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Short communication

Tissue sampling from live blue mussels, *Mytilus edulis*. A field study from the Swedish west coast

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Abstract

Histological techniques are often used to study environmental effects on mussels, but since these techniques include killing of the individuals, rare or endangered populations cannot be studied using conventional tissue sampling. This study is an attempt to find a method that can be used repeatedly with the same mussel individual and which does not affect growth and survival. From 200 mussels, *Mytilus edulis*, tissue was sampled in different ways, such as drilling a hole in the shell or prising apart the shell valves. Two kind of instruments were used, an injection needle and surgery forceps. Some of the drilled mussels had their holes sealed again with cement.

Drilling a hole in the shell, removing tissue sample with surgical forceps and then leaving the holes open did not seriously harm the mussels during the two months the experiment lasted. But if the holes were sealed with cement, both length and weight growth were negatively affected (35% lower length growth and 36% lower weight growth compared to the control mussels). Mortality was highest among the drilled and sealed mussels (80% higher than among the other treatments). The vulnerability of the population, the aim of the study and the duration of the experiment should decide what method to use for tissue sampling. For long-term experiments and repeated sampling, opening the mussels by prizing apart the valves is a better alternative than drilling holes in the shells, but depending on the morphology of the species it could be difficult to sample the anterior part of the mussel body. For a short experiment and to sample anterior parts, drilling the shells, leaving the holes open and using surgical forceps, seems to be an acceptable compromise between the different treatments used.

Keywords: *Mytilus edulis*; Drilling; Growth; Mortality; Sealing; Techniques

1. Introduction

Histological techniques to study environmental effects on mussels, the occurrence of parasites or the health of mussels are widely used and well tested. Studies of parasite infections demand large numbers of mussels. Rare or endangered mussel species (such as the pearl mussel *Margaritifera margaritifera* in Europe) and otherwise vulnerable populations cannot be studied using conventional tissue sampling techniques for which whole individuals are killed. Such populations and species are, however, often the ones most in need of investigation, because environmental and pathological effects on reproduction and growth may be the proximate causes of threat. Methods of sam-

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pling tissue from live animals may be an alternative, important for threatened or rare species, but it may also be desirable in situations where the same individual has to be sampled on several occasions. Live tissue sampling requires methods that do not seriously harm the mussel. Ideally such a method should meet the following demands: (i) no effect on growth, reproduction and behaviour of the mussel, (ii) easy and quick to use, and (iii) allows repeated use on the same individual. One difficulty is to obtain sufficiently large pieces of tissue for further analysis. Berg et al. (1995) sampled 1 cm^2 (approx. 34 mg) mantle pieces from the anterior end of freshwater mussels using biopsy and Naimo et al. (1998) sampled 7.7 mg plugs from the foot tissue of freshwater mussels. In marine mussels biopsy has been used in several studies, but only for cell suspension, not for taking pieces of tissue (e.g. Santarem et al., 1994; Mikhailov et al., 1997). The aim of the present study was to compare the effects on growth and survival using different methods of live tissue sampling of marine mussels *Mytilus edulis*, in order to find optimal conditions for a repeated sampling procedure.

2. Material and methods

Two hundred two-year-old rope-cultured blue mussels (*Mytilus edulis*), 4–5 cm in length, were used in the experiment, which included ten treatments combining drilling and prising apart the valves, and sampling by needle or forceps. Control mussels were weighed and measured and in addition mussels to compensate for the effect of drilling and for the effect of prising apart the shells, so-called procedure controls (Underwood, 1997) were used. Thus the following treatment were used:

1. The shells drilled, the holes sealed, no tissue sampled.
2. The shells drilled, the holes left open, no tissue sampled.
3. The shells drilled, the holes sealed, tissue sampled by surgery forceps.
4. The shells drilled, the holes left open, tissue sampled by surgery forceps.
5. The shells drilled, the holes sealed, tissue sampled by injection needle.
6. The shells drilled, the holes left open, tissue sampled by injection needle.
7. The mussels not drilled or sampled but handled in the same way as drilled ones (PC).
8. The mussels only weighed and measured (C).
9. The shells not drilled but forced to open, tissue sampled by injection needle.
10. The shells not drilled but forced to open, no tissue sampled (PC).

Mussels were placed in a random order in plastic pots (one for each treatment) in the laboratory. The length and wet weight were measured. Seawater was poured into the pots, to avoid the mantle cavity being air filled after the drilling. Since the aim was to test different sampling methods, no pre-determinate tissue or fluid was sampled and no tissue analysis was done. The mussels were supposed to be in the same stage of the gametogenesis, that is the spawning period.

