ECOLOGICAL SCIENCES SERIES

Aquatic Biotechnologies

From Genetic Engineering to Enzymatic or Fermentation Engineering

Joël Fleurence

ISTE

WILEY

Aquatic Biotechnologies

Aquatic Biotechnologies

From Genetic Engineering to
Enzymatic or Fermentation Engineering

Joël Fleurence

iSTE

WILEY

First published 2024 in Great Britain and the United States by ISTE Ltd and John Wiley & Sons, Inc.

ISTE Ltd
27-37 St George's Road
London SW19 4EU
UK

www.iste.co.uk

John Wiley & Sons, Inc.
111 River Street
Hoboken, NJ 07030
USA

www.wiley.com

Library of Congress Control Number: 2023949514

British Library Cataloguing-in-Publication Data
A CIP record for this book is available from the British Library
ISBN 978-1-78630-969-3

Contents

Preface

Since the end of World War II, humankind has been trying to cope with a growing population. The animal protein production system based on terrestrial animal husbandry soon proved insufficient to meet food needs generated by the demographic growth of many Asian countries such as China and India. This food security challenge has been largely met by the development of aquaculture, and in particular fish farming. The breeding of freshwater fish, such as tilapia and trout, is at the root of the worldwide boom in inland fish farming. In marine fish farming, Atlantic salmon, a diadromous species with anadromous behavior, has rapidly established itself as a development and production model for this type of aquaculture. Genetic engineering, a biotechnological process subject to much controversy, has enabled the production of transgenic fish from species commonly used in fish farming.

This book takes stock of the biological characteristics of the fish concerned, their traditional farming methods and the contribution of biotechnologies to the development of a new aquaculture described by its initiators as the "blue revolution".

However, the development of transgenesis is not limited to marine organisms of animal origin. More recently, genetically modified microalgae have been used to create humanized antibodies and recombinant vaccines. This biotechnological approach, which has been little developed at the industrial level, is nonetheless an interesting example of the application of transgenesis. As such, it is discussed briefly in this book.

Marine biotechnologies or blue biotechnologies are not limited to transgenesis applied to marine organisms, whether of animal or plant origin.

The application of technology to living organisms includes all the processes likely to change the characteristics of a biological matrix by modifying behavior, nutritional value or organoleptic quality.

In this context, this book also deals with enzymatic or fermentative transformation processes that can be applied to an animal matrix (fish fillets) or plant matrix (seaweed), in order to improve the nutritional or organoleptic added value of the final product. Particular emphasis is placed on the contribution of enzymatic engineering to the elimination of anti-nutritional compounds in seaweed and the development of new flavors in the case of fish fillets and seaweed.

In addition to the biotechnological aspects, the book also reviews the regulatory obstacles that may apply, depending on the country, to products derived from blue biotechnology and more particularly to genetically modified organisms.

More generally, the aim of this book is to take stock of aquaculture production methods incorporating transgenesis as a biotechnological tool and of biotechnological processing methods applied to marine resources, whatever their mode of production (fishing, traditional aquaculture, “blue revolution” aquaculture).

As such, it is particularly aimed at teacher-researchers, teachers and engineers working in the fields of aquaculture or seafood product valorization. It is also of interest to students and engineering students training in these fields.

November 2023

Introduction

Aquatic resources play an important role in the overall dietary intake of many populations. This is particularly true in China, where fish accounted for just over 21% of animal protein intake in the population diet in the early 2000s (see Figure I.1) (Tacon 2003). On the Asian continent as a whole, fish consumption accounted for 23% of dietary animal protein intake (see Figure I.1).

Since the early 1960s, the annual growth rate in fish consumption has been 3.1%, whereas the annual increase in the human population has been half that (1.6%) (FAO 2020). This annual increase in fish consumption is significantly higher than that of other sources of animal food proteins, such as meat, milk or their derivatives (+2.1%).

Today, this trend translates into a per capita consumption of 30–50 kg of fish per year in Asia (FAO 2020). Maritime Europe, including countries such as France, Spain and Scandinavia, shows a fish consumption rate comparable to those reported for the Asian continent (FAO 2020). In contrast, some countries such as Greenland, Norway and Iceland have fish consumption levels well in excess of 50 kg per capita per year (FAO 2020).

The inclusion of fish as a source of animal protein in the human diet has therefore helped to meet the demographic challenges facing the world's population. This food security challenge has been met by increasing fish catches, and also by the unprecedented development of aquaculture over the last four decades (see Figure I.2).

