

Population dynamics of American horseshoe crabs—historic climatic events and recent anthropogenic pressures

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Abstract

Populations of the American horseshoe crab, *Limulus polyphemus*, have declined, but neither the causes nor the magnitude are fully understood. In order to evaluate historic demography, variation at 12 microsatellite DNA loci surveyed in 1218 *L. polyphemus* sampled from 28 localities was analysed with Bayesian coalescent-based methods. The analysis showed strong declines in population sizes throughout the species' distribution except in the geographically isolated southern-most population in Mexico, where a strong increase in population size was inferred. Analyses suggested that demographic changes in the core of the distribution occurred in association with the recolonization after the Ice Age and also by anthropogenic effects, such as the past overharvest of the species for fertilizer or the current use of the animals as bait for American eel (*Anguilla rostrata*) and whelk (*Busycon* spp.) fisheries. This study highlights the importance of considering both climatic changes and anthropogenic effects in efforts to understand population dynamics—a topic which is highly relevant in the ongoing assessments of the effects of climate change and overharvest.

Keywords: *Limulus*, over-harvest, population decline, population dynamics

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Introduction

Coalescence-based methods allow estimation of the timing of changes of population size, but mostly have been used in studies of terrestrial vertebrates (e.g. Goossens *et al.* 2006; Heller *et al.* 2008; Craul *et al.* 2009; Mondol *et al.* 2009) and fishes (e.g. Koskinen *et al.* 2002; Gold *et al.* 2009; Nikolic *et al.* 2009; Swatdipong *et al.* 2010). Several of these studies showed declines explained by either older climatic change or more recent anthropogenic pressure. However, an important underlying question is the effect of fecundity in relation to inferring changes of population sizes. That is, do high fecundity organisms generally show older climatically driven

population declines (e.g. Gold *et al.* 2009; Karlsson *et al.* 2009; Swatdipong *et al.* 2010; but see Koskinen *et al.* 2002 for an example of an inferred recent decline in a high fecundity organism), whilst recent anthropogenic effects are often found for low fecundity organisms (e.g. Goossens *et al.* 2006; Mondol *et al.* 2009; Craul *et al.* 2009 but see Heller *et al.* 2008 for an example of an inferred old decline in a low fecundity organism). Whilst available results indicate such a pattern, a wider selection of habitats and taxonomic groups is required in order to improve our understanding on how different life history factors may influence demographic inferences. In this study, we analysed effective population size (N_e) changes of a marine invertebrate with high fecundity, the American horseshoe crab (*Limulus polyphemus*).

Limulus polyphemus, occupies a large latitudinal range (from 20 to 45°N) in eastern North America, with

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breeding populations in estuaries and bays along the west Atlantic coast from Maine to Florida, with additional populations in the eastern Gulf of Mexico and around the Yucatán peninsula of Mexico (Anderson & Shuster 2003). The annual average seawater temperatures span from around 7 °C in Hog Bay, Maine to 26 °C in St. Joseph Bay, Florida, Gulf of Mexico, whilst July water temperatures span between 13 °C and 30 °C (Locarnini *et al.* 2006). *L. polyphemus* spawn subtidally or on intertidal beaches with moist, aerobic sandy sediment. Development time decreases with higher temperature (Penn & Brockmann 1994). Moreover, there appears to be a threshold temperature below which eggs do not develop (French 1979; Jegla & Costlow 1982), which could further impact marginal distribution.

The area around the Delaware and Chesapeake embayments is in the centre of their distribution, and holds the largest populations (Shuster 1982). There appear to be otherwise suitable coastal habitats north of their current distribution, and the northern limit of their distribution is generally assumed to be determined by temperature (Sekiguchi & Schuster 2009). There also appears to be suitable habitat south of the current distribution, here temperature may indirectly affect the southern limit of the distribution.

The population dynamics of individual populations of *L. polyphemus* has probably been affected by three major factors: the last ice age; post-Pleistocene climatic fluctuation, e.g. the Little Ice Age; and direct or indirect consequences of increased human populations in the last few centuries.

The temperature of much of the current distribution of *L. polyphemus* was rather similar in the last ice age and only the most northerly populations would have been directly exterminated by the lower temperature (Roche *et al.* 2007). Changed sea levels, however, would have reduced the available habitat in many areas, and many of the current populations were founded after the ice age. Delaware Bay, for instance, formed in the middle of the Holocene and since then has contained suitable habitats for several million horseshoe crabs (Kreamer & Michels 2009). If the populations of *L. polyphemus* in Delaware Bay were founded by a limited number of individuals, this would have caused a bottleneck, resulting in a pattern of "northern purity" in which northern populations show low genetic variation (Hewitt 2000). Such bottlenecks can be recognized both as population declines or expansions depending on the intensity and length of the bottleneck, but population decline is thought to be most likely (Hein *et al.* 2005).

Recent temperature fluctuations in North America show a medieval warm period, followed by a markedly cold period between 1450 and 1600 AD, followed by a

gradual temperature increase (Bradley & Jonest 1993; Cronin *et al.* 2003; Mann *et al.* 2008). In temperature-limited species, one would expect population expansion southwards and a northern population reduction around 400 years ago. As temperatures increased, a concomitant northward expansion by species would be predicted.

