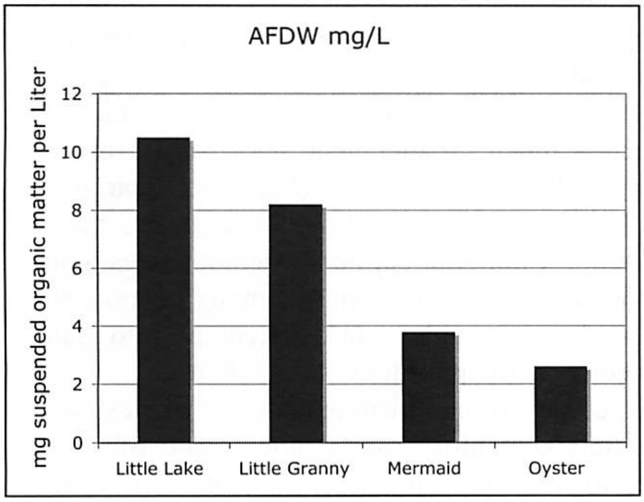

Figure 4. The amount of ash-free dry weight (an estimate of suspended organic material) per Liter of pond water sampled.

Sex determination. *P. longisquamosa* appear to reach sexual maturity by the end of the first year of growth as hinge length exceeds 10 mm. (Among the smallest size class "A", only the largest individuals exhibit mature gonads). We estimated the age for these individuals using demographic-growth data (Swanson, et al., this volume). In Mermaid Pond, juveniles achieve sexual maturity within the first year, and do so predominantly (if not exclusively) as males (Figure 5). Over the next few years of growth (as estimated by hinge length) the population comes to express a near 50:50 sex ratio following the conventional protandrous hermaphroditism seen in other *Pinctada* species. This appears to occur by re-differentiation of testis as ovary (Figure 6).

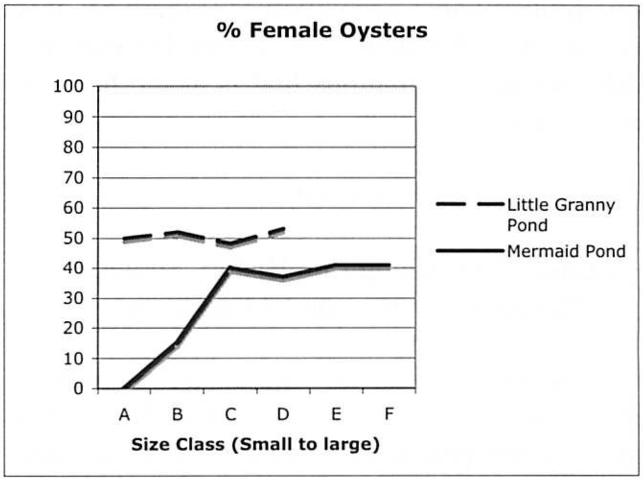

Figure 5. Pinctada gender assignment correlated with age. The smallest size class with developed gonads: "A", has a 5-10 mm hinge length. Size classes go up in 5mm increments. Only oysters with mature gonads were scored.

Figure 6. A mature P. longisquamosa testis with developing oocytes (black arrows) surrounded by developing spermatozoa. Sperm tails are visible in tracts (white arrows).

Oysters from Little Granny Pond, however, exhibit gonochoristic sex determination, producing a 50:50 sex ratio even among the smallest individuals with identifiable gonad tissues (Figure 5). It is this switch in life history trajectory between recently isolated populations that is at the heart of this investigation.

DISCUSSION

A Case of Switching Life History Strategies Based on Environmental Conditions.

Among molluscs, there are numerous well-studied examples of hermaphroditism. Sequential hermaphroditism has been demonstrated in several limpet-like Gastropods (*Patella;* Le Quesne and Hawkins, 2006, and *Crepidula*; Hoagland, 1975, 1978), and sedentary marine bivalves such as the edible oyster (*Crassostrea;* Guo, et al, 1998), the shipworm (*Teredo*; Coe, 1943), and the pearl oyster (*Pinctada;* Tranter, 1958). In almost every case of sequential hermaphroditism within bivalve molluscs the direction of sex-reversal is protandrous, from male to female. This is especially true for sessile molluscs with free-swimming larvae that do not exhibit sexual selection associated with social contests for mates.

In the pearl oysters of the genus *Pinctada*, we typically see a switch from male to female form as individuals mature. This is consistent with evolutionary theory that predicts protandry when there is a reproductive advantage for females with an increase in body size (Ghiselin, 1969, Hoagland, 1978, Charnov, 1982). *Pinctada* species from both the Caribbean, and the Pacific have been shown to switch from male-only juveniles to a 50:50 sex ratio within the older members of a population, or even towards a female bias amongst the oldest, largest specimens (Tranter, 1958, O'Connor & Lawler, 2005). This can have profound demographic consequences when natural catastrophes result in size-dependent mortality (specifically targeting the older, female-rich segment of the population). Such an event has been shown to have shifted sex-ratios in favor of juvenile males in Pacific *Pinctada mazatlanica* whose geographic range has expanded only recently after, presumably, a catastrophic local extinction coupled with immigration and ongoing size-dependent mortality (Arnaud-Haond, et al., 2003).

