Influence of the Environmental Factors on Contamination of Mediterranean Mussels (*Mytilus galloprovincialis*)

Henigman U.[1,*], Biasizzo M.[1], Vadnjal S.[1] ,Knific T. [1], Kirbiš A.[1]

[1]University of Ljubljana, Veterinary faculty, Gerbiceva 60, 1000 Ljubljana, Slovenia

*corresponding author: e-mail: Urska.Henigman@vf.uni-lj.si

Abstract
Mussel harvesting areas in Slovenia are monitored for contamination with *E. coli* as pollution indicator bacteria. According to contamination levels, areas are classified into A, B or C category. An A category means that there is less than 700 *E. coli* MPN/100 g of the shellfish flesh and intervalvular fluid. Mussels from the areas with the established A category are placed on the market directly, whereas shellfish from areas classified as B or C category undergo a depurating process. The aim of our research was to gain detailed insight into the contamination with *E. coli* as well as with some other microorganisms and heavy metals. We also took into account possible factors affecting the contamination of seawater and shellfish (e.g. marine currents, rainfall, tides). During a one-year period 34 samplings at three shellfish harvesting areas and one wild area were carried out (306 samples). Higher levels of contamination were observed in colder parts of the year (spring, winter) and at the time of heavy rainfalls. Correlation between *E. coli* number in mussels and enterococci in seawater was statistically significant. To ensure safety of the mussels it is therefore important to carry out increased number of samplings at critical periods of the year.
Keywords: *mussels, E. coli, safe food*

1. Introduction

Bivalve shellfish are filter-feeding organisms and may accumulate microorganisms and other agents present in the surrounding water. Contamination of shellfish is influenced by different factors, such as pollution sources, rainfall, salinity, temperature and many others. Higher concentrations of *E. coli* in shellfish in autumn and in winter are mainly associated with heavy rains (Riou et al., 2007; Almeida and Soares, 2012). Shellfish harvesting areas in Slovenia: Seča, Strunjan and Debeli rtič are located in the Northern Adriatic, which is characterized by poor circulation of water masses, shallowness and extreme influences of inland waters (Sotlar, 2000). In addition to regional influence, the pollution of the northern Adriatic is due to the economic and urban development of the coastal towns with hinterland.

2. Material and Methods

2.1. Sampling

Samples of mussels were collected at three harvesting areas: at the Seča, Strunjan, Debeli rtič and at one non harvested area near the lighthouse in Debeli rtič. Mussels were transported to the laboratory where bacteriological examination was performed. The number of *E. coli* was determined according to the ISO/TS 16649-3.

2.2. Statistical analysis

For the statistical analysis we used the program R 3.3.0. We tested differences between *E. coli* contamination in different harvesting areas and between months of the year. The preliminary homologous variance test showed that variance between the groups differs. Differences in *E. coli* in bivalve molluscs between harvesting areas and periods of the year were compared with nonparametric Kruskal-Wallis analysis of variance at the sum of ranks. In the case of a statistically significant difference between groups, pairs of groups were compared with each other on the basis of a post hoc test of multiple comparisons. For all tests, the criterion for statistical significance p was <0.05.

3. Results

During the tested period 88.2% of samples did not exceed 230 MPN *E. coli*/100 g. Among the sampling points, the maximum limit value was exceeded in the free living mussel samples, where the highest contamination values were found. There were individual higher peaks of *E. coli* in the autumn period at the Seča and Strunjan harvesting areas, but not at the Debeli rtič. During the main bathing season, in the summer months, no value of 230 MPN/100 g was exceeded in any of the sample. Results of *E. coli* contamination in mussels were collected into trimesters, and in October, November and December 81% of the results exceeded 700 MPN *E. coli*/100g (Figure 1). The seasonal impact is therefore important, but due to the short period of our study it is not possible to confirm this with certainty.

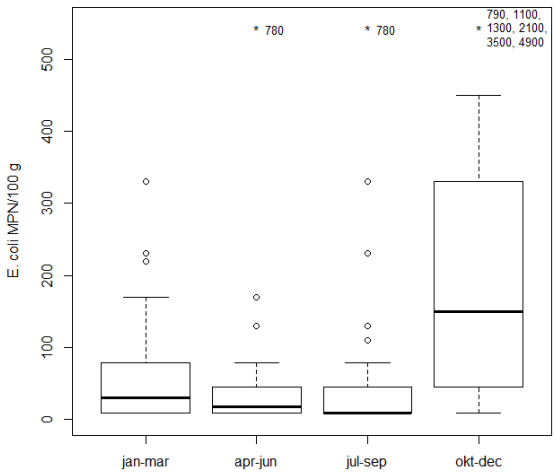

Figure 1. Number of *E. coli* containing in mussels in trimesters: from November 2014 to October 2015 in all harvesting areas

The Debeli rtič harvesting area with the lowest median value (9) was statistically significantly different from the other two. Between the Seča, which had the highest value of the median (45) and the Strunjan harvesting area (20), there was no statistically significant difference (Figure 2). Two municipal wastewater treatment plants; in Koper and in Piran have direct discharges into the sea. Both treatment plants represent the largest point source of pollution, but there are several other technological sources of pollution along the coast, the most important one is port Luka Koper.

Studies (Riou et all., 2007) of the hydrodynamic model showed influence of the rain on the microbiological contamination of the shellfish; due to the higher water level the influx of fecal contaminants to the growing areas was increased. Small influxes, which are due to heavy rainfall, are expected to have a major impact, and large quantities of faecal pollutants are brought into the sea, also due to the overloaded wastewater treatment plants. In particular, local areas close to these tributaries and to the coast are burdened. The degree of contamination within these periods is also associated with the density of population in the coastal area (Glasoe and Christy, 2004).

In this study we find that extreme weather conditions, especially heavy rains, have a strong impact on marine pollution and a consequent increase in the value of *E. coli* in mussels. To conclude, the hydrodynamic behavior of the northern Adriatic Sea is rather complex. During its annual hydrologic cycle, the basin is changing its main hydrodynamic features from homogenous to a stratified system. It is characterized by sinking heavier and denser waters during the winter season, and surface warming during summer, by abundant precipitation and discharges from rivers during spring and fall, associated by different wind regimes (Russo et al., 2012).

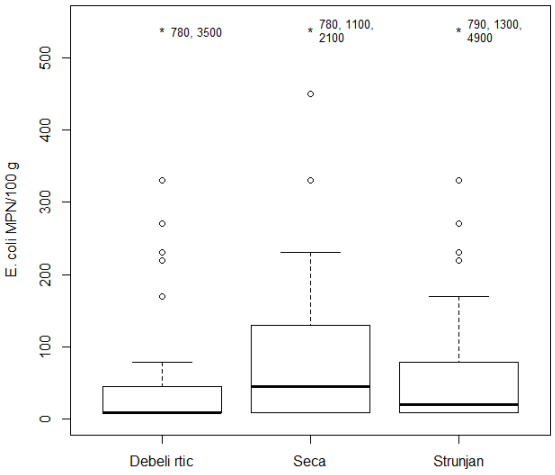

Figure 2. Number of *E. coli* containing in mussels in the harvesting areas from November 2014 to October 2015

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