Limulus polyphemus were intensively harvested for soil fertilizer in the Delaware Bay area from around 1850 to 1970 (Shuster 2001). Harvesting continues today, as *L. polyphemus* are used for bait in American eel (*Anguilla rostrata*) and whelk (*Busycon* spp.) fisheries (Berkson & Shuster 1999). Trawl surveys show that *L. polyphemus* stocks remain relatively low in most states (Atlantic States Marine Fisheries Commission 2009). There are only limited data for the population densities before harvesting began in the nineteenth century, but the yearly harvest in the 1870s in Delaware was over 4 million individuals, a large figure compared to the estimated population size of 20 million adults within the bay in 2003 (Smith *et al.* 2006). Harvest records show a strong decrease in landings beginning in the 1870s, which presumably corresponds to a strong decrease in population size (Shuster & Botton 1985).

Limulus polyphemus population sizes have declined recently (Shuster 2001), which appears to have affected the demographics of other animals. Atlantic loggerhead sea turtles (*Caretta caretta caretta*) mainly foraged on adult *L. polyphemus*, but have changed their diet following a decline in this food item (Seney & Musick 2007); this change to less-suitable food sources has been suggested as contributing to the large decline in loggerheads in Florida, which constitutes one of the largest populations of this species (Witherington *et al.* 2009). Several bird species also depend on *L. polyphemus*, not directly as a food source, but instead feeding on the enormous amount of eggs available on certain beaches during spawning. *L. polyphemus* eggs are the primary food for up to 1 million shorebirds which stage in the Delaware Bay during migration from overwintering locations in South America to breeding locations in the Arctic. Large population declines for *L. polyphemus* may have contributed to strong shorebird declines, especially for the American Red Knot (*Calidris canutus rufa*), which currently is listed as threatened under the Bonn convention (Niles *et al.* 2009).

Limulus polyphemus populations in Florida have not been harvested to the same extent as in the mid-Atlantic region, and the direct effects of fishing are probably unimportant. However, there has been an intense human population increase throughout Florida the last 150 years (approx. 50 000 in 1840, 500 000 in 1900, 5 000 000 in 1960 and 18 250 000 in 2008; University of Virginia 2009, U.S. Census Bureau 2009). This growing human population has led to a decrease in many wild

animals, such as the endemic Florida scrub jay (*Aphelocoma coerulescens*; BirdLife International 2008a), the Florida black bear (*Ursus americanus floridanus*; Garshelis *et al.* 2008) and the Florida panther (*Puma concolor coryi*; Caso *et al.* 2008). The effect of humans on Florida *L. polyphemus* is not known, but the concentration of humans by the sea and habitat modifications in areas used for spawning likely have had an adverse effect for *L. polyphemus*; they are known to have led to a decrease in the population size of the Florida Manatee *Trichechus manatus latirostris* (Deutsch *et al.* 2008).

Population genetics theory suggests that N_e of a population determines the rate of change in the composition of a population caused by random genetic drift. N_e is crucial in determining the level of variability in a population, and the effectiveness of selection relative to stochastic processes (i.e. the dynamic balance between selection and genetic drift). N_e thus becomes of central importance for inbreeding avoidance and for maintaining genetic diversity and evolutionary potential (Charlesworth 2009). Very low population size leads to the potential for inbreeding depression; population sizes a magnitude or two higher than the threshold for inbreeding depression may be necessary to maintain genetic diversity and hence evolutionary potential (Frankham & Franklin 1998; Lynch & Lande 1998). What constitutes minimum viable population size depends highly on the organism in question. Comparisons between species pairs such as the Mauritius Kestrel *Falco punctatus* and the Pink Pigeon *Columba mayeri*, however, indicate that a naturally low population size may decrease the susceptibility to inbreeding depression (Swinnerton *et al.* 2004; BirdLife International 2008b,c).

In this study, we used a Bayesian approach to detect temporal changes in N_e with a single contemporary dataset (Beaumont 1999; Storz & Beaumont 2002) to gain insights into the historical population dynamics of *L. polyphemus*. We thereby analysed the effects of the last ice age temperature fluctuations within the last millennium and anthropogenic factors as well as potential interactions. The results may prove useful for designing a suitable conservation strategy for *L. polyphemus*. The results likely will be comparable to many other co-occurring organisms and therefore will improve our understanding of the evolutionary history of the western North-Atlantic and will give new insights into potential interactions amongst factors influencing population dynamics.

Materials and methods

Data acquisition

A total of 1218 *L. polyphemus* were collected from 28 populations spanning the entire distribution of the

species (Table 1, Fig. 1) and genotyped for variation at 12 microsatellite loci (King & Eackles 2004). Data from 892 of the animals were previously used for an analysis of geographic subdivision of the species (King *et al.* 2005).

The PCR reactions consisted of 100–200 ng genomic DNA, 1 × PCR buffer (10 mM Tris–HCl [pH 8.3] and 50 mM KCl), 2 mM MgCl₂, 0.15% Tween-20, 0.25 mM deoxynucleotide triphosphates, 0.5 μM of both primers and 0.1 U *Taq* DNA polymerase in a total volume of 10–20 μL. The PCR conditions were an initial denaturation at 94 °C; followed by 35 cycles of: 94 °C for 40 s, 58 °C for 40 s and 72 °C for 60 s; and a final extension at 72 °C for five minutes. See King *et al.* (2005) for further details.

