

Incidence of *E. coli* in aquaculture areas of the Adriatic Sea

DAMIR KAPETANOVIĆ, BOŽIDAR KURTOVIĆ,
ZLATICA TESKEREDŽIĆ, EMIN TESKEREDŽIĆ

Laboratory for aquaculture, Department for Marine and Environmental Research, Ruđer Bošković Institute,
Bijenička 54, Zagreb, Croatia

Kapetanović D., Kurtović B., Teskeredžić Z., Teskeredžić E.
Incidence of *E. coli* in aquaculture areas of the Adriatic Sea

Summary

Aquaculture is currently one of the fastest growing food production sectors in the world. An increase in nutrients and organic wastes have led to a general deterioration of water quality. The water quality problem is associated with both physical and chemical factors, as well as microbiological water quality. This study was undertaken to investigate the water quality at five fish farms at the Adriatic Sea. The results indicate variations but not significant differences in the presence of *E. coli* in the surface layers of the sea in relation to the water column depth.

Keywords: aquaculture, *E. coli*, Adriatic Sea

The aquaculture activities may introduce excessive nutrients into surrounding water and the seabed, contributing to local eutrophication and to general deterioration of water quality. At the same time, eutrophication and other harmful impacts due to other pollution sources also pose a threat to the fish farms. The surface layer of the seawater presents a trophic zone of primary production (1). Furthermore this layer is known to contain a lipid film at the air/water interface. It has been shown that there is an accumulation of microorganisms in the surface layer (2). Also, it is well known that an important fraction of the bacterial flora in the sea is absorbed on solid or particulate substrates.

Food- and waterborne diseases are still of major concern regarding human health (3). Most important emerging pathogens in water include viruses, bacteria and protozoa (4). Many of the pathogens may only be present in very small numbers or not at all. Therefore it's important to look for indicator bacteria. Fecal coliforms, mainly comprising *E. coli*, are a subgroup of the total coliform group and they occur almost entirely in faeces. Untreated water sources almost always contain some fecal coliforms. Physical factors and characteristics of the receiving water such as thermal and salinity stratification, water depth, and surface area affect water quality.

The aim of this pilot study was to analyze impact of the aquaculture activities on marine environment in

Adriatic Sea, by determination of physical characteristics and qualitative bacteriological analysis of seawater.

Material and methods

Five locations were selected to represent a large region of the Adriatic Sea. On each location, about 150 m away from fish farm control sample was taken. Ten samples were taken at the surface and six in the water column at depth of 4.0 m. Five out of ten samples from the surface were taken at the fish farm while another five on the control location. At the same time, three out of six water samples at 4.0 m depth were taken at the fish farm while another three on the control location. The fish farm at the South is located in the coastal zones (S1), while the fish farms in the Middle Adriatic are located in the sheltered bays (S2 and S3). The fish farms at the North are located in the bay (S4) and in the estuary (S5). The examination was carried out in December 2003. Salinity, temperature and pH were measured with a hand held refractometer (ATAGO), digital thermometer (UC-12 Kagaku, Japan) and digital pH meter (UC-23 Kagaku, Japan), respectively.

For the purpose of qualitative bacteriological analysis of seawater indicator bacteria were enumerated: total coliforms and *E. coli* using Colilert®-18 Test Kits (IDEXX Laboratories, Inc., Westbrook, ME, USA). Water samples were collected in sterile, transparent vessels. Colilert-18 is the only US EPA-approved 18-hour test of water and is included in Standard Methods for Examination of Water and Wastewater. The significance between data was evaluated by a chi squared test.

Results and discussion

The investigated region had an air distance of 379.3 km between southern (S1) and northern (S5) fish farm. The results are summarized in tab. 1. Temperature and salinity were characterized by rather high seawater temperature and low salinity in the South Adriatic. Differences among locations on the North (S4 and S5) probably exist due to different hydrographic conditions in environment of channel. Strong wind and waves on location 4 led to features changes (diluting effect), i.e. flushing rate and lower fluctuation of pH in water samples.

A total of 16 strains were sampled for microbiological examination. Results of the qualitative bacteriological testing indicate that there is no variability in total coliforms between locations.

Of the 8 strains collected from the fish farm, 5 strains were from surface layer (0.5 m) and 3 strains from water column (4.0 m). *E. coli* was detected in all surface samples except on location S 1A. Considering water column, *E. coli* was detected in one out of three samples, at the fish farm S 4A.

Of the 8 strains collected from the control points, 5 strains were from surface layer (0.5 m) and 3 strains from water column (4.0 m). *E. coli* was detected in all surface samples. Considering water column, *E. coli* was detected in one out of three samples, at the fish farm S 4B.

From these findings, *E. coli* was detected in nine out of ten surface samples and in two out of six samples in water column from fish farm and control point.