It is likely, that the literature underestimates the lability of sex-determination as a life history trait. Tranter (1958) reported rare cases of female-to-male transitions amongst a predominantly protandrous species of *Pinctada.* Hoagland (1978) also comments on conditions that favor lability of sex-determination: "Environmental influence in the timing of sex change allows the size and age of an organism at sex change to be tailored to suit very localized conditions, even if the genetic constitution of local individuals reflects a much broader gene pool from a broader range of environments." (See also Charnov & Bull, 1977, Charnov 1982). The sessile marine annelid, *Capitella capitata*, demonstrates gonochoristic life histories unless females become rare. In such cases, males can differentiate into simultaneous hermaphrodites (P.S. Petraitis, 1988). There is even one documented case of freshwater mussels switching from gonochoristic life histories to hermaphroditism in populations that have become isolated from the main breeding population (Weisensee 1916).

In *Pinctada longisquamosa* we document a remarkable divergence in life history associated with local habitat. In Mermaid Pond, we see a traditional protandrous hermaphrodite, yet in a different location on the same island (Little Granny Pond), we see the same species following a gonochoristic pathway, directly developing a 50:50 sex ratio. This represents a unique opportunity to explore the developmental or evolutionary factors that drive sex determination in wild populations. We propose two sets of hypotheses: 1) differences observed in life history trajectories are due to genetic changes resulting in evolutionary adaptation and 2) these differences represent adaptive phenotypic plasticity to differing environmental cues.

The Hurricane Rebound Hypothesis.

We suspect that hurricanes have played a frequent and periodic role in devastating the oyster population of Little Granny Pond, while sparing those of the relatively protected Mermaid and Oyster Ponds. We estimate (based on modern records) that a hurricane appears approximately every 5-10 years (See Figure 7). As Little Granny Pond recovers from hurricane impact, it is covered with settling juveniles within a half-year's time. Storm activities in September may actually trigger spawning due to sudden drops in temperature or salinity (Cole, et al., 2007). By the end of the first growing season, (if these *Pinctada* follow a protandrous sex determination pathway), all the juveniles will have differentiated into males. If one supposes that periodic hurricane events trigger spawning resulting in a near monoculture of juvenile male offspring against an otherwise decimated adult population (including most of the females), then any genetic variant exhibiting precocious feminization (or elimination of the maphrodite pathway in favor of direct female development) would find selection working in its favor. A mature female would find itself the sole (or rare) beneficiary of all resident male spawning activity. Most importantly, in the absence of competition from other females, this individual would have a preponderance of resources available to allocate to offspring production. With repeat performances, one can imagine that an allele (or suite of alleles) favoring gonochoristic reproduction over protandry would reach some equilibrium level of penetrance within the population. The added possibility of storm-driven genetic bottlenecks might even lead to fixation of such a genotype. This suggests a genetic model to account for the prevalence of protandry within our protected pond, and a switch to gonochoristic sexual development within ponds experiencing periodic catastrophic reductions. A genetic basis to sex determination could be evaluated by raising oysters from each pond under controlled laboratory conditions. If juveniles follow the same sex determination trajectory regardless of the habitat provided, this would provide support for a genetic basis of pathway divergence.

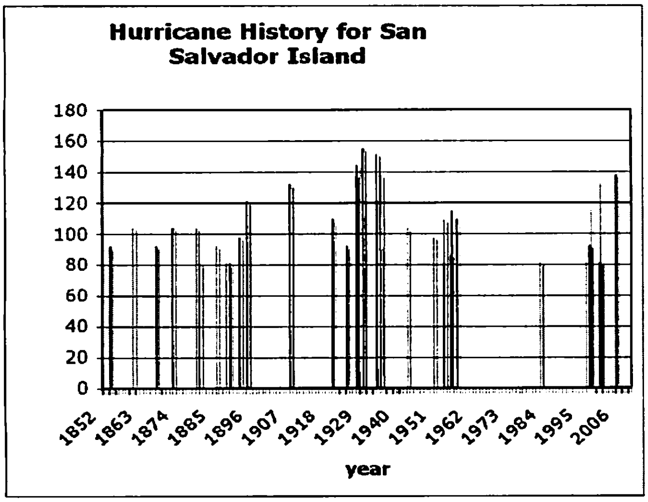

Figure 7. A graph showing every storm to hit San Salvador (directly) with winds exceeding 70 mph since 1852.

The Developmental Adaptation Hypothesis.

