



1. GENERAL INTRODUCTION

Aquaculture has been practiced for centuries, but the industry, as we know it today, has grown dramatically in the last half century, and has matured into a major industry worldwide (Davenport *et al.*, 2003).

According to FAO statistics, aquaculture is one of the fastest growing food producing industries. Global aquaculture production is estimated at nearly 60 million metric tons annually, and contributes to more than 50% of the total consumed fish and shellfish (Subasinghe, 2006). Although the importance of the global aquatic production is unquestionable, in national terms, the relative importance of aquaculture varies considerably from country to country.

European sea bass, *Dicentrarchus labrax*, is one of the most economically important species in the Mediterranean mariculture. The species has been in the focus of research for the last three decades (Conides & Hunter, 2000), and several reports consider disease outbreaks as a significant constraint to the development of the sea bass aquaculture sector (Christiakov *et al.*, 2007).

Until recently, one of the most frequent approaches used to prevent and control the aquatic microbial environment, relied on the administration of chemotherapeutic and antimicrobial disinfectant compounds (Vine *et al.*, 2004). However, this practice promoted the emergence and dissemination of antibiotic-resistant bacteria, in both livestock and throughout aquaculture facilities. Drug-resistant bacteria are becoming increasingly difficult to avoid and eradicate (Marques *et al.*, 2005).

As a consequence, several alternative strategies to the use of antimicrobials in disease prevention, have been proposed, and have already been applied successfully in aquaculture production systems (Olafsen, 2004; Gómez *et al.*, 2007). Among the various options under scrutiny (immunostimulants, quorum sensing analysis, antimicrobial peptides, etc), one of the approaches, which is receiving considerable recognition within the aquaculture industry, is the use of beneficial probiotic bacteria (Verschuere *et al.*, 2000a; Irianto *et al.*, 2003).

Although promising results have already been obtained, mostly through empiric approaches, it is now obvious that further progress can only be expected through a more fundamental approach. A better understanding of the mechanisms involved in host-microbial interactions seems crucial (Tinh, 2007).

The gnotobiotic animal models, offer considerable potential as fundamental tools in the study of such host-microbial relationships (Gordon & Pesti, 1971).

The *Bijzonder Onderzoeksfonds* (BOF) research project, financed by Ghent's University – “Development of a gnotobiotic food chain for the study of host-microbiota interactions” – has as its main objective, a better understanding of the interactions between fish larvae and microorganisms, when fed live feed. A standard operating procedure is presently being developed, in order to establish such a gnotobiotic food chain for aquaculture research purposes.

In view of the complexity of such a programme, various proposals began to arise for the model system to be developed in three distinct parts.

This thesis in particular, represents a small step forward at a very early stage of the project. Considerable efforts were made in an attempt to combine the presently available information with the most feasible and effective procedures, so as to establish a standardized culture system for *D.labrax* gnotobiotic larvae (yielding predictable and reproducible results).

The techniques necessary to obtain and culture axenic micro-algae, baker's yeast (Marques *et al.*, 2004b), *Brachionus* (e.g. Rombaut *et al.*, 1999) and *Artemia* (e.g. Verschuere *et al.*, 2000b), being already available, the present study was designed in order to develop a reliable approach, which would provide the essential framework conditions to achieve healthy gnotobiotic sea bass larvae. In brief, an innovative methodology with appropriate standard procedures and holding apparatus, capable of supporting axenic hatching, and of enabling further successful larvae performance, under gnotobiotic conditions.