A stratified comparison of *E. coli* incidence indicates that the changes in surface layer and water column did not differ significantly.

This pilot study was carried out to compare the water quality at five marine fish culture locations with different hydrographic conditions. Environmental impacts vary considerably between locations, and were significantly reduced at locations with good water circulation and low stocking density. Physical water properties were characterized with higher seawater temperature and lower salinity in the South Adriatic.

It was revealed that bacteria in water column and surface were present, with presence of total coliforms in all samples, whereas presence of *E. coli* was observed in the surface layer with a decreasing trend, but not significant, toward to the depth of the water column. This is in accordance with previous observations (1, 2). Finally, our results provide a basis for further a long-term program initiated to monitor incidence of the bacteria on the year round basis.

References

1. Egli T., Köster W., Meile L.: Pathogenic microbes in water and food: changes and challenges. FEMS Microbiol. Rev. 2002, 26, 111-112.
2. Liu C. C. K., Sou I. M., Lin H.: Artificial upwelling and near-field mixing of nutrient-rich deep-ocean water plume. Marine Environ. Engin. 2003, 1, 1-14.
3. Kjelleberg S., Norstrom B., Löfgren H., Larsson K.: Surface balance study of the interaction between microorganisms and lipid monolayer at the air/water interface. Appl. Environ. Microbiol. 1976, 4, 609-611.
4. Kurtović B., Teskeredžić E.: Zoonosis of aquatic organisms. Ribarstvo 2001, 59, 159-169.

Author's address: Damir Kapetanović DVM, Laboratory for aquaculture, Department for Marine and Environmental Research, Ruđer Bošković Institute, Bijenička 54, Zagreb, Croatia; e-mail: kada@irb.hr

Tab. 1. Water quality at sampling sites in the Adriatic Sea

Site	Temperature (°C)	pH	Salinity (‰)	Total coliforms	<i>E. coli</i>
1A (0.5 m)	18.5	8.12	37	+	-
1B (0.5 m)	18.5	NA	37.7	+	+
2A (0.5 m)	16.1	8.31	38	+	+
2A (4.0 m)	16.2	8.34	38	+	-
2B (0.5 m)	16.6	NA	38	+	+
2B (4.0 m)	16.6	NA	38	+	-
3A (0.5 m)	16.3	8.39	38	+	+
3A (4.0 m)	16.8	8.39	38	+	-
3B (0.5 m)	16.7	NA	37.9	+	+
3B (4.0 m)	16.7	NA	37.7	+	-
4A (0.5 m)	13.4	8.32	38	+	+
4A (4.0 m)	13.0	8.35	38	+	+
4B (0.5 m)	13.4	8.43	38	+	+
4B (4.0 m)	13.4	8.42	38	+	+
5A (0.5 m)	15.8	8.21	38	+	+
5B (0.5 m)	15.9	8.20	38	+	+

Explanations: A – location at the fish farm, B – control location, 150 m from the fish farm, NA – not analysed

MYLONAKIS M. E., BOURTZI-HATZOPOULOU E., KOUTINAS A. F., PETRIDOU E., SARIDOMICHELAKIS M. N., LEONTIDES L., SIOCHU A.: Seroepidemiologia leptospir w populacji kotów w szpitala zwierząt w Grecji. (Leptospiral seroepidemiology in a feline hospital population in Greece). Vet. Rec.156, 615-616, 2005 (19)

Badano serologicznie w kierunku zakażenia różnymi serowarami leptospir 96 kotów pacjentów Kliniki Medycyny Zwierząt w Salonikach pochodzących z różnych środowisk. W grupie S było 51 kotów z chorobami miejscowymi i ogólnymi, w grupie H 48 zdrowych kotów. Ogółem zbadano 61 kotów trzymany w domu, 3 koty wolno żyjące, 35 kotów o mieszanym trybie życia. 26 kotów miało stały kontakt z psami, 27 miało okazjonalne kontakty z gryzoniami, 10 z ptakami, 1 ze zwierzętami na fermie. Osiemnaście kotów (35,3%) S i 15 (31,3%) z grupy H reagowało dodatnio w teście aglutynacji mikroskopowej z jednym lub dwoma serowarami z 33 serowarów leptospir użytych w badaniach. Najczęściej występowały wyniki dodatnie z *L. interrogans* grupa serologiczna *autumnalis* serowar *Rachmati*. W grupie S reagowało 9, a w grupie H 8 kotów. Nie występowały zależności pomiędzy wynikiem dodatnim odczynów serologicznych a wiekiem lub płcią kotów. Większy odsetek reagentów na *L. autumnalis Rachmati* występował u zwierząt starszych. Występowanie ujemnej korelacji pomiędzy dodatnim wynikiem odczynu serologicznego na *L. autumnalis Rachmati* u kotów kontaktujących się z psami świadczy o małej roli psów w epidemiologii zakażeń kotów tym serowarem.