1. Drilling the shells (n = 120): A drilling-machine ('SKIL 2125H') with a speed of 230 r. p. m. was used and the holes were made in the same shell-half in all mussels, near the anterior end. The holes were made with a metal drilling bit (∅ 3 mm). From 80 of the drilled mussels a small piece (approx. 3 mm^3) of tissue was removed through the hole, in 40 mussels with surgical forceps, and in another 40 mussels with an injection needle. The holes of 50% of the drilled mussels (n = 60) were sealed with 'Justi Resin Cement' from Ivoclar, Lichtenstein; a 'non-toxic' cement used by dentists.

2. Prizing apart the shells (n = 40): The shells were opened at the posterior end by inserting a thin piece of cork between the valve-halves when the mussels already had their shells slightly open. This is fairly easy to do if the mussels are placed with the anterior end towards the bottom of a pot and the siphons turned upwards. In 50% of the opened mussels a sample was taken from the posterior end of the mussel mantle using an injection needle (n = 20). The other 50% of the mussels were handled in the same way but no samples were taken (n = 20).

All the mussels were placed in a randomised order in baskets, each mussel surrounded by a marked net-tube. There were five baskets, with 40 mussels each and covered with a net. The baskets were placed in the sea.

Every second week the mussels were checked and fouling algae and barnacles were removed from the baskets with a hard brush. After two months the experiment was terminated, and the baskets were transferred to the laboratory. Growth was estimated from weight and length increase and shells of the mussels were examined.

3. Statistical analysis

As some of the mussels died during the experiment, the data sets became unbalanced. However, the different null hypotheses were first tested using all the observations in an unbalanced data set. If a null hypothesis was rejected, the same hypothesis was tested again with a data set that had been balanced by either random elimination or by addition, reducing data to a proper replicate number. The mortality however, was tested using the result from the original 200 individuals.

The results of the experiment were analysed by a two-factor ANOVA, testing for effects of the fixed factor 'treatment' with ten levels and the random factor 'basket' with five levels, A–E. Post-hoc, the Student-Newman-Keuls (SNK) (Underwood, 1997) tests, were used to assess differences among levels of a significant factor.

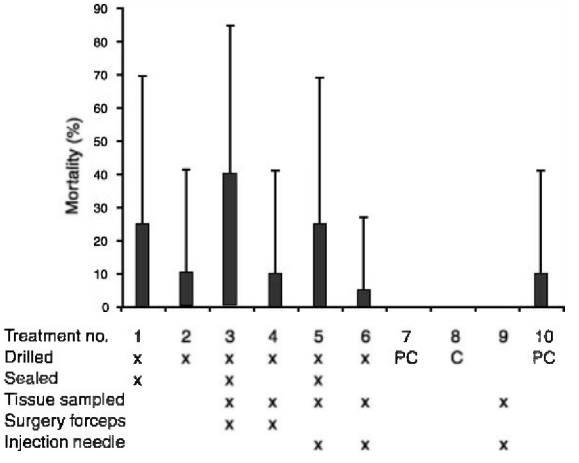

Fig. 1. Mortality prevalence in 200 individuals of *Mytilus edulis* exposed to ten different treatments (20 individuals in each) for a period of two months. C is the control group and PC is the procedure group (see text). Error bars: 95% confidence interval.

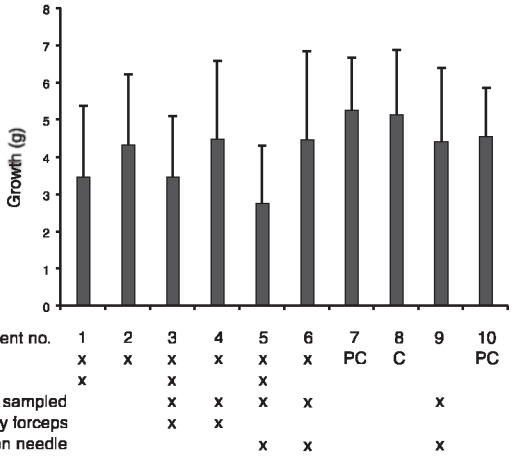

Fig. 2. Effects of ten different treatments on the weight growth of 150 individuals of *Mytilus edulis* (15 individuals in each treatment) after a period of two months. C is the control group and PC is the procedure group (see text). Error bars: 95% confidence interval.

