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## MORTALITY OF THE VEINED RAPA WHELK, *RAPANA VENOSA*, IN RELATION TO A BLOOM OF *ALEXANDRIUM MONILATUM* IN THE YORK RIVER, UNITED STATES

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**ABSTRACT** Veined rapa whelks (*Rapana venosa*), carnivorous marine gastropods experienced significant mortality during an *Alexandrium monilatum* bloom in the lower York River, VA in September 2007. Rapa whelks stopped feeding as dissolved oxygen and chlorophyll concentrations increased with the development of the bloom. Whelk mortality was preceded by external signs of stress including reduced ventilation, inability to attach to hard substrates, periodic pumping of the opercular plate, and increased mucus production over a period of 24–48 h prior to death. High concentrations (2–7  $\mu\text{g g}^{-1}$  tissue) of gonioidinum A, a toxin produced by *A. monilatum*, were observed in bivalves attached to the shells of rapa whelks. Concentrations of gonioidinum A in whelk foot tissue ranged from 0.02–8.39  $\mu\text{g g}^{-1}$ . Mortality of rapa whelks was 100%. Mortality of oysters (*Crassostrea virginica*) and northern quahogs (*Mercenaria mercenaria*) in the same flow through system was 0%. The symptoms displayed by the rapa whelks in the 24–48 h prior to death were indicative of paralysis and followed a similar time course documented for other molluscs exposed to toxic *A. monilatum*.

**KEY WORDS:** Veined rapa whelk, *Rapana venosa*, *Alexandrium monilatum*, gonioidinum A, Chesapeake Bay, toxicity

### INTRODUCTION

Veined rapa whelks (*Rapana venosa*) are large carnivorous marine gastropods that are native to Asian waters near Japan and Korea (Tsi et al. 1983). This invasive species is now established in the Chesapeake Bay, United States with a known range extending from Tangier Island south to the Bay mouth (Harding & Mann 1999, Harding & Mann 2005). Rapa whelks eat bivalves (Morton 1994, Zolotarev 1996, Savini et al. 2002, Harding et al. 2007) including oysters (*Crassostrea virginica*), northern quahogs (*Mercenaria mercenaria*), and mussels (*Geukensia demissa*, *Modiolus*, *Mytilus*).

The dinoflagellate *Alexandrium monilatum* (Howell, Balech; previously *Gonyaulax* spp) is a chain forming species that has been associated with “red” tides and the mortality of fish and invertebrates (e.g., Sievers 1969) typically on the US Gulf coast (Connell & Cross 1950, Williams & Ingle 1972, Perry et al. 1979). This toxic dinoflagellate has also been observed on the east coast of Florida (Howell 1953, Norris 1983) and the York River in Chesapeake Bay (MacKiernan 1968) and produces gonioidinum A, a lipophilic toxin, that is released when the cell ruptures (Hsia et al. 2006). This toxin has effects on muscle (e.g., Furukawa et al. 1993). Gonioidinum A, or another, as yet undescribed, toxin produced by *A. monilatum*, has demonstrated hemolytic (Bass et al. 1983) and neuroactive properties (Bass & Kuvshinoff 1983). Dinoflagellate cells may rupture in response to mechanical stimulation, influx of fresh water (precipitation; Connell & Cross 1950), or as a bloom dies or collapses (Aldrich et al. 1967).

Exposure to *A. monilatum* toxin(s) results in paralysis and death of fish (e.g., Gates & Wilson 1960, Sievers 1969) as well as molluscs (Ray & Aldrich 1967, Sievers 1969) including green mussels (*Perna viridens*, May et al. In review) and oysters (Sievers 1969) after 24–48 h of exposure. Wardle et al. (1975) also noted moribund gastropods on the beaches in association with *A. monilatum* blooms in Galveston Bay during August

1971 and 1972 including *Polinices duplicata* and *Stramonita* (= *Thais*) *haemastoma*.

