In their study on the impact of the zebra mussel in the St. Lawrence River, Ricciardi *et al.* (1996) had shown that a few large *D. polymorpha* may impair unionids by interfering with valve closure. They also demonstrated that mean infestations as low as ten *D. polymorpha* per native mussel can result in a sharp decline in unionid abundance after only a few years. This suggests that although the number of *D. polymorpha* per unionid at Andrewsville is currently low (1 to 2 fouling zebra mussels per native mussel), such an infestation may nonetheless result in the decline and possible extirpation of this community of unionids. The decline will also occur sooner than expected if numbers of *D. polymorpha* keep increasing annually, as they have in the past few years. The case of the native mussel community of the Rideau River, with its ongoing decline following the introduction of *D. polymorpha* by humans over 15 years ago, points to the importance of public awareness and education as an absolute necessity if we want to conserve regional biodiversity as well as ecosystem functions in our river systems.

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Hemolymph as a nonlethal and minimally invasive source of DNA for molecular systematic studies of freshwater mussels

By Morgan E. Raley, Jay F. Levine & Arthur E. Bogan

North America is home to more than 300 species of freshwater mussels in the family Unionidae (Williams *et al.*, 1993). These freshwater invertebrates play an essential role in maintaining water quality in aquatic ecosystems. However, more than 70 % of North American freshwater mussels are imperiled. This imperiled status functionally limits the number of individuals that can be used for genetic analysis as individuals are often sacrificed in the normal course of research on the group. Research groups have begun to propagate mussels with the goal being to augment many of these critically threatened species (O'Beirn *et al.*, 1998). As such, the emphasis has been to develop culturing systems for these species, a daunting task itself. However, without requisite extensive genetic sampling of these groups, only minimal *a priori* genetic knowledge exists for these species and in some instances, augmentation efforts could fail in a manner similar to those witnessed in the salmon fisheries (Waples, 1991). Nonlethal procedures for acquiring DNA for genetic analysis are needed to facilitate phylogenetic and population level genetic studies so that previous kinds of augmentation failure can be avoided. These new techniques will facilitate appropriate sampling and aid researchers and wildlife managers to develop accurate estimates of genetic diversity without adversely impacting the population in question.

Previous efforts by our lab have led to the development of nonlethal protocols used to monitor the health of freshwater mussels via biochemical standards routinely employed in veterinary medicine (Gustafson *et al.*, 2005a, b). Blood chemistry panels can now be conducted using hemolymph, the circulatory fluid of invertebrates, instead of whole organisms or tissues to monitor the impacts of environmental contaminants on freshwater mussels. These techniques have proven no more lethal than a typical veterinary blood draw so long as individuals are sampled only once a month. Hemolymph contains several classes of hemocytes, all potential DNA sources for genetic studies. To investigate the utility of hemolymph as a reliable non-lethal source of DNA for molecular investigations, we collected 30 *Elliptio complanata* Lightfoot, 1786 from a site in Wake County, North Carolina (Richland Creek, Neuse River drainage), and drew hemolymph before sacrificing them for phylogenetic examination as part of our ongoing research examining relationships among southeastern *Elliptio* species. Sterile tuberculin syringes (1.0 ml, 25 guage x 5/8 inch [[16 mm]) were inserted into the sinus in the anterior adductor muscle following the protocol outlined by Gustafson, *et al.* (2005a). Depending on the size of the individual, between 0.1 and 0.5 ml of hemolymph was drawn, placed in sterile microcentrifuge tubes and held at -20 °C until the samples could be processed. After sacrificing the specimens, mantle tissue was clipped and held in 95 % ethanol, which is one current standard practice of obtaining tissue samples in freshwater mussels. All voucher specimens were deposited with the North Carolina State Museum of Natural Sciences (NCSM 28211).