Demographic analyses

The existence of linkage disequilibrium or deviations from Hardy-Weinberg proportions were analysed in all populations (groups of sample sites) and the degree of geographic differentiation between sample sites was estimated by calculating F_{ST} values between all sites using GENEPOP 4.0.10 (Raymond & Rousset 1995). Sample sites were grouped into regions, defined as a group of sample sites where the F_{ST} values between neighbouring sample sites were lower than 0.01, and the demographic analyses were performed on these regions. Populations were characterized this way instead of using automatic procedures such as STRUCTURE (Pritchard *et al.* 2000), as this programme is known to have problems with datasets with strong isolation by distance (Boulet *et al.* 2007), as is found in *L. polyphemus* (King *et al.* 2005).

Recent population bottlenecks were inferred using the maximum likelihood-based software BOTTLENECK 1.2 (Cornuet & Luikart 1996) assuming an infinite allele model (IAM) or a two-phase model of mutation (TPM, with 70% Stepwise Mutation Model, SMM).

Historical population decline or expansion was inferred using the Bayesian MSVAR (Beaumont 1999). We performed the analyses for *L. polyphemus* from all regions assuming an exponential decline/expansion, as this model is valid for short-term strong declines (Beaumont 1999). Using multilocus microsatellite genotypes, the frequency distribution of allelic length variants can be viewed from a genealogical perspective to make inferences about historical changes in N_e . The programme assumes a stepwise mutation model, closed populations, and populations in Hardy-Weinberg equilibrium. It estimates the posterior probability distribution of several genealogical and demographic parameters using Markov Chain Monte Carlo simulations based on the observed distribution of microsatellite alleles and their repeat numbers (Beaumont 1999). The

Table 1 Supporting information for 28 collections of the American horseshoe crab (*Limulus polyphemus*). See Fig. 1 for a map showing the individual localities

Name	State	Locality	Region	Sample size
MEH	Maine	Hog Bay, Franklin	Hog Bay	47
MET	Maine	Thomas Point Beach	Upper Gulf of Maine	45
MEM	Maine	Middle Bay, Brunswick	Upper Gulf of Maine	48
NHS	New Hampshire	Chadman's Landing, Squamscott River	Lower Gulf of Maine	47
MAP	Massachusetts	Pleasant Bay	Greater Delaware Bay	48
RIN	Rhode Island	Green Island, Narragansett Bay	Greater Delaware Bay	48
CTH	Connecticut	Housatonic River, Milford Point	Greater Delaware Bay	48
NYP	New York	Great Peconic Bay, Long Island	Greater Delaware Bay	48
NJF	New Jersey	Fortescue Beach	Greater Delaware Bay	48
NJR	New Jersey	Reeds Beach	Greater Delaware Bay	48
NJH	New Jersey	Highs Beach	Greater Delaware Bay	49
DEK	Delaware	Kitt's Hummock Beach,	Greater Delaware Bay	36
DEB	Delaware	Big Stone Beach	Greater Delaware Bay	31
DEF	Delaware	Fowler Beach	Fowler Beach	47
MDT	Maryland	Turkey Point, Chesapeake Bay	Turkey Point	30
MDF	Maryland	Flag Pond State Park, Chesapeake Bay	Greater Chesapeake Bay	29
MD5	Maryland	Ocean City–2005	Greater Chesapeake Bay	45
MD6	Maryland	Ocean City–2006	Greater Chesapeake Bay	47
VKI	Virginia	Kiptopeke St. Park, Chesapeake Bay	Greater Chesapeake Bay	48
VAI	Virginia	Tom's Cove, Assateague Island	Not analysed	48
NCS	North Carolina	Shackleford Banks	Greater Chesapeake Bay	54
SBU	South Carolina	Bulls Bay, South Carolina	South Atlantic	53
SBE	South Carolina	Beaufort, South Carolina	South Atlantic	48
GSA	Georgia	Savannah, Georgia	South Atlantic	46
FLM	Florida	Mosquito Lagoon, Florida	Florida Atlantic	45
FLC	Florida	Seahorse Key, Cedar Keys NWR, Florida	Florida Gulf	46
FLJ	Florida	St. Joseph Bay, Florida	Florida Gulf	23
MXY	Yucatan, MX	Río Lagartos and San Filipe	Mexico	20

Bayesian methods provide, by using historical or subjective data about the probability distribution (prior distribution) of one or more input parameters of the model, a probability distribution (posterior distribution) of the following parameters: r , which is the ratio between the current and ancestral population size, (N_0 and N_1); tf , which is the ratio between the number of generations since the population size change (ta) and N_0 ; and θ ($2\mu N_0$), where μ is the mutation rate. The probabilities of expansion or decline are estimated from the simulated chain by counting the proportion of iterations in which the population has expanded and then dividing this by the proportion of iterations in which it has contracted (Storz & Beaumont 2002). The programme was run on each population ten times with different starting points for the Markov chains. Each analysis was run for 650 million–1 000 million iterations on the BioHPC cluster (BioHPC 2010). Every 50 000th sample point was stored, giving a total of 13 000–20 000 sample points, with the first 3 000–10 000 sample points discarded as burn-in. The average likelihoods of the last 10 000 datapoints in each analysis were calculated, and the five chains which had converged with the highest

average likelihood were discarded in order to remove the potential effect of chains trapped in local optima. The remaining five runs were combined and the reported results are based on these runs.