In the presence of toxic *A. monilatum* in laboratory experiments, mussels lost the ability to close their valves (moribund) within 24 h of exposure (May et al. In review), whereas oysters became moribund within 48 h (Sievers 1969). Filter feeding oysters and northern quahogs survived exposure to *A. monilatum* in laboratory experiments (May et al. In review) and may use behavioral responses including selective feeding (Ray & Aldrich 1967, Shumway 1990, Bricelj et al. 1991), valve closure (Ray & Aldrich 1967), or burrowing (*Mercenaria*, Shumway 1990) to avoid toxic algae. Field observations for *C. virginica* during *A. monilatum* blooms include reports of no effects (Wardle et al. 1975, Perry et al. 1979) as well as oyster mortality (Wardle et al. 1975).

Here we describe the mortality of cultured and wild veined rapa whelks *Rapana venosa* in relation to an *Alexandrium monilatum* bloom in the lower York River, Virginia during September 2007. The bloom was first detected on September 4, 2007 (T. Leggett, Chesapeake Bay Foundation, pers. com.) as a large patch of reddish water in the lower York River offshore of the VIMS campus extending into Sarah’s Creek. The reddish area of water increased in area (coverage) and became darker red from 9/5 through 9/10/07. *Alexandrium monilatum* was definitively identified from York River water samples collected 9/4 through 9/7 on 9/10/07 (K. Reece and W. Jones, VIMS, pers. com.).

### MATERIALS AND METHODS

#### Rapa Whelks

All rapa whelks were maintained in flow through tanks at the Virginia Institute of Marine Science (VIMS, Gloucester Point, VA, 37°14' 51" N, 76°29' 58" W) at ambient York River conditions. Wild whelks ( $n > 100$ , shell length [SL, maximum dimension from the spire to the end of the siphonal canal] range 34–165 mm) had been at VIMS since at least July 2007. Four-year classes (1999, 2000, 2002, 2005) of cultured whelks were hatched from egg capsules, settled, and maintained at

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ambient York River conditions throughout their lives. In September 2007, these cultured whelks ( $n = 98$  whelks) ranged in shell length from 65–110 mm SL.

All rapa whelks were maintained in tanks with at least 3 bivalve prey (*Crassostrea virginica*, *Geukensia demissa*, *Mercentaria mercenaria*) per individual whelk. Whelk tanks were checked twice weekly for food consumption and consumed food items were replaced with specimens of similar size from the same species as part of other ongoing feeding and growth experiments (Harding, unpublished data). The number of food items removed from each cultured whelk holding tank was recorded when food was replaced. The number of bivalves consumed whelk<sup>-1</sup> week<sup>-1</sup> was calculated by dividing the total number of bivalves eaten by all cultured whelks from one Thursday to the next. Feeding rates were assigned to the date corresponding to the first Thursday, thus food consumed between 8/16 and 8/23/07 was assigned a consumption date of 8/16/07 to facilitate comparisons with environmental data.

Rapa whelks were declared dead when there was no visible retraction response to physical contact with the mantle tissue around the opercular opening and/or the siphon and no visible water currents/mucus strings emerging from the mantle cavity. Wild whelks were removed from tanks and destroyed after death. Cultured whelks were frozen immediately post mortality. Mussels (*Geukensia demissa*, SL approximately 15–30 mm) and oysters (*Crassostrea virginica*, SL < 30 mm) attached to the exterior of cultured whelk shells were also frozen.