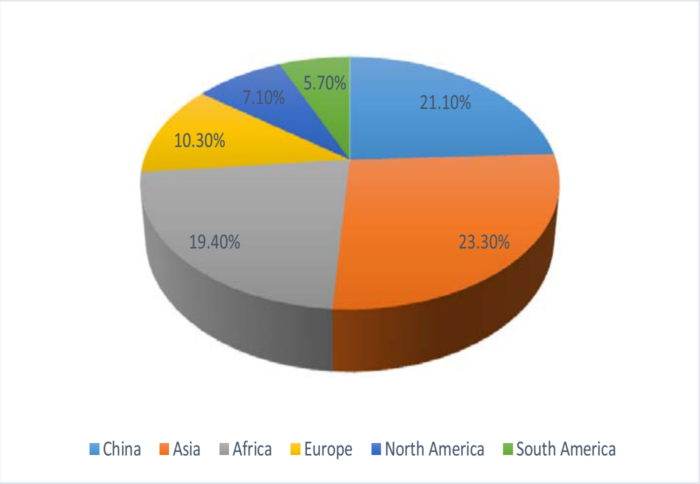

Figure I.1. *Distribution of animal protein intake through fish consumption in different regions of the world (according to Tacon (2003)). For a color version of this figure, see www.iste.co.uk/fleurence/aquatic.zip*

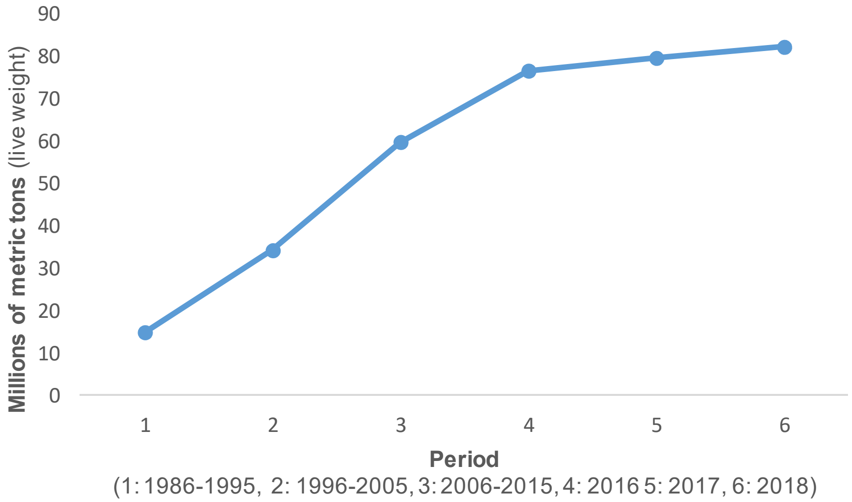

Figure I.2. *Trends over the last four decades for world aquaculture production of fish, including shellfish (according to FAO (2020))*

Fish production by aquaculture, also known as fish farming, today accounts for 52% of the volumes destined for human consumption (FAO 2020). The farming of freshwater species, known as continental fish farming, mainly produces various species of carp and one species of tilapia (Nile tilapia) (see Table I.1). Aquaculture production of marine species is distinctly classified as marine fish farming.

Fish species	Live weight tonnage (thousands of tons)
Grass carp (*Ctenopharyngodon idella*)	5704.0
Silver carp (*Hypophthalmichthys molitrix)*	4788.5
Nile tilapia (*Oreochromis niloticus*)	4525.4
Common carp (*Cyprinus carpio)*	4189.5
Atlantic salmon (*Salmo salar*)	2435.9
Rainbow trout (*Oncorhynchus mykiss*)	848.1
Marine fish	767.5

Table I.1. *World production in 2018 of the main fish species (according to FAO (2020))*

The rise of fish farming has been based on the breeding of species such as tilapia, carp and certain salmonids such as trout or salmon. Alongside traditional fish farming, a new concept called the "blue revolution" based on the genetic transformation of these species has also been developed over the past 30 years. Genetic engineering, a tool for transgenesis, can also be applied to other aquatic resources such as microalgae for the purpose of producing molecules of therapeutic interest, for example for antivirals (Fleurence 2021a).

