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SURVIVAL AND GROWTH OF MUSSELS SUBSEQUENT TO HEMOLYMPH SAMPLING FOR DNA

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ABSTRACT With the increasing use of molecular genetic techniques in ecology and evolution, it has become apparent that methods of non-destructive DNA sampling must be developed. In this study we collected 50 blue mussels (*Mytilus* spp.) in each of three size categories: small (10–20 mm), medium (20–30 mm), and large (30+ mm). Hemolymph was extracted from 25 mussels in each size category and the remaining 25 mussels served as controls. The hemolymph was extracted and control mussels were monitored for 384 days, during which time no significant differences in survival or growth were found. We extracted DNA from the hemolymph and successfully polymerase chain reaction-amplified the ITS and Glu-5′ species-specific markers from 81% and 92% of the samples, respectively, and determined that all mussels were *Mytilus trossulus* (Lamarck). The extraction of hemolymph for DNA analysis allows for molecular investigations of populations or species which are either rare or in limited numbers, and for life history investigations where survival of the organism is necessary.

KEY WORDS: non-destructive, *Mytilus*, PCR, DNA, survival, growth, hemolymph

INTRODUCTION

Molecular genetic techniques and the genetic characterization of individuals have become common in the study of the ecology and evolution of marine invertebrates, particularly bivalves (Mitton 1994). The benefit of the polymerase chain reaction (PCR) is that very little DNA is required, thus making analysis possible when the quantity and/or quality of DNA is limited. PCR-based species markers have been used within the *Mytilus* species complex for conservation, ecological, and evolutionary applications (Heath et al. 1995, 1996, Rawson et al. 1996). Molecular genetic characterization has also clarified population genetic structure in a variety of other bivalves (Sarver and Foltz 1993, Manuel et al. 1996, David et al. 1997, Suchanek et al. 1997, Herbinger et al. 1998), as well as aiding in investigations into the ecology and life history of bivalves with planktonic phases (Toro 1998).

Typically, shellfish are destructively sampled in order to sample tissue for DNA extraction. Destructive sampling involves killing the animal to obtain the necessary tissue for genetic analysis (Taberlet et al. 1999). Although this is acceptable for some studies (Sarver and Foltz 1993, Heath et al. 1995, Hare et al. 1996, Heath et al. 1996, Suchanek et al. 1997, Herbinger et al. 1998, Toro 1998), destructive sampling is clearly not acceptable for studies involving growth or survival measurements, or for investigations of small or rare populations. For such studies there is a need for a technique that would allow the collection of DNA without harming the organism. Non-destructive sampling generally involves capturing the target organism, taking an invasive sample without killing it, and then releasing it (Taberlet et al. 1999). It is, therefore, important that any potential technique be tested for even minor adverse effects on the survival or growth of the target organism.

Here we describe sampling hemolymph for DNA extraction from shellfish. Specifically, we sampled hemolymph from three size categories of blue mussels (*Mytilus* spp.), extracted DNA, and amplified fragments using PCR with species-specific markers. Hemolymph is made up of mostly water, but does contain cells, including nucleated hemocytes (Morse and Zardus 1997) and is responsible for the transportation of digestion products throughout the body (Brusca and Brusca 1990), among other functions. We followed the survival and growth of hemolymph-extracted and control mussel groups for over 1 y to ascertain whether this technique resulted in decreased survival and/or growth.

MATERIALS AND METHODS

Fifty mussels in each of three size categories were collected from the western coast of Quadra Island, located near Campbell River, British Columbia, Canada. The mussels were measured with calipers to the nearest 0.01 mm and sorted into small (10–20 mm), medium (20–30 mm), and large (30+ mm) size categories. In each group, hemolymph was extracted (50–200 μL) from 25, while the other 25 (control) mussels were handled, but not sampled. A 1-cc syringe (22-gauge, 1.5-inch needle) was inserted through the rear hinge joint and hemolymph was extracted until no more fluid could be removed. The extracted hemolymph was expelled into 1.0 mL of 95% ethanol and stored at room temperature. After sampling, mussels were placed in six cages (3 hemolymph-extracted and 3 control) and hung approximately 1 m below the surface at the original collection site. The cages were 5 × 5 × 10 cm and were slotted to enable free water flow through the cages, but excluded potential predators.