The priors (all on a \log_{10} scale) were set as follows: The prior mean and standard deviation for mutation rate were -3.0 and 1.0 for all regions, and the prior mean time since the population started changing in size was 1.0 for all, with a standard deviation of 1.0 . Priors for the standard deviations were set with a mean of zero for all parameters and standard deviations of 0.5 for current and ancestral population sizes, and the time interval. The shortest continuous interval representing 70% or 90% of the distribution was calculated, as was the mode which was considered the point estimate. The analyses initially converged towards high mutation rates (μ often $>10^{-2}$) and converged towards different mutation rates in different populations (range: $10^{-2.24} < \mu < 10^{-1.76}$). This result is not likely, and we therefore recalculated the results based on a mutation rate of 10^{-3} . Whilst this is close to the usual microsatellite mutation rate for many animals, we acknowledge that the mutation rate for microsatellites is unknown for

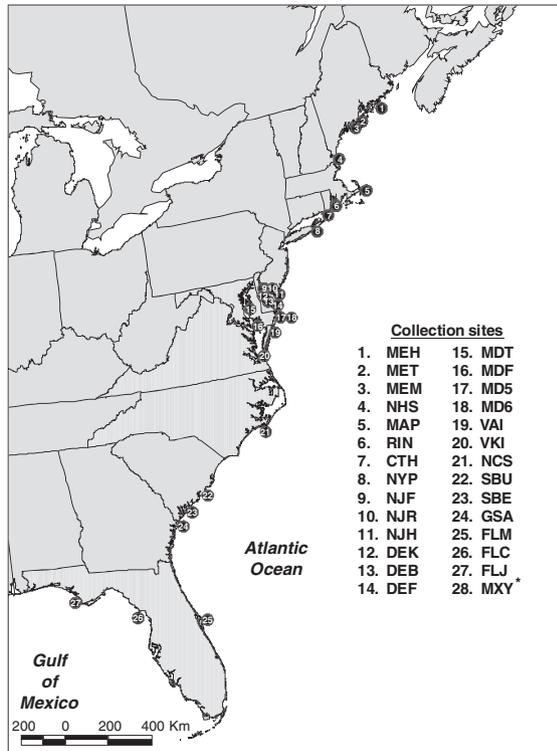


Fig. 1 Sampling sites for American horseshoe crabs (*Limulus polyphemus*). See Table 1 for location abbreviations.

horseshoe crabs and may deviate from this estimate (Weber & Wong 1993; Ellegren 1995; Sibly *et al.* 2001).

The generation time of *L. polyphemus* is complex to specify. The animals mature in 9–11 years for males and females, respectively, but may reproduce several times in their lifespan of at least 17–19 years (Shuster & Sekiguchi 2003). Whilst we believe a generation time of 13 years to be a reliable estimate, because of the uncertainties of this estimate, we present the data both as number of generations and years.

Results

Two sampling sites, the northern-most in Hog Bay, Maine and the southern-most in Mexico were highly diverged from the remaining sampling sites (F_{ST} values generally >0.2), whilst the remaining sampling sites were more closely related (all F_{ST} values <0.12) (Appendix S1). The remaining sampling sites were grouped into nine regions: Upper Gulf of Maine (two sample sites in Maine), Lower Gulf of Maine (one sampling site in New Hampshire), Greater Delaware Bay (nine sampling sites from Massachusetts, Rhode Island, Connecticut, New York, New Jersey and Delaware), Fowler Beach (one sampling site from Delaware), Upper Chesapeake Bay (one sampling site from Maryland), Greater

Chesapeake Bay (five sampling sites from Maryland, Virginia and North Carolina), South Atlantic (three sampling sites from South Carolina and Georgia), Florida Atlantic (one sampling site), and Florida Gulf (two sampling sites) (see Table 1 and Fig. 1). A single sampling site (Toms Cove in Virginia) was discarded. It had high F_{ST} values relative to both neighbouring clusters and had heterozygote deficiencies at four of the 12 microsatellite loci and likely represents a mixed population comprising *Limulus polyphemus* from both adjacent populations (Shuster 1985). It should be noted that although the Greater Chesapeake Bay and Greater Delaware Bay were defined as separate regions in our analysis, this was due to the single intermediate site of Fowler Beach, which was distinct from both regions, and there was very little genetic differentiation between Delaware and Chesapeake individuals.

Only one deviation from Hardy–Weinberg proportions (a heterozygosity deficiency at locus *LpoA26* in Upper Gulf of Maine) remained significant after Bonferroni correction (results not shown). There is no indication of systematic linkage disequilibrium since only approximately 5% of the uncorrected comparisons between all loci by population was significant at $P < 0.05$ (36 out of 726 tests; results not shown). Furthermore, significant linkages were observed between different pairs of loci in different populations, indicating that the results were not caused by physical linkage.

The results of the Cornuet & Luikart's (1996) procedure showed evidence of strong bottlenecks, with significant heterozygosity excess ($0.0006 < P < 0.0024$) assuming an Infinite Allele Model (IAM), and non-significant effects assuming a Two-Phase model (TPM) in all regions except Mexico. In Mexico, a population expansion was found instead (significant heterozygosity deficiency assuming a TPM $P = 0.00064$, and non-significant effects assuming an IAM).

The results from *MSVAR* (Beaumont 1999) were concordant with those of the Cornuet & Luikart (1996) procedure for all populations from Hog Bay, Maine to the Florida Gulf (Figs 2–4). *MSVAR* failed to converge for Mexico, probably because of the low sample size.

The recent N_e s (Fig. 2) were very low in all populations and can roughly be divided into three groups. The estimated N_e was lowest in Hog Bay, Maine ($N_e = 4$); intermediate in Lower Gulf of Maine, Fowler Beach (DE), Turkey Point (MD), and Florida Atlantic ($N_e = 23$ –72); and highest in the Upper Gulf of Maine, Greater Delaware Bay, Greater Chesapeake Bay, South Atlantic, and Florida Gulf ($N_e = 126$ –200).