Bivalve tissue samples from whelk epifauna (12 mussels and 1 oyster) and whelk foot muscle tissue samples (ranging from 0.26–0.89 g tissue) from 10 individual cultured whelks were analyzed for the presence of goniodomin A toxin produced by *A. monilatum* using the assay developed by Hsia et al. (2006) at the National Ocean Service Hollings Marine Laboratory. Foot tissue samples from all whelks were rectangular (approximately 2 mm × 2 mm × 2 mm). Frozen tissue samples from 5 whelks (collected 2/19/08, SL 70–105 mm) included the exterior surface of the foot, whereas frozen tissue samples from five additional whelks (collected 3/9/08, SL 70–105 mm) included only tissue from the interior of the foot muscle. Dissecting tools were dipped in a 10% bleach solution and dried between sample collection from individuals to avoid cross-contamination of tissue samples. Foot muscle was used for these analyses because whelk stomachs were empty (see below) and the foot tissue could be removed without compromising the areas of the whelk body needed for statolith, radula, and gonad recovery (in support of ongoing studies that were the reason the whelks were cultured and maintained).

#### Environmental Conditions

Water temperature, salinity, and dissolved oxygen measurements at the intake for the seawater system (approximate depth = 3 m) are made every 15 min each day and reported from 8/15/07 through 10/2/07 to describe conditions before, during, and after the bloom. Average daily values presented are thus the average of 360 data points. Chlorophyll data are from the Chesapeake Bay Observing System monitoring buoy maintained approximately 300 m offshore of the VIMS seawater intake ([http://chsd.vims.edu/realtime/YRK005.67B/Chlorophyll+\(Flourescence\)](http://chsd.vims.edu/realtime/YRK005.67B/Chlorophyll+(Flourescence))). The daily averages for the 8/15/07 through 10/2/07 period are calculated from readings made every 15 min.

## RESULTS AND DISCUSSION

Average daily York River water temperatures were 27°C to 28°C from 9/2/07 through 9/12/07 (Fig. 1A) with average daily salinities of 22.4–22.8 ppt (Fig. 1B). Whereas these water temperature and salinity values are within the lower portion of the range of temperature and salinity conditions observed during *A. monilatum* blooms on the coasts of Florida, Mississippi, and Texas (summarized by Juhl 2005). These conditions are also similar to those observed in the York River, Virginia during September 1966 and in the York River as well as at Wolf Trap Light during late August and September 1967 when *A. monilatum* blooms were described by MacKiernan (1968). These water temperatures and salinities are also within the window of optimal growth conditions for this species in laboratory experiments performed by Juhl (2005).

The variation in salinities observed between days in the 8/31 through 9/12/07 period corresponding to thunderstorms and locally heavy precipitation within the York River watershed (Climatological Data: Virginia, August and September 2007). Runoff from locally heavy precipitation events may provide a source of nutrients for *A. monilatum* blooms (Connell & Cross 1950) and a stimulus for cell lysis and toxin release.

Average daily oxygen values were abnormally high (>5.5 mg/L) from 9/5 through 9/14/07 (Fig. 1B) corresponding to the highest period of bloom activity. Connell & Cross (1950) also reported abnormally high oxygen values (200% saturation) in relation to an *Alexandrium* bloom in Offatt Bayou on Galveston Island, Texas from July through September 1949. Chlorophyll values in the 42–59 µg L<sup>-1</sup> range were observed on 9/5/07 between 4:15 and 6:15 PM (Fig. 1C). The highest chlorophyll values associated with the bloom, 80–102.4 µg L<sup>-1</sup>, were observed on 9/10/07 between 10:00 and 11:15 AM.

Cell counts of *A. monilatum* from York River water samples collected near the VIMS water intake on 9/7/07 were approximately 40,000 cells/mL (W. Jones and K. Reece, VIMS, unpublished data) with average daily chlorophyll values of 13.51 µg L<sup>-1</sup> (standard error = 0.64, Fig. 1C) but with an observed daily maximum of 40.2 µg L<sup>-1</sup> (12:45 PM). Marshall et al. (2008) report *A. monilatum* cell counts from the York River in 2007 of 1200 cells mL<sup>-1</sup> presumably from early September 2007, but they do not provide an exact date for sample collection.