The hemolymph and alcohol were transported to the laboratory where they were centrifuged (13,000 rpm, 15 min), the liquid was removed, and the pellet dried (LABCONCO Centrivap Concentrator) at 60 °C for 8 min. The dried cells were digested overnight in 200 μL of lysis buffer (10 mM Tris-HCl, pH 8.0, 15 mM ethylenediamine tetra acetate, and 0.5% sodium dodecyl sulphate) and 125 μg of proteinase K at 37 °C. The solution was then extracted once with an equal volume of phenol:chloroform: isoamyl alcohol (24:24:1), followed by isopropanol precipitation (Heath et al. 1995). The extracted DNA was resuspended in 100 μL of double-distilled water and was then PCR-amplified following the ITS protocol described in Heath et al. (1995) and the

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Between April 3, 1998 and April 22, 1999, the mussels in this experiment were measured and the survivors were counted at three sampling times after transfer to the experimental cages (58, 140, and 384 days). Student's *t* test (shell length) and chi-square (survival) were used for statistical analysis to determine whether differences existed between the two groups at day 58 and day 384. Day 58 comparisons were made to test for short-term effects, while day 384 comparisons were made for long-term effects.

RESULTS

DNA was successfully PCR-amplified from 61 of the 75 samples (81%) for ITS, and from 69 of the 75 samples (92%) for GLU (Fig.1). There was no consistent effect of mussel size (and hence hemolymph volume) on the success of the PCR amplification. All mussels were determined to be *Mytilus trossulus*. The hemolymph technique was found to have little effect on either survival or growth (Fig. 2). At day 58 and day 384, the survivorship of the hemolymph-extracted mussels was not found to be significantly different than the survivorship of the control mussels in any of the size categories ($P > 0.10$). At day 58, the control mussels were slightly larger than the hemolymph-extracted mussels in the small size category ($P = 0.018$), but there was no significant difference in either the large or the medium size categories ($P > 0.50$). At day 384, the hemolymph-extracted mussels were larger than the control mussels in the large size category ($P = 0.033$), but there was no significant difference in the medium or small size categories ($P > 0.10$).

There were also no consistent differences found in survival or growth among the mussel size categories. The smallest mussels we sampled were between 10 and 20 mm and had mortality and growth similar to the larger size categories.

DISCUSSION

We sampled a wide range of sizes of mussels using a nondestructive method of DNA sampling and successfully extracted DNA for PCR purposes from most of the mussels, including those in the small category. Our PCR success rates were comparable to those of Heath et al. (1995) who used destructive tissue sampling methods. The technique described here is a useful tool for fieldwork, as it does not require the killing of the organism under study. Furthermore, we found no consistent effect of hemolymph sampling on either survival or growth of the mussels. Although this is not surprising for the larger mussels, it is unexpected for the small animals, as the extraction of a large portion of the organism's body fluid would be expected to negatively effect the organism's growth and/or survival.

Although we used mussels, our technique is applicable to other bivalve species. For example, Manuel et al. (1996) used a similar technique on scallops (*Placopecten magellanicus*); however, they extracted approximately 5 to 10 times the volume of hemolymph and did not test for potential growth or survival effects of their sampling method. Other researchers have reported hemolymph sampling in bivalves for various purposes, including DNA extraction (Marsh et al. 1995), hemocyte pathology and function (Moore et al. 1991; Oliver & Fisher 1995), and ploidy analysis (Komaru et al. 1988). However, no attempt was made to determine the effect of that sampling on the viability of the animals. Our study also

