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DETERMINATION OF THE BACTERIAL CONTAMINATION IN LIVE FOOD PRODUCTION SYSTEMS IN MARINE FISH HATCHERIES IN SOUTHERN EUROPE

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Introduction

Due to the rapid expansion of fish farming, the increasing production of larvae leads to the intensification of production techniques. As a consequence more disease problems arise in larval rearing. Most of them seem to be related to bacterial infections. As often the live food is suspected to be a source of contamination (Tanasomwang and Muroga, 1990), a sampling campaign was organized in three different marine hatcheries in southern Europe. The aim was to study the microbiological environment quantitatively in the live food production units of the hatcheries and to compare the levels of contamination in various hatcheries, using different live food production techniques.

Materials and methods

Samples of *Artemia* and rotifers were taken at different stages of culturing and kept refrigerated for maximum 2h before freezing. The *Artemia*/rotifer samples were separated from the tank water by filtering through a sterile nylon filter. The samples were quickly frozen (CO_2/methanol or liquid nitrogen), using glycerol as a cryoprotectant, and transported to the laboratory in dry ice or liquid nitrogen. In the laboratory, the tank water samples were plated directly and also in a 1:500 dilution onto the media. Excessive seawater was removed from the *Artemia*/rotifer samples, a 1% suspension in seawater was made and shaken for 10min at 28°C. The organisms were removed from the wash water by filtration through a sterile nylon filter. The filtrate was plated directly in 1:500 dilutions onto the media. The samples were plated on Marine agar (MA, Difco), BTB and TCBS (Oxoid) medium using a spiral plater.

Hatcheries A and B both use a flow-through water circulation system, while hatchery C applies a recirculation water system.

Results

The results on *Artemia* hatching and enrichment are given in Tables I and II. The hatching started with low bacterial numbers on all media; decapsulated or disinfected cysts contained a maximum of 10^3 bacteria/g. The microbiological quality of the seawater in all hatcheries was satisfactory ($<10^2$-10^3.ml^{-1} on MA). During the hatching process (*i.e.* about 14h after incubation) an important bacterial flora developed, and remained more or less constant throughout the rinsing and enrichment steps. The total count in the hatching water after 24h hatching (t_{24}) ranged from 10^6 to 10^8.ml^{-1} on MA and 10^4 to 10^6.ml^{-1} on BTB and TCBS. From hatched as well as from enriched nauplii similar numbers were recovered, *i.e.* 10^6 to 10^7.g^{-1} on MA, 10^5 to 10^7.g^{-1} on BTB and 10^4 to 10^5.g^{-1} on TCBS, by Tanasomwang and Muroga (1990).

Rinsing had no effect on removing this flora from the nauplii. Rinsing had only a diluting effect on the water surrounding the nauplii as numbers on BTB decreased a 10^4- and 10^5-fold on TCBS just after rinsing. In hatchery A, the overall number of bacteria recovered was 10 to 100 times higher when unfiltered, instead of UV-treated seawater was used.

The results on the culturing of rotifers are presented in Table III. The numbers of Vibrionaceae recovered from the algal cultures were generally low ($<10^2$-10^3.ml^{-1}), whereas total counts on MA ranged between 10^4 and 10^6.ml^{-1}.

The bacterial numbers in semi-continuous rotifer culture tanks were very stable, regardless of the rotifers' age and density in the tanks (10^4.ml^{-1} on MA, 10^2.ml^{-1} on BTB, and 10-10^2.ml^{-1} on TCBS). In the batch-production culture tanks ($2m^3$), the overall contamination was 10 times higher as compared to the semi-continuous cultures. The bacterial numbers isolated from the rotifers were in the same range for both culture techniques: 10^7-10^8.g^{-1} rotifers (wet weight) on MA, 10^5.g^{-1} on BTB and 10^4.g^{-1} on TCBS. Rinsing did not seem to have an effect on bacterial numbers.

Conclusions

The bacterial contamination levels found in this broad survey are similar to those found in the literature. In *Artemia* production, the bacterial blooms are associated with the breaking of the cysts during hatching. The flora developed remains rather constant during the following steps. Rinsing has no significant effect on removing this flora. Batch culturing and semi-continuous rotifer culturing result in comparable bacterial loads on the rotifers.

In order to evaluate the importance of the contaminating microflora in the live food production process it is necessary to proceed to its identification. This is the subject of an ongoing parallel study (Verdonck *et al.*, 1991).