DNA was extracted from all samples (hemolymph and mantle tissue) using the DNeasy® tissue kit (Qiagen, Inc.) following the manufacturer's recommended protocols. Samples (5.0 µl) were loaded on 1-2 % agarose check gels to visually inspect the integrity of all genomic preparations. All mantle tissue preparations showed a visible band while all hemolymph preparations lacked such a band. PCR amplifications of three mitochondrial gene regions (COI, ND1, cytochrome-*b*) were run for all samples, following published protocols and using standard primers (Folmer *et al.*, 1994; Merritt *et al.*, 1998; Serb *et al.*, 2003). Successful reactions were cleaned using QIAquick® spin columns (Qiagen, Inc.), sequenced (in both orientations) with BigDye® ver. 3.1 (Applied Biosystems, Inc), cleaned with DyeEx® spin columns (Qiagen, Inc.) and

electrophoresed on an ABI PRISM® 377 XL automated sequencer (Applied Biosystems, Inc). Sequences were edited and compiled independently using Sequencher™ ver. 4.2 (Gene Codes Corp.). Resultant sequences from hemolymph and mantle tissue from the same individual were compared for sequence identity in each of the three mitochondrial genes to determine the concordance between tissue types (hemolymph and mantle). In all instances, hemolymph-derived sequences were determined to be identical to mantle-derived sequences and proved to be a reliable source of DNA for genetic analysis. In general, differences between tissue types were minimal and assumed to be a result of the lower concentration of DNA in the hemolymph samples. These minor differences, which only affected the intensity of the sequencing signal occasionally, could probably be compensated for by using a polymerase specifically designed for low copy number samples or by simply increasing the concentration of template used in the initial PCR reactions.

Our previous studies have shown that sampling hemolymph from freshwater mussels is non-lethal if contact is limited to sampling once a month. The present study demonstrates that this same technique can be used to sample DNA for a variety of genetic studies. We have also shown that hemolymph genetically matches somatic tissues, thus avoiding the issue of doubly uniparental inheritance exhibited in gonadal tissues of many mussel taxa (Hoeh *et al.*, 2002). Widespread use of this method with appropriate photodocumentation of potentially confusing species could prove invaluable for conducting genetic studies involving threatened or endangered species of this critically pressured invertebrate group. This method will also facilitate large scale genetic sampling schemes necessary to ensure that augmentation programs minimize the effects of unintentional genetic biasing of their propagated stocks.

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MARINE MATTERS

New marine species recorded for the Mediterranean fauna of Israel

By Henk K. Mienis

During 2005, six species have been recorded for the first time from the Mediterranean waters off the coast of Israel.

Gastropoda

Two rock snails of the family Muricidae were recorded from the northern part of the Mediterranean coast: *Thais sacellum* (Gmelin, 1791) from the Akhziv-Rosh Haniqra area and Qiriyat Yam, and *Ergalatax obscura* (Houart, 1996) from the islands of Rosh Haniqra and in shallow water near Akhziv (Singer, 2005).

Bivalvia

The diminutive oyster *Nanostrea exigua* Harry, 1985 (family Ostreidae) was recorded from Al Manara Island (= Isle of the Flies), off Akko (Lubinevsky & Mienis, 2005). Another exotic oyster *Alectryonella crenulifera* (Sowerby, 1871) (Ostreidae) was found living on the pillars of the coal conveyor belt of the power plant at Hadera (Sharon *et al.*, 2005); this first record has since been confirmed by two additional finds.

Cephalopoda

The bobtail squid *Rossia macrosoma* (Delle Chiaje, 1830) (family Sepiolidae) was recorded from a depth of 375 m off Ashqelon (Mienis, 2005a). A small octopus species, *Octopus aegina* Gray, 1849 (Octopodidae), was recognized among unidentified material in the National Mollusc Collections of the Hebrew University of Jerusalem and the Tel Aviv University from the following Mediterranean localities off Israel: Atlit (collected in 1934) and at a depth of 20 m off Tel Aviv (Mienis, 2005b).

All of these species, except the bobtail squid, *Rossia macrosoma*, belong to the group of so-called Lessepsian or