4. Results

Twenty-five individuals (12.5%) died during the experiment. There was no interaction between the factors treatment and basket, on the mortality, weight or length and no effect of basket alone. The factor treatment, however, showed to be highly significant

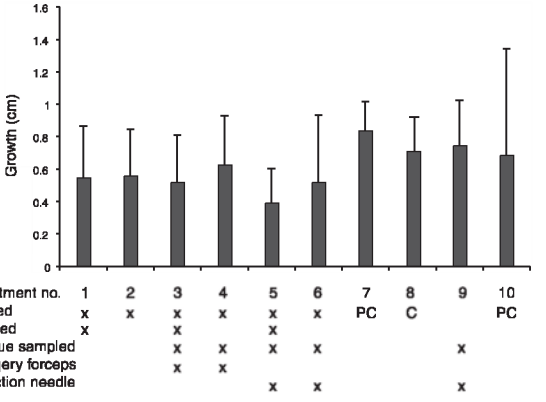

Fig. 3. Effects of ten different treatments on the length growth of 150 individuals of *Mytilus edulis* (15 individuals in each treatment) after a period of two months. C is the control group and PC is the procedure group (see text). Error bars: 95% confidence interval.

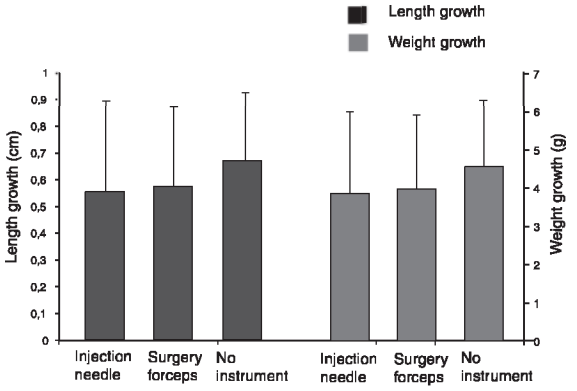

Fig. 4. Effects of instrument used on the growth of 150 individuals of *Mytilus edulis* (15 individuals in each treatment) after a period of two months. Error bars: 95% confidence interval.

and the SNK test revealed a tendency of higher mortality in the mussels with drilled and sealed holes than for mussels from the other treatments and a significantly higher mortality ($p = 0.02$) for drilled and sealed mussels which were sampled with surgery forceps (Fig. 1). The mussels with drilled and sealed holes also had low growth values (Fig. 2, $p_{weight} = 0.01$ and Fig. 3, $p_{length} < 0.001$).

The holes in the shells were repaired in all live mussels and in the shells with sealed holes, the cement string seemed to be unaffected. There was no significant difference between mean growth as effects of the instrument used ($p_{weight} = 0.11$ and $p_{length} = 0.07$), though the mussels in which no instrument was used had the highest growth (Fig. 4). No significant interaction was shown between instrument used and sealed/open holes ($p_{weight} = 0.60$ and $p_{length} = 0.74$).

5. Discussion

Drilled, sealed mussels grew less than the control mussels, and the use of cement decreased both weight and length growth. Kideys (1994) studied the effects of tagging *Buccinum undatum* L. using an electric drilling machine to make a hole (∅ 2 mm) for tagging in the shell. Tagging decreased the growth rate, but the effects of drilling were not separated from other tagging effects in his study. All of the individuals were drilled, and the holes were observed to be repaired and the inner shell surface to be covered with new shell material. Kideys (1994) suggested that a hole in the shell causes the animal to invest extra energy in repair of the shell, which delays growth. My results support his suggestion. Empty shells were found in all baskets, and the fact that mussels from the drill treatments with their holes sealed died at a higher rate than mussels of other treatments (72% of the dead mussels were from this treatment) indicates that the cement was harmful to the mussels after all. Many studies describe the method of using an injection needle to inject a solution into a mussel (e.g. Cancio et al., 1998) or to withdraw blood cells (e.g. Santarem et al., 1994). The methods are widely used and nothing in the literature indicates that they are harmful to the animals. My study supports the use of surgical forceps as an alternative to injection needles; among the treated mussels growth was not affected by the type of instrument used. The advantage of using surgical forceps is the possibility to sample larger tissue pieces.

This study suggests that the vulnerability of the population and the aim of the study should decide what method to use for tissue sampling. Also the duration of the study will be of importance. For long-term experiments and repeated sampling, opening the mussels by prizing apart the valves is better than drilling holes in the shells. For a short experiment drilling, leaving the holes open and using surgical forceps, seems to be an acceptable compromise between the different treatments investigated.

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