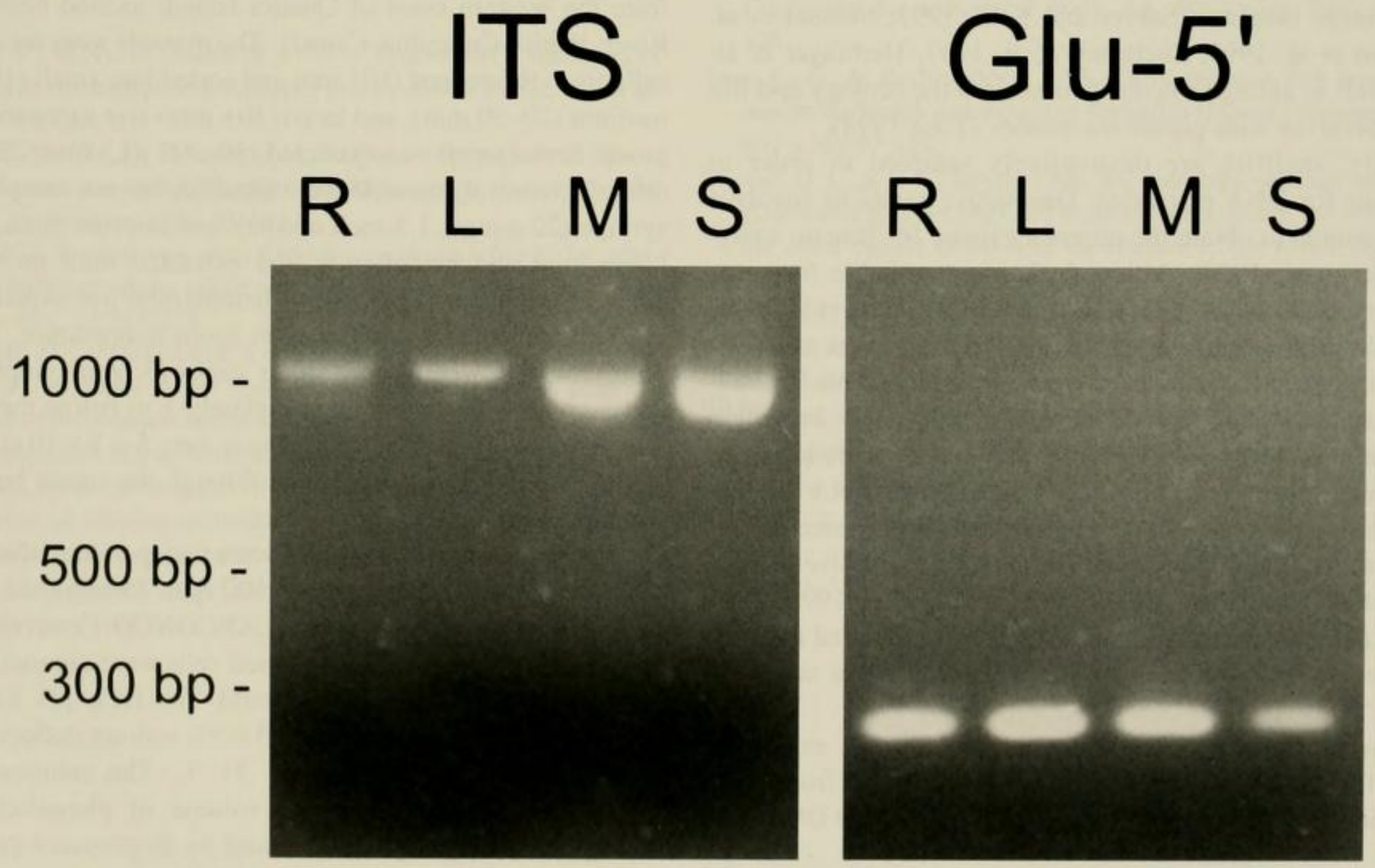

Figure 1. Agarose gel electrophoresis of PCR-amplified DNA fragments using the ITS and Glu-5′ species-specific primer sets. The various lanes are PCR results using DNA from a regular extraction method (R) and DNA extracted from hemolymph taken from mussels in three size classes (L, large; M, medium; S, small).