Table I. Microbiological contamination in *Artemia* hatching and enrichment. Number of CFU.ml^{-1} medium or .g^{-1} *Artemia* nauplii on MA, BTB, TCBS after 2 to 4 days incubation at 28°C. (t_x=moment at which samples are taken, x=hours after hatching)

Sample		CFU.ml^{-1} or .g^{-1} on MA	CFU.ml^{-1} or .g^{-1} on BTB	CFU.ml^{-1} or .g^{-1} on TCBS
Cysts*		$<10^2$-10^3	$<10^2$	$<10^2$-10^2
Hatching water at t_{24}	hatchery A**	10^5-10^7	10^5-10^7	10^4-10^7
	hatchery B	10^6	10^5	10^4
	hatchery C	10^8	10^5	10^5
Hatched nauplii in A, B, C		10^6-10^7	10^4-10^6	10^4-10^5
Enrichment tank water t_{20}-t_{24}:	hatchery A**	10^4-10^7	10^3-10^6	$<10^2$-10^6
	hatchery B	10^7	10^6	10^4
	hatchery C	10^8	10^6	10^4
Enriched nauplii in A, B, C		10^6-10^7	10^5-10^7	10^4-10^5

* Desinfected or decapsulated cysts.

** The *Artemia* section of hatchery A was sampled at two different periods. The numbers vary due to the difference in water quality (UV *versus* unfiltered seawater).

Table II. The microbiological contamination during the hatching process at different time intervals (t_x; x=hours after hatching) in hatchery A and the effect of rinsing on the flora present

Sample	CFU.ml^{-1} or .g^{-1} on MA	CFU.ml^{-1} or .g^{-1} on BTB	CFU.ml^{-1} or .g^{-1} on TCBS
Hatching water t_0	10^3	10^2	$<10^2$
t^7	10^4	10^3	10^2
t_{20}	10^7	10^7	10^7
t_{24}	10^7	10^7	10^7
Hatched naupli			
before rinsing	10^7	10^6	10^5
after rinsing	10^6	10^5	10^5
Water surrounding rinsed nauplii	10^5	10^3	$<10^2$

Table III. Microbiological contamination in rotifer culture. Number of CFU.ml^{-1} medium or .g^{-1} rotifers (wet weight) on MA, BTB TCBS after 2 days incubation at 28°C. Comparison between semi-continuous and batch culturing

Sample	Semi-continuous culture		
	CFU.ml^{-1} or .g^{-1} on MA	CFU.ml^{-1} or .g^{-1} on BTB	CFU.ml^{-1} or .g^{-1} on TCBS
Rotifer culture water			
1-day-old (1 600l)	$4x10^4$	$2x10^2$	20
5-day-old (2 500l)	$4x10^4$	$4x10^2$	40
Rotifers			
before rinsing	$2x10^7$	$2x10^5$	$2x10^4$
5' freshwater rinsing	$5x10^6$	$2x10^5$	
seawater rinsing	$2x10^7$	$1x10^6$	$2x10^5$
	Batch-culture		
Master-culture water	10^5-10^7	ND	$<10^2$
10 l culture water	10^5	10^3-10^4	10^2-10^3
2m^3 culture water	10^4-10^5	10^3	10^2
Rotifers			
before rinsing	10^8	10^5	10^4
after rinsing	10^8	10^6	10^5

ND: Not determined

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Organochlorines in Different Fractions of Sediments and in Different Planktonic Compartments of the Belgian Continental Shelf and the Scheldt Estuary

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ABSTRACT

PCB levels in sediments (bulk and fraction <63 μm), suspended matter and zooplankton from the Belgian continental shelf of the North Sea and the Scheldt estuary were evaluated in relation to their organic carbon content, their lipid content and, for sediments, their particle size distribution. PCB accumulation mechanisms are discussed, considering the importance of direct contamination (adsorption onto the cell surfaces, absorption through the cell walls and partitioning into the cell lipids) for suspended matter and sediments, and of indirect contamination through the food for zooplankton. Geographical and seasonal variations are described.

INTRODUCTION

The ecotoxicological significance of organochlorine residues such as PCBs is a well-known fact. The contamination mechanisms of PCBs in marine and estuarine environments have been investigated during the past few years by various research teams. An equilibrium distribution of PCBs between water and suspended matter has been shown to be based on adsorption on cell surfaces and/or absorption into the cells and subsequent partitioning between the cell constituents (lipids) and the surrounding water (e.g. Bruggeman, 1982; Duursma *et al.*, 1986, 1989; Delbeke & Joiris, 1988).

For bulk sediments, adsorption on the organic and silt fractions has been

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