Alternatively, this suite of ponds and their inhabitants might be exhibiting environmentally driven changes in development resulting in disparate sex-determination pathways. Juvenile *P. longisquamosa* may possess a repertoire of potential sex-determination itineraries that are triggered by specific environmental cues such as 1) nutrient availability (Rosenfeld, C. D. & Roberts, R. M. 2004, Kudo & Nakahira, 2005, Robertson, et al., 2006), 2) a preponderance of one gender over another in the extant adult population {see annelid example: *Capitella capitata*, (P.S. Petraitis, 1988)}, or even 3) the absence of an extant adult population (a gonochoristic colonization strategy vs a protandrous standing population strategy). A final hypothesis falling into this category is that 4) sex determination is being altered by "environmental pharmacology" within these habitats. Little Granny Pond has several features that make this hypothesis tenable. First, without an outlet, and by draining a relatively large forested catchment, organic leachates from forest litter could be concentrating phyto-estrogens in the water supplying Little Granny Pond. Alternatively, the dense monoculture of *Batophora oerstedi* that serves as the ***exclusive*** substrate for oyster colonization in Little Granny Pond, might be producing phyto-chemicals that effect sex determination in juvenile oysters. Last, San Salvador is in the habit of broadcast spraying insecticides to control mosquitos. It is conceivable that, due to its disproportionately large drainage basin, Little Granny pond is concentrating xeno-estrogens from such a source.

Nutrient-driven sex determination. The ocean waters surrounding San Salvador are oligotrophic. Mermaid Pond has conduits that most-likely open into the deep waters facing the Atlantic with no nearby sea-grass beds, estuaries, harbors or mangroves to subsidize the nutrient poor condition of this reservoir. The terrestrial topography surrounding Mermaid also suggests that it drains a relatively small area of scrub jungle following rainfall with surface and subsurface runoff. Little Granny Pond, on the other hand, exhibits no detectable exchange or flushing with ocean waters. The pond drains a considerable acreage of hilly forest-land, and we have detected algae-covered freshwater seeps all along its shoreline stained with iron-rich residues, evidence of sub-surface leaching through the surrounding red paleosols. Furthermore, Little Granny supports an enormous bed of macro-algae, specifically *Batophora*, whereas Mermaid Pond has a relatively barren carbonate and sediment floor covering. This enormous macro-algae community argues for an abundance of nutrients unique to Little Granny Pond. Preliminary analysis (weighed filtrates) shows that Little Granny Pond possesses twice the suspended biomass of that in Mermaid Pond. Histological analysis suggests that oysters from Little Granny Pond may have more diatoms and other micro-algae within their guts than do those of Mermaid Pond. Finally, preliminary growth rate comparisons suggest that Little Granny Pond Oysters have a slightly faster growth rate than those in Oyster Pond. All these observations argue that Little Granny Pond may be richer in nutrients when compared to the smaller, tidally flushed mangrove ponds fed by underground conduits, though further work is needed to properly assess this.

It is conceivable that in habitats characterized by nutrient-rich conditions, *Pinctada longisquamosa* are programmed to alter their developmental pathway to produce an early-female (non-hermaphrodite) developmental trajectory. Again, the argument is that oogenesis is a more energy demanding process, and it would be adaptive to be able to turn to egg-making under nutrient rich circumstances. This hypothesis suggests a physiologically labile rather than a strict genetic form of adaptation resulting in protandry and gonochorism in two nutritionally disparate habitats. If so, this would represent an unique case of environmental sex-determination, a wholesale switch from protandrous hermaphroditism to gonochorism, triggered by nutritional resources.

This hypothesis yields two eminently testable predictions. First, Little Granny Pond should show evidence of elevated nutritional subsidies when compared with Mermaid Pond, (which it does). Second, individuals from both populations, when raised under identical conditions and feeding regimens, should adopt a common developmental trajectory (presumably gonochoristic if they are well fed), while individuals from the same population should exhibit divergent developmental pathways when reared under contrasting conditions.

Adult influence over sex determination. A second form of environmental determinism is that settling larvae (or developing juveniles) might take their cue from the abundance of adults or adults of a specific sex when adopting their own sex determination pathway. This too is testable by raising juveniles in the presence or absence of adults, (preferably of specified sex), and assessing their sex determination trajectories. One might predict that in the presence of adult females, protandry would be favored, while in the absence of such, or in the absence of any adults, a gonochoristic pathway would be followed.

Environmental estrogens and sex determination. A third possibility is that environmental compounds are altering sex determination in these two populations. Nice et al., (2003), demonstrated that exposure to man-made nonylphenolic compounds resulted in both a dramatic feminization within a Pacific population of edible oysters, and an increase in a tendency towards hermaphroditism. This compound is a xeno-estrogen, capable of bringing about dramatic changes in sex determination in a number of aquatic species. It occurs to us, that there could be naturally occurring (or man-made) estrogenic compounds within our test sites that differentially affecting our various populations. Specifically, it seems possible that the oysters of Little Granny Pond might be experiencing such unusual exposure. First, its waters are turning over very slowly and the pond serves as a concentration catchment for the surrounding terrain with no egress. If rainwater is leaching through decaying plant tissues and extracting biologically active compounds (or leaching man-made compounds such as insecticide derivatives) these might become concentrated in this pond to a degree that effects development. Second, since the oyster colony is imbedded within a dense monoculture of *Batophora* algae, we wonder if these might provide an even more local source of developmentally significant compounds. In either case, there are sensitive assays for estrogen-like compounds, and our institution is well equipped to pursue these possibilities.

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