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Age Structure of the Pleasant Bay Population of *Crepidula fornicata:* A Possible Tool For Estimating Horseshoe Crab Age

Sara P. Grady, Deborah Rutecki, Ruth Carmichael, and Ivan Valiela (Boston University Marine Program, Marine Biological Laboratory, Woods Hole, Massachusetts 02543)

Crepidula fornicata, the common slipper shell, lives on rocks, horseshoe crabs (*Limulus polyphemus*), and other hard surfaces, often in stacks of one animal atop another. Unlike many other gastropods, they tend to remain sessile, and as they grow, their shells contour to the substrate (1). The association between horseshoe crabs and *C. fornicata* offers the possibility to use the slipper shell as a tool to determine the ages and average lifespan of horseshoe crabs (2). Knowing this information would be helpful for trying to understand horseshoe crab ecology for use in conservation efforts.

It is difficult to directly estimate horseshoe crab age because horseshoe crabs lack any hard parts that could be sectioned and analyzed for growth rings. Their chitinous exoskeleton is molted with decreasing frequency until a theoretical "terminal molt" (3). There are also a variety of sizes within visually estimated age classes because growth is very slow or stops in adults (3).

Other methods have been suggested for aging horseshoe crabs, including qualitative aging criteria and tagging studies. From the results of tagging studies it has been estimated that horseshoe crabs live 9 to 12 years before maturity and 5 to 7 years as adults, for a total lifespan of 14 to 19 years (4). These age spans are consistent with the prediction of Botton and Ropes (2) based on laboratory work using *C. fornicata* as a proxy for horseshoe crab age.

C. fornicata could indicate age of host horseshoe crabs if 1) horseshoe crabs have a terminal molt or do not molt often as adults, 2) *C. fornicata* remain on the same horseshoe crab, and 3) *C. fornicata* age can be determined with some degree of accuracy (5). It is also assumed that *C. fornicata* attach to a host horseshoe crab as soon as the new cuticle hardens.

Botton and Ropes (2) used a regression proposed by Walne (1) of *C. fornicata* length to age to quantitatively estimate the ages of horseshoe crabs. These *C. fornicata* were used to formulate this regression without comparison to a local population of horseshoe crabs, since the *C. fornicata* data was from England and horseshoe crabs were not measured at all.

In this study we measured shell length of *C. fornicata* and prosomal width of *Limulus polyphemus* in Pleasant Bay, Chatham, Massachusetts. We measured 496 crabs and their corresponding *C. fornicata,* with the number of *C. fornicata* per crab ranging widely from 1 to 30, with an average of 4 per crab. From these data we fitted cohorts of *C. fornicata* to a size-frequency distribution. We also related size of *C. fornicata* to prosomal width of *L.*

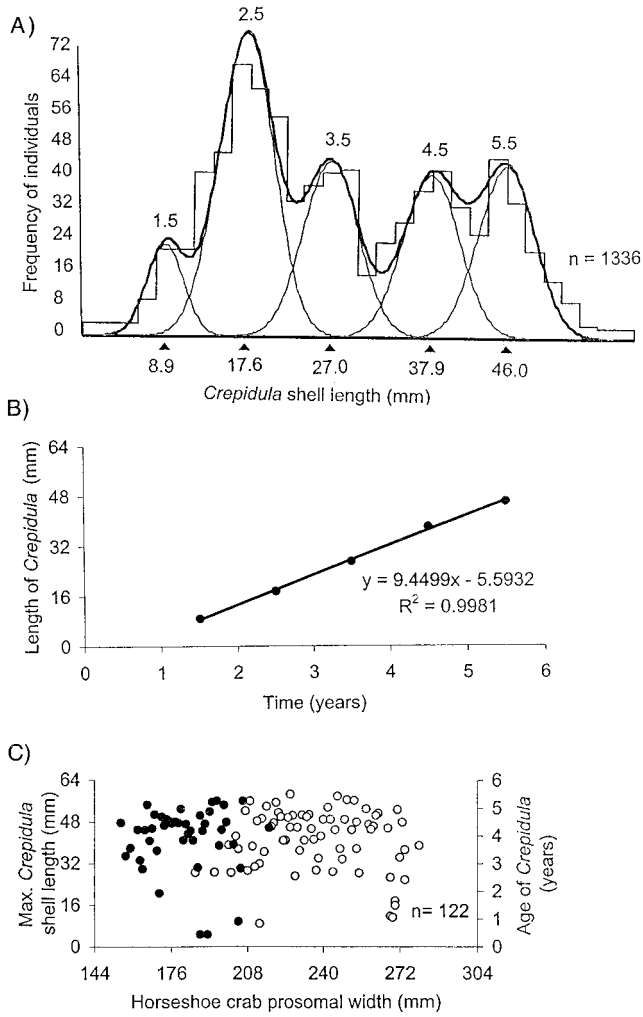

Figure 1. *(A) Cohorts of Pleasant Bay population of* Crepidula fornicata: *8.9 mm (~1.5 y), 17.6 mm (~2.5 y), 27.0 mm (~3.5 y), 37.9 mm (~4.5 y), and 46.0 mm (~5.5 y). (B)* C. fornicata *length* vs. *age; extrapolation data from Botton and Ropes (2). (C) Length of largest* C. fornicata *on horseshoe crabs of different prosomal width. Filled circles (●) represent males,open circles (○) represent females.*

polyphemus to see if *C. fornicata* could provide a proxy for *L. polyphemus* size and age.

The analysis of cohorts demonstrated that *C. fornicata* in Pleasant Bay can be divided into 5 size cohorts (Fig. 1A), with *C. fornicata* of approximately 4–6 mm in length appearing to represent the most recent spatfall. The cohorts differed in abundance, reflecting different magnitudes of recruitment from year to year. Growth rates in this study did not decrease with increased size and age (Fig. 1B). This may be due to low numbers of larger (50 mm +) and older *C. fornicata.* Published data of sizes and ages (1, 2) match those found in this study, and thus confirm the conversion from size to age of the *C. fornicata.* The largest *C. fornicata* found resident on a horseshoe crab was 58 mm. This size *C. fornicata* could be from 8–11 years old (2).

There was no evident relationship between maximum length and age of *C. fornicata* and size of the host horseshoe crabs (Fig. 1C). Male horseshoe crabs were consistently smaller than females, but in both sexes the length and presumed age of *C. fornicata* varied greatly, and was independent of the size of the crab.

It is not possible to establish a strong relationship between true horseshoe crab length and the length of the *C. fornicata* upon it. At most the data of Figure 1C support that a minimum age can be calculated by adding the maximum *C. fornicata* length on a given horseshoe crab to the minimum age of horseshoe crabs at maturity. Using 9 years as the age at maturity (4), the crabs in this study were from 12 to 17 years old.

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Hydrogen Peroxide: An Effective Treatment for Ballast Water

Alan M. Kuzirian, Eleanor C. S. Terry, Deanna L. Bechtel, (Marine Biological Laboratory, Woods Hole, Massachusetts 02543), and Patrick L. James[1]

Introduced species have been a problem in the marine and coastal environments for centuries. Historically, many of these introductions have a strong geophysical component often associated with natural disasters. However, in more recent times, "man, the supreme meddler" (1) has dramatically changed the rate, number, and geography of exotic species invasions through importation, transportation, intentional releases related to agriculture or aquaculture, as well as unintentional escapes. During the last century, the problem has dramatically accelerated with the advent of modern high-speed freighters and their methods of ballast water exchange.

Transport and discharge of biocontaminated ballast water constitutes a major route (29%) by which potentially invasive species—from plants and algae to fish, invertebrates, planktonic and bacterial micro-organisms, and even potential pathogens—are introduced into coastal waters worldwide. It is estimated that 3000 species are transported daily *via* ballast water (National Research Council, 2000). The Great Lakes have experienced the introduction of at least 129 non-indigenous species (2), while the San Francisco Bay estuary has recorded 234 exotic species with at least an additional 125 cryptogenic species (3). At the current estimated rate, a new species is introduced into the ecosystem every 14 weeks (3).

The problem is not confined to the United States but occurs worldwide. One noteworthy example is the introduction of the western Atlantic ctenophore, *Mnemiopsis leidyi,* into the Black and Azov Seas in 1987 and 1988, respectively. This invader has been blamed for a 20-fold decrease in zooplankton biomass, the subsequent sharp decline in anchovy and other pelagic fish stocks, and a marked disruption in these ecosystems (4).

The United Nations International Maritime Organization (IMO), established in 1991, developed a voluntary ballast water exchange (BWE) at sea policy that has now become mandatory (5). BWE is carried out either by draining and refilling the ballast tanks or by continuous flushing equivalent to three volume exchanges. The policy is based upon the rationale that coastal organisms will not survive at sea and vice versa, so BWE is simpler, less costly, and thus preferable to controls implemented before departure or upon arrival (*i.e.,* land-based treatments). Unfortunately, BWE is only 90%–95% effective, and the exchange itself can be dangerous in foul weather or can produce excessive hull stress. Therefore, alternative ballast water treatments are being sought.

Some current technologies available for ballast water treatment include filtration, cyclone or hydrotech-drum settling, UV, ultrasonics, and heat. Additional secondary treatment methods include biocides, ozone, electric pulse or pulse plasma, deoxygenation, and biological. Some of the biocidal methods involve the storage of dangerous chemicals and cause unacceptably high levels of corrosion (*e.g.,* hypochlorite). However, hydrogen peroxide, generated on-site at low (safe) concentrations, precludes these hazards and is more cost-effective than the sophisticated and high-energy-demanding equipment necessary for ozone generation. Neutral hydrogen peroxide has been effective in a number of studies, but only at moderately high concentrations (10–50 ppm; [6]), for planktonic and some small neustonic organisms. Because most marine organisms and bacteria cannot tolerate pH extremes (7), hydrogen peroxide combined with elevated pH (alkaline hydrogen

[1] Eltron Research, Inc., Boulder, CO.