Rapa whelks stopped feeding on 9/2 or 9/3/07 (Fig. 1D). No empty bivalve shells were observed when tanks were checked on 9/6/07 for the collection interval 9/3 through 9/6/07. Whelks began to show external signs of stress including reduced ventilation, inability to attach to hard substrates, periodic pumping of the opercular plate, and increased mucus production on 9/6/07. The frequency of water changes and tank flushing was increased to twice daily and the symptoms neither increased nor decreased in intensity on 9/7 and 9/8/07. All bivalves were transferred from whelk flumes to a bivalve holding flume on 9/7/07 to reduce oxygen demand in the whelk flumes. Bivalves showed no signs of distress at the time of transfer or at any time during the bloom. Bivalve mortality between 9/5 and 10/1/07 in flow through conditions was 0%. On 9/9/07, all wild rapa whelks and all but 3 cultured rapa whelks were moribund (dead). The three living cultured whelks were immediately transferred to individual 20-L containers of 5 micron filtered seawater with aeration but these last rapa whelks

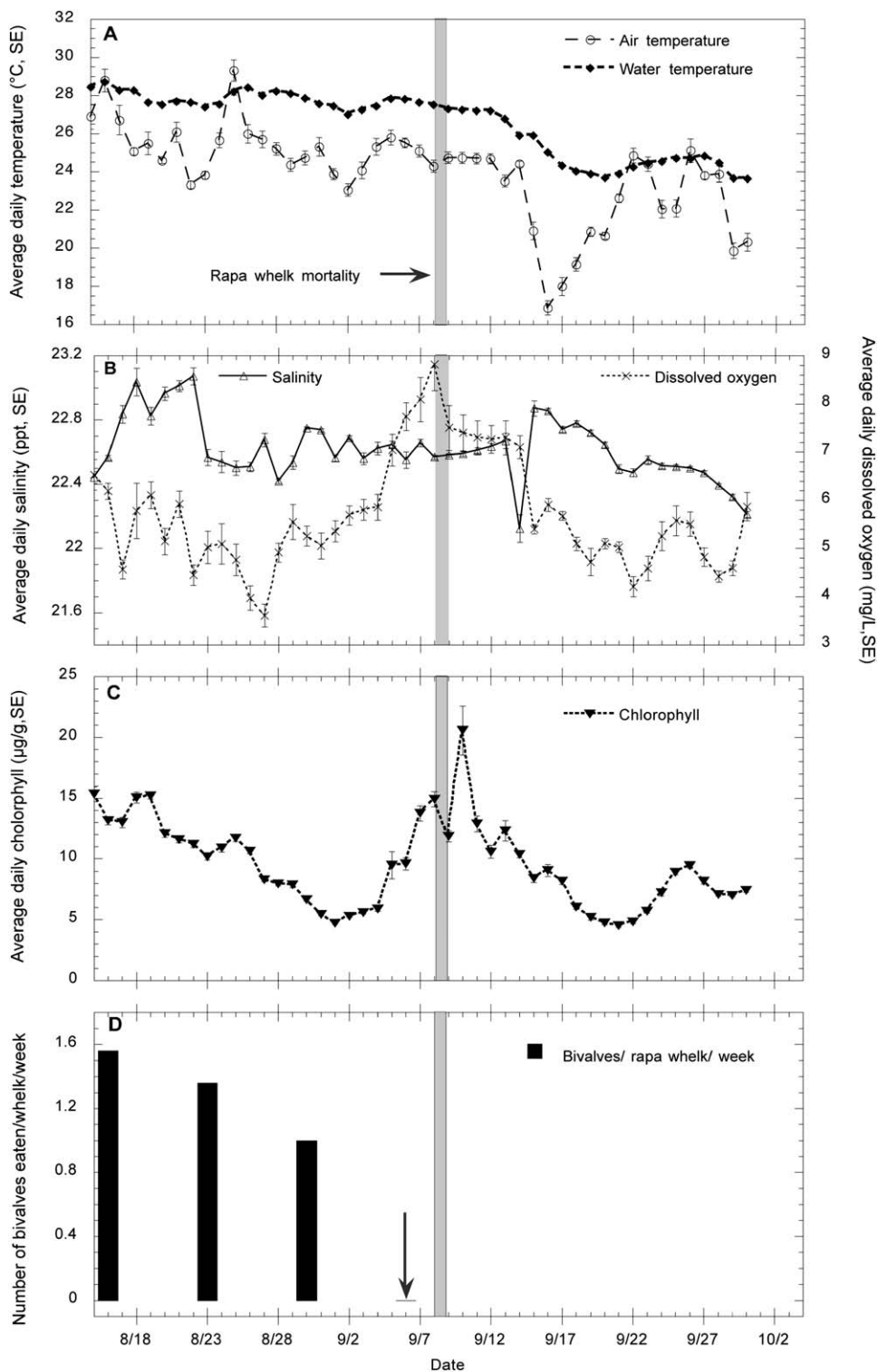
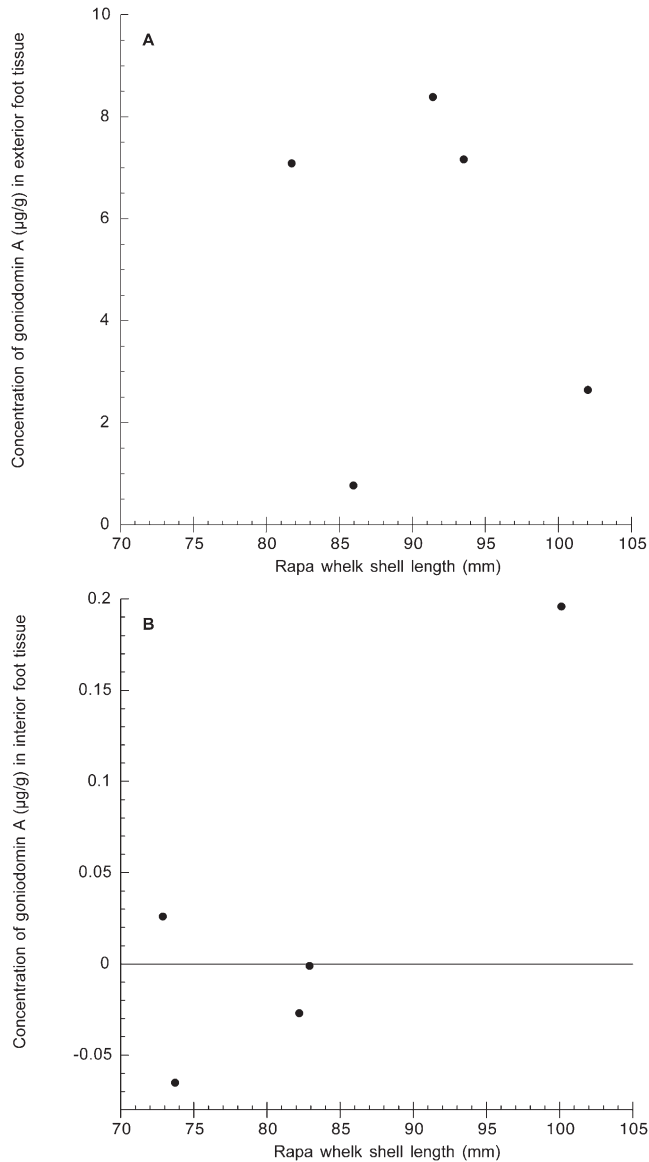


Figure 1. Environmental conditions recorded in the York River 8/15/07 through 10/2/2007 including (A) air and water temperature ( $^{\circ}\text{C}$ ), (B) salinity (ppt) and dissolved oxygen ( $\text{mg L}^{-1}$ ), (C) chlorophyll ( $\mu\text{g L}^{-1}$ ) in relation to (D) rapa whelk bivalve consumption rate during the same time period. The black arrow highlights a bivalve consumption rate of zero observed between 9/3 and 9/6/2007. The shaded box for the 9/8–9/9/07 period represents the time when all but 3 of the rapa whelks died. All rapa whelks were dead by 9/10/07.

were dead on 9/10/07 for an overall rapa whelk mortality of 100%.