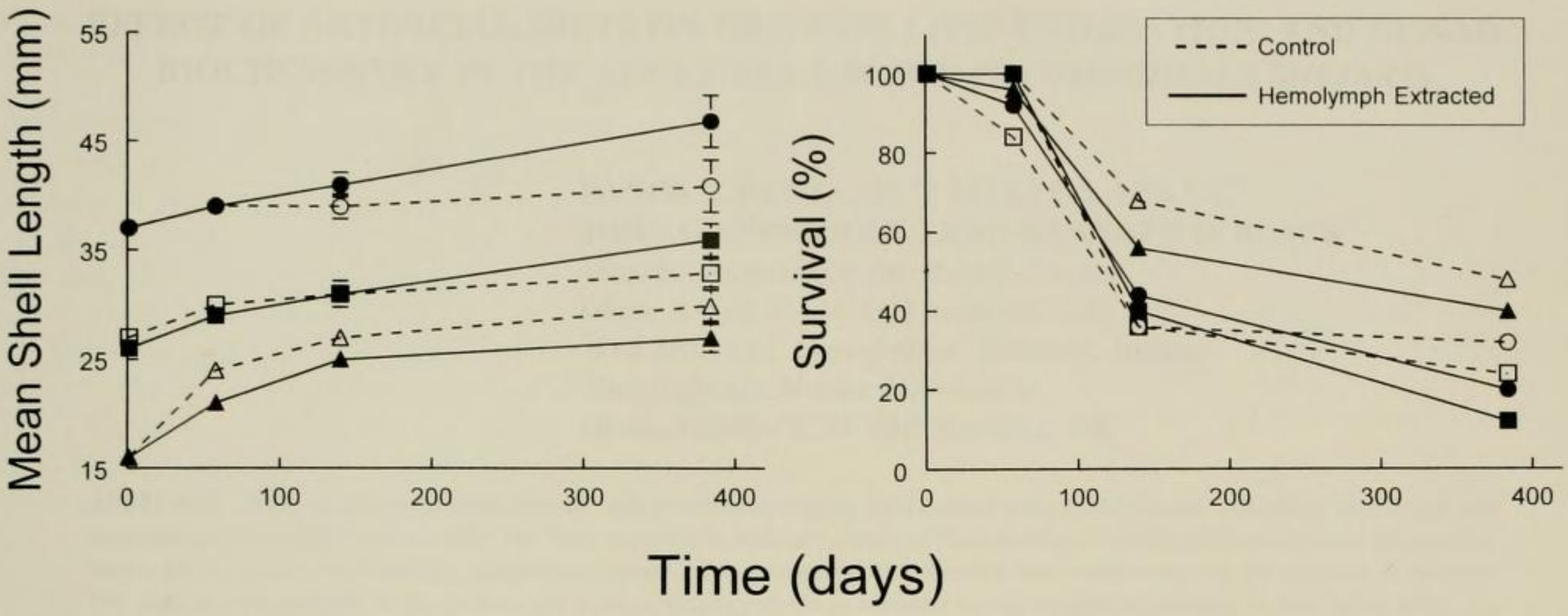

Figure 2. Comparison of mean shell length (±1 SE) and survival for hemolymph-extracted and control mussels from three size classes over a 385-day period, post-treatment. The circles represent the large size class, the squares represent the medium size class, and the triangles represent the small size class. The filled symbols with the solid lines are the hemolymph-extracted mussels, while the open symbols with the dashed lines are the control mussels.

showed the syringe-extracted hemolymph consistently provides PCR-quality DNA from large numbers of animals sampled under field conditions. Hemolymph extraction clearly has considerable potential for studies requiring the non-destructive sampling of DNA from bivalves, and thus has applications for growth and survival studies. This technique will also be useful for studies of the ecology and population dynamics of bivalves where destructive sampling of the organism is either not permitted or not desirable for the experimental design.

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ITS

Glu-5'

RLMS RLMS

1000 bp-

500 bp -

300 bp -

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(L, large: M, medium; S, small).

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Control

Hemolymph Extracted

400

Time (days)

Figure 2. Comparison of mean shell length (±1 SE) and survival for hemolymph-extracted and control mussels from three size classes over a

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