All populations showed very intense declines (Fig. 3). The population decline was most intense in Hog Bay, Maine ($r = 2.1 \times 10^{-5}$). The declines in the other

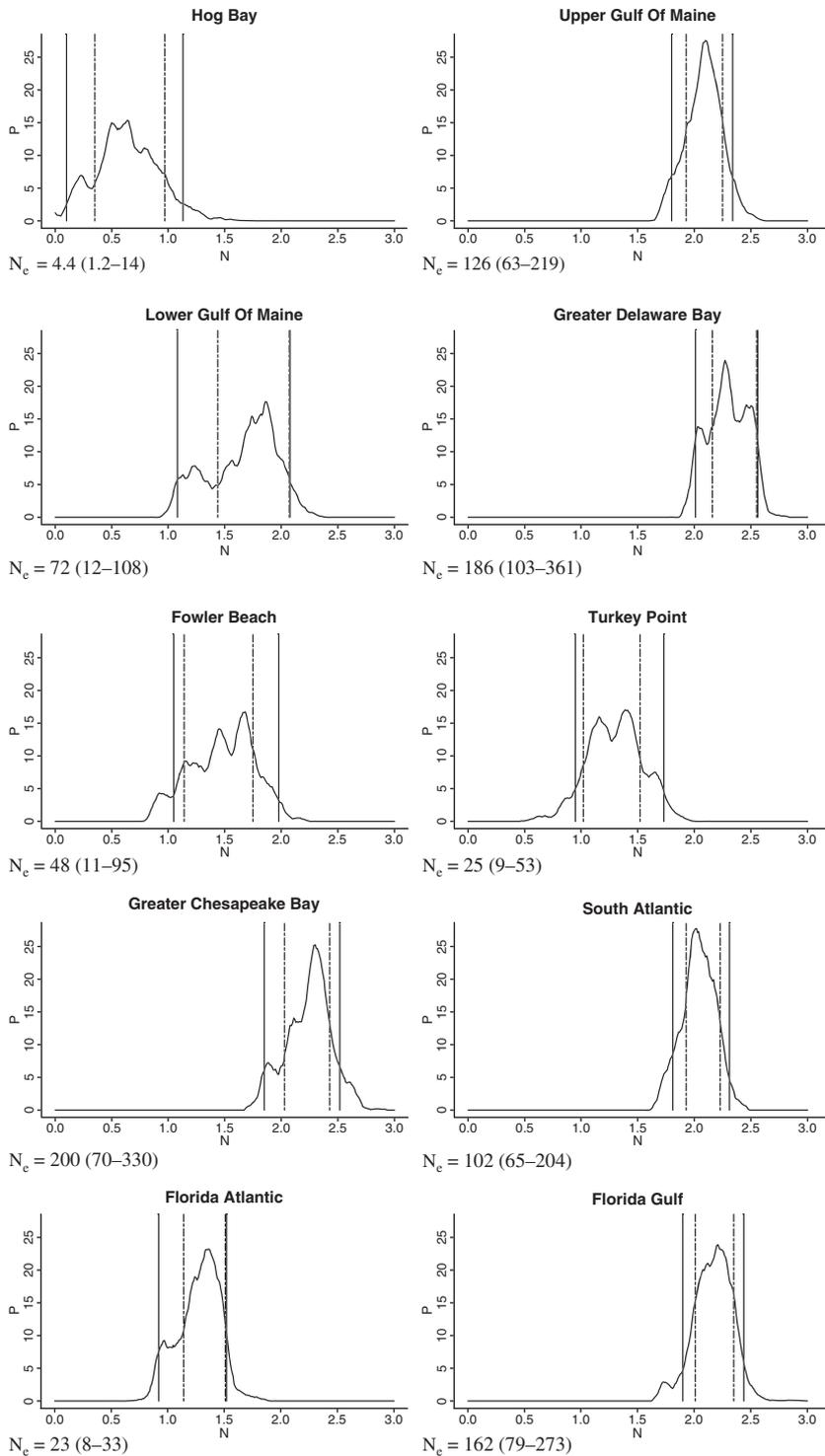


Fig. 2 Estimated time since population size changes in the respective populations. The graphs show the posterior distribution of the logarithm of the number of generations since the initiation of the population size change in bins of 0.01. The lines are smoothed by taking a running average of 10 points centred on each bin. The stippled and full lines are the shortest interval representing 70% or 90% of the distribution. The transformed mode and the 90% interval of each population are given below each graph, with an estimated generation time of 13 years.

populations were roughly similar, although the decline in the Florida Atlantic ($r = 2.5 \times 10^{-4}$) appeared more intense than in the Florida Gulf ($r = 1.6 \times 10^{-3}$). All population declines were inferred to have occurred between 832 and 6200 years ago (Fig. 4). The declines appeared more recent in Hog Bay (ME), Turkey Point (MD) and

Florida Atlantic ($T = 832\text{--}1290$ years ago); intermediate in Fowler Beach (DE) and Lower Gulf of Maine ($T = 2820\text{--}3550$ years ago); and older in the remaining populations ($T = 4170\text{--}6200$ years ago). The most recent and most intense declines and the lowest current N_e generally were found for the same populations.