This biotechnological approach involving marine or freshwater organisms is referred to as "blue biotechnology". However, it is not limited to genetic engineering applied to transgenesis. Indeed, the use of enzymatic or microbiological engineering to transform the nutritional or organoleptic properties of a marine food resource are also considered biotechnological approaches and are included in the concept of blue biotechnologies. The latter are notably applied to macroalgae or fish to improve the nutritional or taste qualities of products offered for human consumption.

1

The Biological Characteristics of Organisms Involved in Blue Biotechnologies

1.1. Fish

1.1.1. *Tilapia*

The genus *Tilapia* includes some 40 species from East Africa. In the vernacular, the term “tilapia” is used to designate species belonging to the genus *Tilapia* and also to the genera *Oreochromis* and *Sarotherodon*. Two species are mainly exploited for human consumption: Nile tilapia (*Oreochromis niloticus*) (see Figure 1.1) and Mozambique tilapia (*Oreochromis mossambicus*). Tilapia are freshwater fish with an omnivorous diet, but with a strong herbivorous tendency.

Nile tilapia is the most consumed species and contributes to the diet of many populations on the African and Asian continents. This species, raised in Ancient Egypt, was able, after introduction, to adapt to different ecosystems far from its continent of origin (Japan, Thailand, Vietnam, Brazil, China). It is a tropical fish that lives in shallow waters and has a strong aptitude for colonizing ponds and streams. However, the Nile tilapia is very sensitive to water temperature. Temperatures below 11°C and above 42°C prove to be lethal for this organism. Water depth is also a parameter that plays a significant role in mortality (El-Sayed et al. 1996). A depth of 200–300 cm reduces mortality observed for fish living at a depth of 50 cm by approximately 50% (see Figure 1.2).

This is an important criterion, particularly for breeding this species. In addition to habitat depth, temperature has a major influence on growth (see Figure 1.3). It also plays a role in the reproductive process, since spawning, after sexual maturation, is only possible when water temperature reaches 24°C.

Figure 1.1. Oreochromis niloticus *or Nile tilapia (photo credit © Tørrissen B.C. 2012, Wikimedia/CC BY-SA 3.0)*

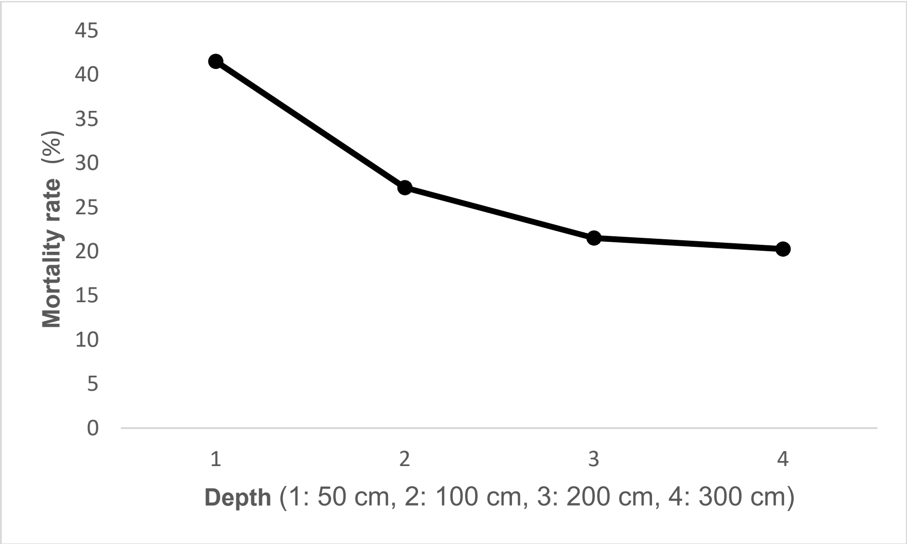

Figure 1.2. *Impact of pond depth on the survival of Nile tilapia (*Oreochromis niloticus*) (average over 10 months of rearing) (from El-Sayed et al. (1996))*