Concentrations of goniiodomin A from the mixed epibiont bivalve tissue sample (12 mussels, 1 oyster spat) attached to the

shells of cultured rapa whelks ranged from 2–7  $\mu\text{g g}^{-1}$  of tissue. Goniiodomin A concentrations from the rapa whelk foot tissue samples that included tissue from the exterior surface ranged from 0.77–8.77  $\mu\text{g toxin g}^{-1}$  of whelk tissue (Fig. 2A). Two out



**Figure 2.** Measured goniiodomin A toxin concentrations in relation to rapa whelk shell length (mm) for foot muscle tissue including exterior surfaces (A) and interior foot muscle tissue (B).

of 5 interior rapa whelk foot tissue samples contained measurable concentrations of goniiodomin A ( $0.03$  and  $0.20 \mu\text{g g}^{-1}$ , Fig. 2B). The three remaining interior foot tissue samples yielded results that were unresolved or below the detection limit.

Prior to the summer of 2007, the only documented occurrences of *A. monilatum* in the York River and the Chesapeake

Bay of which we are aware were blooms in the York River at Gloucester Point (1966 and 1967, MacKiernan 1968) and in the Bay in the vicinity of Wolf Trip Light (late August-September 1967, MacKiernan 1968). Whereas other species of *Alexandrium* that produce goniiodomin A (*A. pseudogoniaulax*) are found in Japanese coastal rock pools, adult rapa whelks are typically found in deeper subtidal benthic habitats during warmer months (e.g., Chung et al. 1993, Chung et al. 2002) and may not co-occur with *Alexandrium* blooms in their native waters.

In this instance, it is unlikely that rapa whelk mortalities were related to consumption of bivalves contaminated with *A. monilatum* cells or toxins. Although rapa whelks can bioaccumulate paralytic shellfish poisons including gonyautoxins and saxitoxins through consumption of contaminated bivalves (Ito et al. 2004), *A. monilatum* has not been associated with paralytic shellfish poisoning (Owen & Norris 1982). The whelks examined by Ito et al. (2004) were live when collected and showed no obvious effects of the high gonyautoxin and saxitoxin concentrations observed in their tissues. Symptoms of distress in the VIMS rapa whelks were not observed until 3–4 days after they stopped eating. Rapa whelk mortality was 24–48 h after symptoms of distress were noted. The symptoms displayed by the rapa whelks in the 24–48 h prior to death were indicative of paralysis and followed a similar time course documented for green mussels (moribund 24 h post exposure, May et al. In review) and oysters (24–48 h, Sievers 1969).

These rapa whelks were probably killed by *A. monilatum* through exposure to water-borne toxin(s) including goniiodomin A. The lipophilic nature of goniiodomin A limits the solubility of the toxin molecule in water and encourages toxin molecules to adhere to organic substrates including whelk tissue. The high concentrations of toxin from the exterior of the rapa whelk foot tissues are indicative of the high toxin concentrations that were present in the whelk tanks. The mantle cavity and gill tissue of the whelks were exposed to similar external toxin concentrations. Although the toxin molecules are suited for transport across cell membranes, contact with respiratory surfaces by toxin molecules is likely sufficient to initiate respiratory paralysis leading to death.

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Mr. Todd Nelson, VIMS Physical Science, provided expert assistance in establishing the intake monitoring system as well as access to chlorophyll data. Mr. William Jones and Dr. Kimberly Reece (both VIMS Environmental and Aquatic Health) identified *A. monilatum* and provided information on cell counts. This is Contribution Number 3301 from the Virginia Institute of Marine Science, Gloucester Point, Virginia.

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