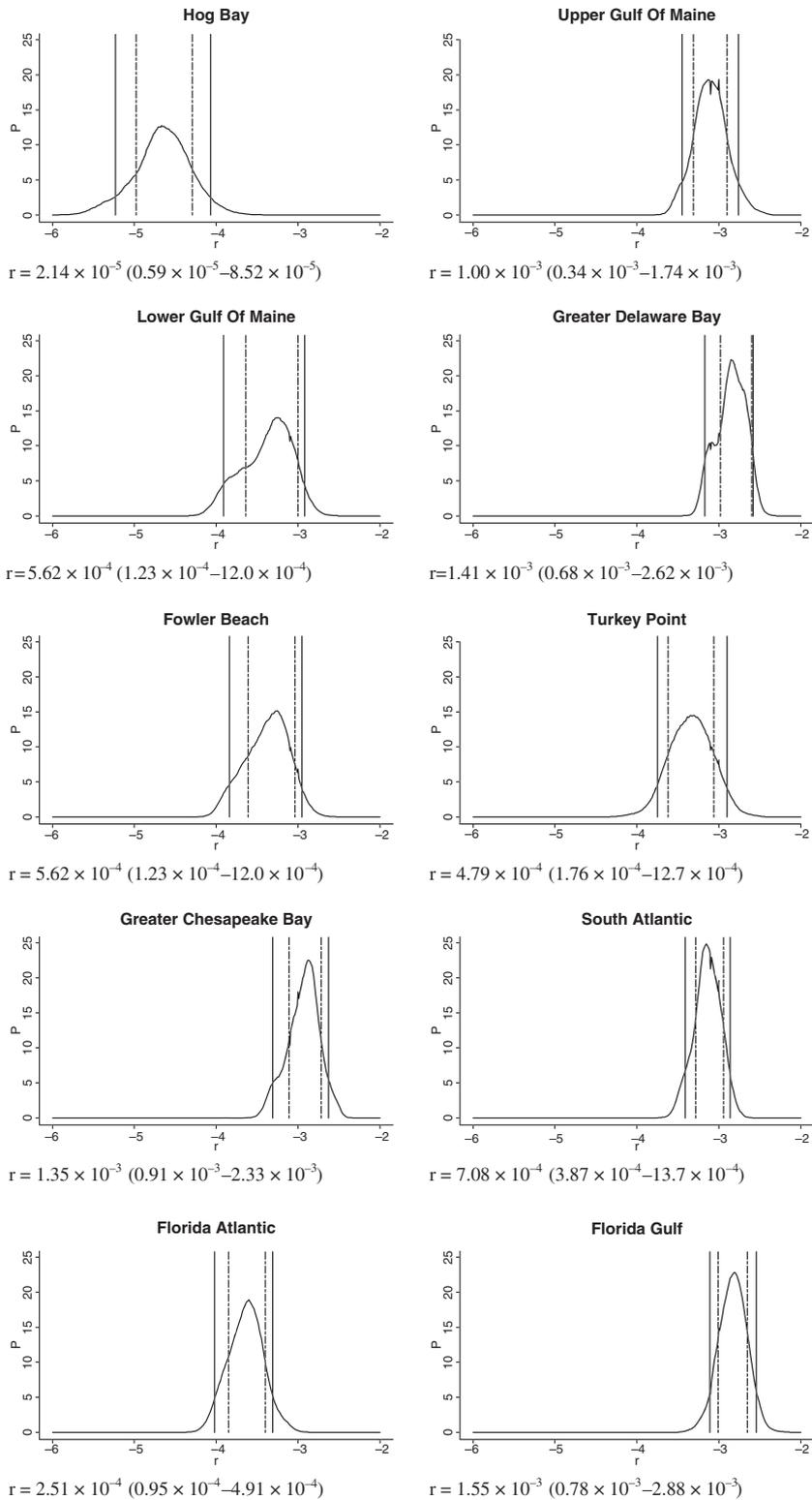


Fig. 3 Estimated current population size in the respective populations. The graphs show the posterior distribution of the logarithm of the current effective population size change in bins of 0.01. The lines are smoothed by taking a running average of 10 points centred on each bin. The lines are smoothed by taking a running average of 10 points centred on each bin. The stippled and full lines are the shortest interval representing 70% or 90% of the distribution. The transformed mode and the 90% interval of each population are given below each graph.

Discussion

Two independent demographic analyses using BOTTLENECK (Cornuet & Luikart 1996) and MSVAR (Beaumont

1999) suggested population declines in all horseshoe crab populations with the exception of Mexico, where a population expansion was inferred using BOTTLENECK.