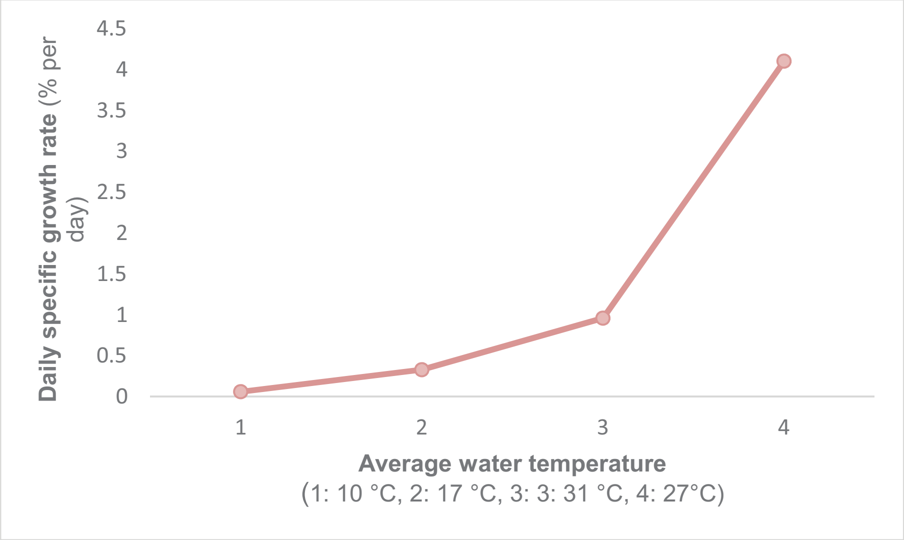

Figure 1.3. *Impact of pond temperature on the specific growth rate of Nile tilapia (*Oreochromis niloticus*) at a depth of 3 meters (from El-Sayed et al. (1996))*

1.1.2. *Trout*

"Trout" is the common name for several species of fish belonging to the Salmonidae group. Two distinct taxa or genera, the genus *Oncorhynchus* and the genus *Salmo*, are listed principally. A third, the genus *Salvelinus*, including the species *Salvelinus fontinalis*, known as speckled or blue trout, is included in the common trout group. The common trout found in whitewater rivers generally belong to the *Salmo trutta* species (see Figure 1.4). In contrast, rainbow trout, which is used worldwide in aquaculture, is listed under the scientific name of *Oncorhynchus mykiss*. Other trout species, or even subspecies, are also harvested or exploited in aquaculture but are more limited than the previous two species. They live in a wide variety of environments, including whitewater rivers, lakes and the marine environment (see Table 1.1). Like salmon, some trout species are anadromous. This is particularly true of rainbow trout (*Oncorhynchus mykiss*), which leave the marine environment to reproduce in freshwater rivers. Trout are carnivorous fish, feeding on aquatic and aerial insects and various invertebrates. Examples of cannibalism by trout towards their fry have also been reported for some species and under some conditions

(Lestage 1937). This is particularly true of *Salmo trutta* and *Salvelinus alpinus* (Arctic trout) (Klemetsen et al. 2003).

Figure 1.4. Salmo trutta fario *or common trout (photo credit © Engbretson E. 2006, Wikimedia/public domain)*

Trout species or subspecies	Common name	Living environment
Oncorhynchus mykiss	Rainbow trout or salmon	Well-oxygenated seas and rivers
Oncorhynchus mykiss aguabonita	Golden trout	High-altitude lakes and rivers (> 2,100 m)
Oncorhynchus clarkii	Cutthroat trout	Seas (Alaska) and rivers (Northern California)
Salmo trutta trutta	Sea trout	Seas (Atlantic and Mediterranean)
Salmo trutta fario	Brown trout	Mountain streams, rivers, brooks
Salmo trutta lacustris	Silver trout	Lakes
Salmo trutta ferox	Ferox trout	Lakes (Iceland, Scotland)
Salmo trutta labrax	Black Sea salmon	Sea (Black, Azov, Caspian and Aral seas)

Table 1.1. *Examples of different trout species and their habitats (based on FAO (2020) and Wikipedia)*

1.1.3. *Atlantic salmon*

The term "salmon" is applied to various species belonging to the Salmonidae group. There are three main genera (*Oncorhynchus*, *Hucho*, *Salmo*), with varying numbers of species (see Table 1.2). The genus *Salmo* is represented by one species, *Salmo salar* (see Figure 1.5), known by its common name of Atlantic salmon. This species occurs naturally in the northern Atlantic zone and along its watershed. However, the significant development of aquaculture activity around this species has led to its presence in fish farms in the Southern Hemisphere, such as in Chile, which is one of the three main producers of Atlantic salmon, along with Norway and the United Kingdom. The genus *Hucho* comprises two species found mainly in the rivers of eastern Europe and the Far East (Siberia). Finally, the genus *Oncorhynchus* comprises eight species found in the northern part of the Pacific Ocean and its watershed. