The Effect of Salinity on Crystalline Style Occurrence in the Estuarine Snail, *Ilyanassa obsoleta* (Say), (Mollusca : Neogastropoda) and its Possible Significance with Respect to Local Distribution

BY

LAWRENCE A. CURTIS AND L. E. HURD

Program in Ecology, School of Life and Health Sciences, and College of Marine Studies, University of Delaware
Newark, Delaware 19711

(1 Text figure)

INTRODUCTION

THE PROSOBRANCH NEOGASTROPOD, *Ilyanassa obsoleta* (SAY, 1822), is an abundant organism in many coastal habitats along the eastern seaboard of North America. It also occurs on the Pacific coast, but is not native there. Fluctuating salinity is a major environmental factor in many of these habitats. *Ilyanassa obsoleta* has been shown to be affected in various ways by lowered salinity: for example, DIMON (1905) noted that snails were restricted to the higher salinity regions of estuaries. She also showed that snails are rendered inactive by freshwater, but can survive many hours of exposure to it. NAGABHUSHANAM (1968) and BERGMANN & GRAHAM (1975) showed that long-term exposure to a salinity of approximately 6‰ is lethal. CRISP (1969) pointed out that lowered salinity affects behavioral responses.

A crystalline style occurs in this species (NOGUCHI, 1921; JENNER, 1956), and has been recognized as an important adaptation for digestion (JENNER, 1956; SCHELTEMA, 1964; BROWN, 1969; CURTIS & HURD, 1979). BROWN (1969) noted that the style is temporally inconstant and suggested that presence or absence is related to type of food ingested. ROBERTSON (1979), working in Georgia, within a 24-hour period, collected data and correlated style absence with low tide. Since all of Robertson's snails that lacked a style also lacked food in the gut, he inferred a tidally regulated feeding rhythm. Work done in Delaware during the summers of 1978 (CURTIS, in press) and 1979 suggests a different pattern to style occurrence. Extensive, continuous day and night sampling showed that styles tended to be absent from snails in the hours after dawn (0800-1000 h), regardless of tidal stage. Moreover, other data resulting from this work suggest that style occurrence is not consistently related to presence or type of food in the gut (Curtis & Hurd, in prep.). During these studies styles were occasionally low in occurrence or absent in snails collected out of association with the post-dawn period. On several occasions this was associated with low salinity in the water overlying the snails collected. Since lowered salinity is known to affect *I. obsoleta* we ran the following experiments to test whether lowered salinity could bring about a loss of crystalline style.

MATERIALS AND METHODS

All specimens of *Ilyanassa obsoleta* used in this work were collected from the Cape Henlopen sandflat in Delaware, located just inside the mouth of Delaware Bay (75°6′W, 38°47′N). Snails ranged in shell height from 17 mm to 26 mm. Salinity of the water over the sandflat can vary between about 10‰ (low tide; heavy rain) and 31‰ annually. The normal range of salinity is between 25‰ and 30‰.

All control and treatment groups employed consisted of 10 snails each. The efficacy of this sample size was determined from the results of other work carried out at the same location on this sandflat during the summers of 1978 and 1979, in which the temporal occurrence of the crystal-

line style was examined. In these studies, 10 snails were collected each hour, over periods of time ranging from 18-28 consecutive hours. A total of 1650 snails was thus examined. We found that the overall probability of finding a snail with a style (chosen at random) was 62 ± 4%, indicating a reliable consistency in this population. The relative probabilities of collecting 10 snails from this population with 10/10, 9/10 ... 0/10 having styles was calculated through expansion of the binomial expression: $(p+q)^k$, where p = probability of possessing a style (= 0.62), q = probability of not having a style (= 0.38), and k = number of snails in the sample (= 10). This procedure revealed that 10 snails is an adequate sample size for this population, since the probability of 10/10 snails having a style by chance alone is < 1%. The criterion for style loss in the population is ≤ 3/10 snails, since random collections of 10 snails with 3 or fewer having styles would occur by chance alone with a probability of ≤ 1%

At 1400 h on 19 July 1979, 40 snails were collected randomly from the sandflat population and taken immediately to the nearby laboratory for treatment, where they were randomly separated into four groups of 10 snails each. These four groups were placed in separate finger bowls containing different dilutions of baywater: 28‰ (full strength baywater at the time of collection), 20‰, 15‰, and 10‰. Dilutions were made by the addition of distilled water to baywater, and all salinities were measured with a refractometer. The time of immersion was 1410 h, and all snails in this experiment were forced to remain submerged for a period of 60 minutes, during which time their behavior was observed. At the end of this time, snails were removed (all at once) from the finger bowls and examined by dissection under a dissecting microscope as rapidly as practicable, for condition of the crystalline style (fully formed, partially formed, or absent). All dissections and observations were completed within 50 minutes following removal from the finger bowls (i.e., by 1600 h). Previous experience has shown us that removal of the snails from water for this length of time will not by itself cause style loss (Curtis, in press).

In addition to the four treatment groups described above, three field control groups of 10 snails each were also collected and examined by dissection during the course of the experiment. The first of these groups (collection control) was collected at the same time as the 40 laboratory treatment animals (1400 h), and dissected immediately to determine style occurrence in the field at the beginning of the experiment. A second group (collection + 50 min. control) was collected and dissected just prior to the removal of snails from the finger bowls in the laboratory (1450 h). The third control group (collection + 135 min. control) was collected after all observations had been made on the laboratory snails (1615 h). This third group served as an overall measure of changes in style occurrence in the field population while the experiment was in progress.

The direct effect of different concentrations of baywater on style dissolution time was also tested. Fully formed styles were removed from sandflat snails collected separately from those above. Upon removal, styles were immediately transferred to separate vials containing either 28‰ (full strength baywater), 20‰ or 10‰ baywater. As before, dilutions were made by the addition of distilled water to baywater. Dissolution time for each style was determined by observing its gradual disappearance with the aid of a dissecting microscope. Ten styles were tested at each concentration. All tests were performed at room temperatures.

RESULTS

During the 60 minute immersion in experimental baywater dilutions, there were obvious differences in behavior among the four groups of living snails. Snails in the 28‰ group actively crawled about the finger bowls, but did not attempt to escape. The groups immersed in 20‰ and 15‰ attempted to crawl up and out of the water, and had to be pushed back repeatedly. Snails in the 10‰ dilution simply pulled into their shells and lay quiet throughout the period of immersion.

Figure 1 shows the number of snails having either a fully formed or partially formed crystalline style in each of the four experimental (A) and three control (B) groups. All snails from the three control groups (collection control, collection + 50 min. control, and collection + 135 min. control) had styles present. Therefore, style occurrence in the field population did not change over the course of the experiment.

Since all control snails collected from the field had styles (Figure 1B), the expected probability of style occurrence in the treatment groups during the course of the study is 1.0 (10/10 snails). We compared this expected value with observed value frequencies in our laboratory treatment groups (Figure 1A) in a chi-square, and found that snails immersed in 15‰ and 10‰ dilutions were significantly affected by treatment ($\chi^2 = 11.3$, df = 1, $P < 0.01$), whereas those immersed in 20‰ and 28‰ were not ($\chi^2 = 1.3$, df = 1, $P > 0.10$). Further, where styles were present at 10‰ or 15‰, they were always of a flimsy consistency (partially formed).

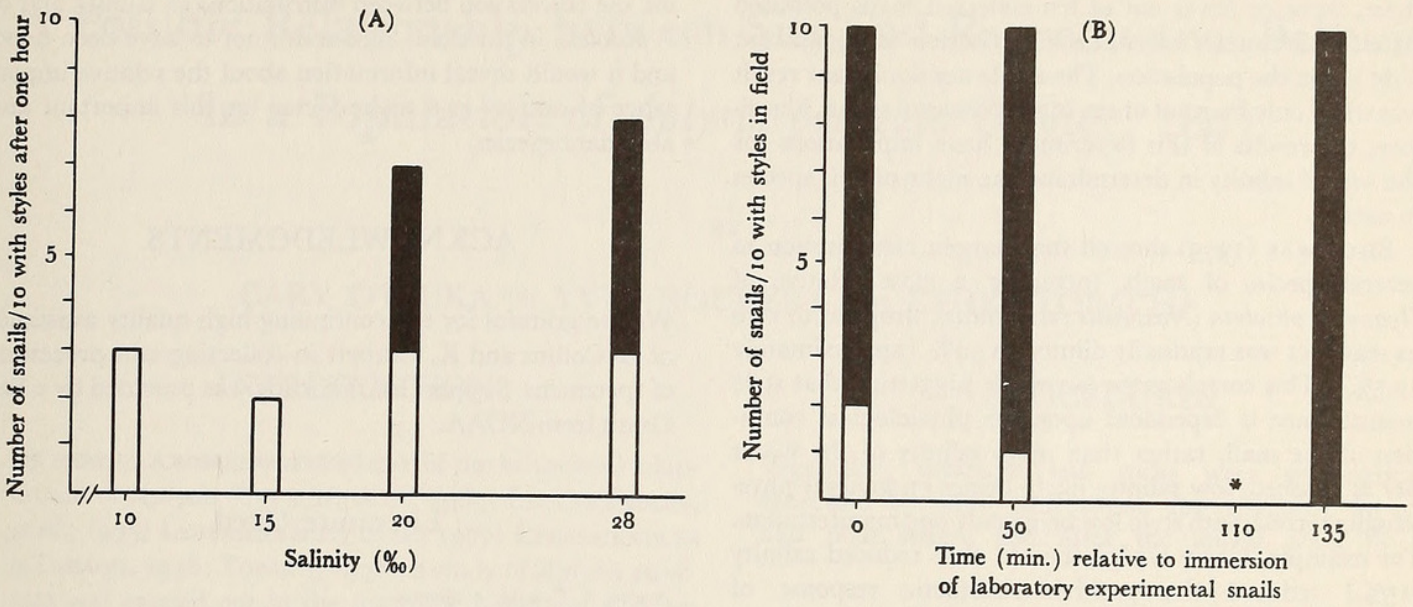

Figure 1

The number of individuals of *Ilyanassa obsoleta* with crystalline styles for each group of 10 snails examined: (A) snails collected at same time and immersed in different salinities for one hour; (B) snails collected from field at various times relative to immersion of laboratory snails, as controls. Shaded areas of bars represent the number of snails with fully formed styles, and unshaded areas represent those with partially formed styles. For (B), salinity at time 0 was 28‰, and was 30‰ thereafter. * indicates time of completion of dissection for all laboratory snails.

The results of this experiment led us to ask whether the effect of diluted baywater on crystalline style occurrence shown by the snails exposed to 10‰ and 15‰ was direct, or actively mediated in some way by the snails. To answer this question, fully formed styles were extracted from recently collected snails and placed in three of the same dilutions used in the snail immersion experiment (see Materials and Methods).

Mean dissolution times for styles removed from snails and immersed in various dilutions were: 16 minutes (10‰), 13 minutes (20‰) and 12 minutes (28‰). One-way ANOVA indicates no significant difference among these groups ($F_{2,27} = 2.21$, $P > 0.10$). Therefore, baywater salinities between 10‰ and 28‰ do not directly affect the rate of dissolution for styles removed from the snails. Indeed, the trend (though nonsignificant) for *in vitro* styles is the opposite of that exhibited by styles *in vivo* for these same salinities.

DISCUSSION

Our results indicate that crystalline style occurrence in *Ilyanassa obsoleta* is affected by ambient salinities of 10‰ and 15‰, but relatively unaffected by salinities of 20‰ and 28‰. Further, this effect is not due to the direct action of saline water on the style at these salinities, but is mediated in some manner by the living snails. The dilutions used in this experiment represent the range of naturally occurring environmental salinities in the sandflat habitat, as well as in other nearby habitats such as salt marshes. Although 28‰ to 30‰ is more common, salinity drops to 15‰ and 10‰ upon the occasion of heavy rainwater input at low tide. That snails in the field are actually affected in this way by salinity of overlying water is supported by additional observations made during the 1978-1979 studies (Curtis, in prep.). There were five occasions when salinity dropped well below 20‰ (range 16‰-10‰). On four of

these, three or fewer out of ten collected snails possessed styles, which meets our statistical criterion for significant style loss in the population. The single nonsignificant result was when only four out of ten snails possessed styles. Therefore, the results of this experiment have implications for the role of salinity in determining the niche of this species in nature.

SHUMWAY (1979) showed that oxygen consumption in several species of snails, including a close relative of *Ilyanassa obsoleta* (*Nassarius reticulatus*), dropped to zero as seawater was gradually diluted to 30% (approximately 10.5‰). This corroborates our result suggesting that style maintenance is dependent upon the physiological condition of the snail, rather than upon salinity of the water *per se*. Indeed, low salinity likely brings on general physiological stress, with style loss being only one manifestation. For example, CRISP (1969) showed that reduced salinity (15‰) reduced the positive rheotactic response of *I. obsoleta*.

It has long been known that *Ilyanassa obsoleta* is adversely affected by low salinity waters (DIMON, 1905; NAGABHUSHANAM, 1968; CRISP, 1969; KASSCHAU, 1972; BERGMANN & GRAHAM, 1975), and the inference has been that lethal salinities in large measure determine local spatial distribution in estuaries. This seems a reasonable inference, since gastropod mollusks apparently do not osmoregulate (AVENS & SLEIGH, 1965; PROSSER, 1973). Lethal salinity for *I. obsoleta* seems to be around 6‰ (NAGABHUSHANAM, 1968; BERGMANN & GRAHAM, 1975). Our results suggest that snails are severely affected at a salinity level considerably above 6‰, somewhere between 15‰ and and 20‰. Since the style is an important aspect of *I. obsoleta's* digestive apparatus (JENNER, 1956; SCHELTEMA, 1964; BROWN, 1969; CURTIS & HURD, 1979), its loss likely will curtail nutrient procurement activities. Thus, local distribution in estuarine habitats will not be set by the perimeter of lethal salinities for *I. obsoleta*. DIMON (1905) showed that these snails can survive exposure even to fresh water for a limited time (18 h). The important factor in setting a perimeter for distribution, with regard to salinity, may be the amount of time potential habitat areas are subjected to salinities below the critical level for style maintenance, relative to the total amount of time required for adequate nutrient procurement.

As a matter of general observation, *Ilyanassa obsoleta* does seem to be restricted to higher salinity regions of estuaries (DIMON, 1905). It would be interesting to examine the correlation between distributions of salinity and of *I. obsoleta* in the field. This seems not to have been done, and it would reveal information about the relative importance of salinity as a niche factor for this important and abundant species.

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Literature Cited

AVENS, A. C. & M. A. SLEIGH
1965. Osmotic balance in gastropod molluscs - I. Some marine and littoral gastropods. Comp. Biochem. Physiol. 16A: 121 - 141

BERGMANN, J. R. & M. G. GRAHAM
1975. Salinity as a factor defining the habitat of the mud snail, *Nassarius obsoletus*. Proc. Malacol. Soc. London 41: 521 - 525

BROWN, STEPHEN CLAWSON
1969. The structure and function of the digestive system of the mud snail *Nassarius obsoletus* (Say). Malacologia 9 (2): 447 - 500 (20 July 1970)

CRISP, MARY
1969. Studies on the behavior of *Nassarius obsoletus* (Say). (Mollusca: Gastropoda). Biol. Bull. 136: 355 - 373

CURTIS, LAWRENCE A.
in press. Daily cycling of the crystalline style in the omnivorous, deposit-feeding estuarine snail, *Ilyanassa obsoleta* (Say): Mar. Biol.

CURTIS, LAWRENCE A. & L. E. HURD
1979. On the broad nutritional requirements of the mud snail, *Ilyanassa (Nassarius) obsoleta* (Say), and its polytrophic role in the food web. Journ. exp. mar. Biol. Ecol. 41: 289 - 297

DIMON, A.
1905. *Nassa obsoleta*. Cold Spring Harbor Monogr. 5: 1 - 48

JENNER, CHARLES EDWIN
1956. Occurrence of a crystalline style in *Nassarius*. Biol. Bull. 111: 304

KASSCHAU, M. R.
1972. Temperature-salinity interactions on *Nassarius obsoletus*. Bull. So. Carol. Acad. Sci. 34: 104

NAGABUSHANAM, R.
1968. Effect of low salinity on the mud snail, *Nassarius obsoletus* (Say) (Mollusca: Gastropoda). Sci. & Cult., India 34: 132 - 133

NOGUCHI, H.
1921. *Cristispira* in North American shellfish. A note on a spirillum found in oysters. Journ. exp. Medic. 34: 295 - 315

PROSSER, CLIFFORD LADD
1973. Comparative animal physiology. 3rd ed.; 966 pp. W. B. Saunders Co., Philadelphia, Penna.

ROBERTSON, J. R.
1979. Evidence for tidally correlated feeding rhythms in the eastern mud snail, *Ilyanassa obsoleta*. The Nautilus 93: 38 - 40

SCHELTEMA, RUDOLF S.
1964. Feeding and growth in the mud-snail *Nassarius obsoletus*. Chesapeake Sci. 5: 161 - 166

SHUMWAY, S. E.
1979. The effects of fluctuating salinity on respiration in gastropod molluscs. Comp. Biochem. Physiol. 63A: 279 - 284