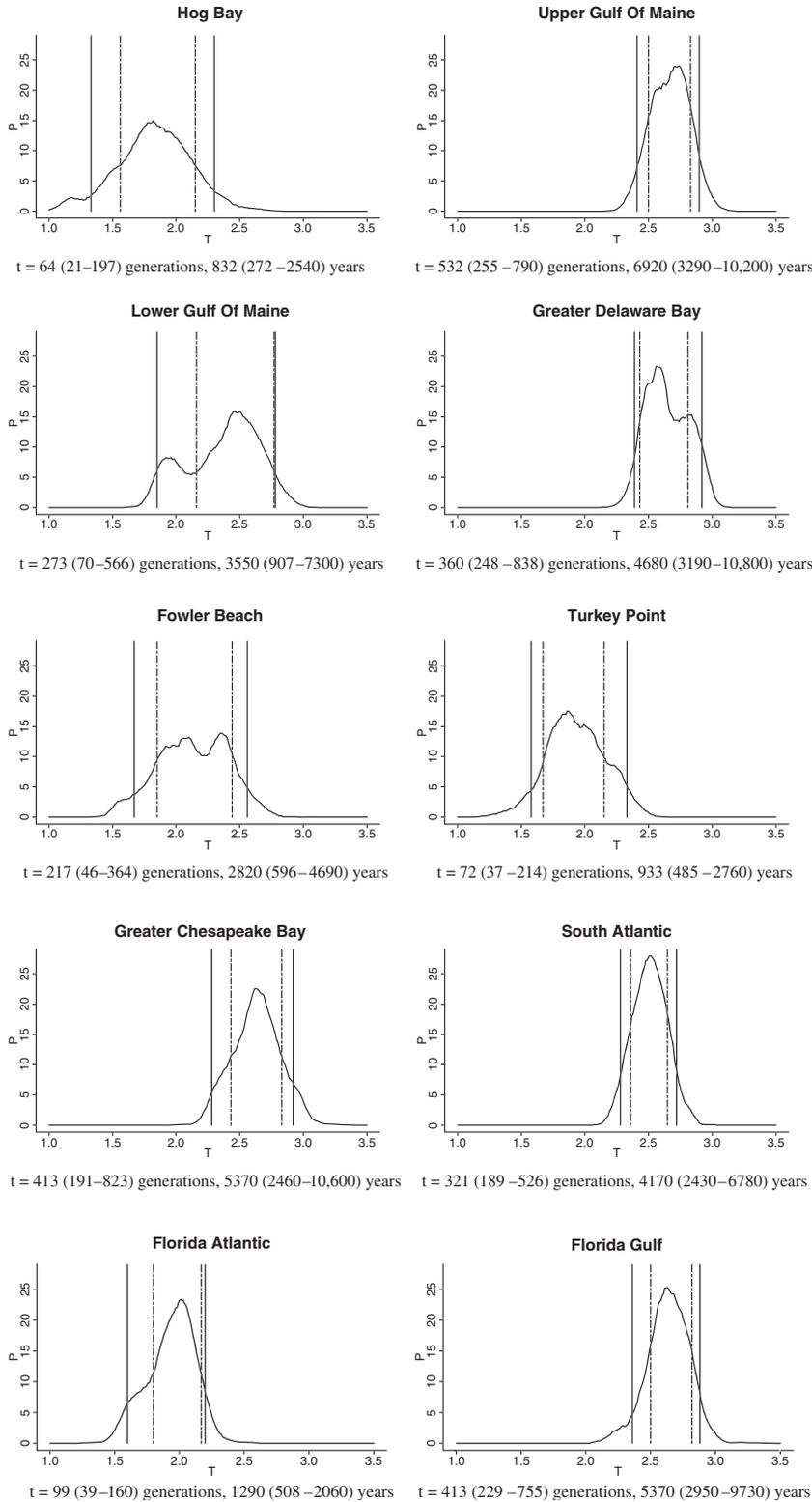


Fig. 4 Estimated population size changes in the respective populations. The graphs show the posterior distribution of the logarithm of the population size changes in bins of 0.01. The lines are smoothed by taking a running average of 10 points centred on each bin. The stippled and full lines are the shortest interval representing 70% or 90% of the distribution. The transformed mode and the 90% interval of each population are given below each graph.

The population expansion in Mexico indicated by BOTLENECK was perhaps surprising and contrasted with the view that numbers of *Limulus polyphemus* have

decreased markedly in recent decades (Gomez-Aguirre 1979, 1987; Gómez-Aquirre 1993). Note, however, that our result was based on a sample of relatively few

individuals from a small geographic range. Future studies should include more individuals from different Mexican localities to test our findings.

The oldest demographic declines were inferred in five regional populations: Upper Gulf of Maine, Greater Delaware Bay, Greater Chesapeake Bay, South Atlantic and Florida Gulf. Given the variance in the time estimate and the uncertainties in generation time, these declines could have co-occurred at the end of the last Ice Age, although it should be noted that all point estimates are more recent. The declines in the more northerly populations can be explained by northern purity (Hewitt 2000), but this pattern cannot easily explain the decline in the southern populations, especially Florida Gulf. The result could be explained by the specific demographic patterns in *L. polyphemus*. The largest current populations are in areas uninhabited during the Ice Age and all populations are linked with rather large gene flow, as suggested by the low F_{ST} values between them. It is therefore possible for the larger populations to swamp genetic uniqueness of the southern populations. This can create a pattern where N_e in the southern populations decreases as a consequence of the increased amount of immigrants with low variation, even though there is no decline in the populations themselves. It would, however, require migration against present oceanic currents, and similarly old declines have also been inferred for two species of snappers (*Lutjanus* sp.) from the Mexican Gulf (Gold *et al.* 2009; Karlsson *et al.* 2009). The declines in the southern populations may therefore also be a direct consequence of rising sea level, which could have affected many populations as previously discussed for Delaware Bay.

Since evidence for very old population declines was found for both northern and southern populations, it seems likely that the Ice Age and following colonizations have impacted all populations similarly. The populations may therefore not have reached new equilibria before recent factors influenced them again. The study by Okello *et al.* (2008) on the population dynamics of African elephants (*Loxodonta africana*) showed how $MSVAR$ (Beaumont 1999) responds to such conditions. They found that when there was a strong effect of both climatic fluctuation and poaching upon the population, the estimated time of population decline did not co-occur with either of them, but rather was estimated to have occurred intermediate between them. If similar factors are working here upon horseshoe crabs, the timing of the decline in the five other populations Hog Bay (ME), Turkey Point (MD), and Florida Atlantic, and perhaps also Lower Gulf of Maine and Fowler Beach—cannot be inferred, but can only be suggested to be more recent than in the other populations. Although not discussed in a study by Pruett *et al.*

(2010) on Rock ptarmigans from the Aleutian Islands, their analysis of the population size changes could be interpreted in a similar way. They found evidence of population decline on three islands. The island with the weakest decline had the decline co-occurring with the end of the last ice age, whilst the decline on two other islands were much more intense and shifted towards more recent times, likely as a consequence of introduced predators which led to an additional population decline.

In the present study, it seems likely that different factors were responsible for additional decline in the different populations. The northern-most population in Hog Bay, Maine is isolated genetically from the other populations. The population density in the surrounding areas is low compared to other *Limulus* populations and no deliberate fishing of the population is known. The population, therefore, is unlikely to have been negatively affected by humans and the additional decline is likely to have been caused by an additional founder effect, as this also could explain the genetic distinctiveness. The timing of the population founder event is unknown. The population was not known prior to the genetic analysis of King *et al.* (2005), and it is possible that the population represents the leading edge of the distribution founded as a consequence of recent global warming.

The additional decline in the other populations is, however, likely to be anthropogenically induced. The intense and rapid urbanization of the Atlantic coast of Florida has led to the coastal areas being highly affected by humans (Halpern *et al.* 2008) and has likely led to the destruction of habitats for horseshoe crabs. The decline in the southern Gulf of Maine population could potentially be linked to harvesting for medical uses, since one of the major blood collection facilities is situated close to this population, but the lower Gulf of Maine is also heavily populated and the decline could also be a consequence of increased coastal population size of humans as in Florida.

It is interesting that additional declines were inferred only in some of the populations which have been affected negatively by humans in the last two centuries. This finding is probably related to the respective population sizes. All the populations where additional declines were inferred consisted of only a single sample site and had lower N_e than the populations where such declines were not inferred. It is possible that population fluctuations or the effects of the initial founding event have reduced the correlation between census size and N_e . If this is the case, fluctuations in census population will effectively be independent of N_e until the census population size becomes lower than a certain threshold, which would happen faster for smaller isolated populations. That is, even though the data from the single

Delaware Bay site Fowler Beach and not the Greater Delaware Bay population indicated additional declines, it is therefore not possible to state that the decline in census population size was more intense at Fowler Beach than in the Greater Delaware Bay.

The variation of N_e differed between populations and all populations had very low N_e . Our estimate of N_e in the Mid-Atlantic region is 186 individuals (103–361). This estimate stands in sharp contrast to estimates of 7.1 million adult *L. polyphemus* in the coastal Atlantic region from New Jersey to Maryland (Hata & Berkson 2003) and 20 million adults within Delaware Bay (Smith *et al.* 2006). The ratio between effective and census population N_e/N in this case is therefore only around 2.62×10^{-5} based on Hata & Berkson (2003) or 9.3×10^{-6} based on Smith *et al.* (2006), which tend to make the estimate of N_e/N even more dramatic. Similarly low N_e/N ratios have been reported in the marine fishes *Sciaenops ocellatus* and *Pleuronectes platessa* (Turner *et al.* 2002; Hoarau *et al.* 2005). In these cases, the authors suggested that the main reason for the low N_e/N was due to high variance in reproductive success. A theoretical study supported the view that N_e/N ratios can be very low when a few individuals contribute predominantly to the offspring pool, even when a large proportion of the adults also make smaller contributions (Hedrick 2005). Variation in reproductive output between individuals caused by the high fecundity [21 300 eggs per female, Leschen *et al.* (2006); 88 000, Shuster & Botton (1985)] will be of importance in *L. polyphemus*, but we do not believe that this is the only driving factor. Instead, we believe the massive decline in population size associated with the founding of populations after the Ice Age is also an important factor. Furthermore, fluctuating population sizes probably also contributed to the very low N_e/N ratio for *L. polyphemus*. Recent modelling has shown that under some circumstances, these fluctuations may be so intense that N_e may in fact decrease with increasing census population size (Per-toldi *et al.* 2007).

Low N_e has been linked to inbreeding in other marine species (e.g. plaice, Hoarau *et al.* 2005). Even though N_e estimate for all regions are very low, the slow rate of decrease suggests that the risk of inbreeding depression in these populations may not be as large as their small current population sizes suggest, but the low N_e s of individual populations may yet pose problems for their demographic viability. A further genetic hazard posed by small N_e is loss of genetic variation by means of random genetic drift, with consequent loss of adaptive potential. Authors disagree upon what N_e is needed to maintain genetic variability and adaptive potential. Recent assessments based on both theoretical and

empirical arguments suggest that the critical N_e would be in the range of 500–5000 individuals (Frankham & Franklin 1998; Lynch & Lande 1998). Estimated current N_e for *L. polyphemus* (Table 2) fall within or below that range, suggesting that loss of genetic variability could affect the adaptation of populations to changing ecological regimes. Such concerns have been discussed for other marine populations (e.g. New Zealand snapper, Hauser *et al.* 2002).

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Supporting information

Additional supporting information may be found in the online version of this article.

Appendix S1 Pairwise F_{st} estimates (below diagonal) and associated probabilities (after 1,000 permutations; above) among 28 collections of *Limulus polyphemus*. See Table 1 for explanation of collection site abbreviations.

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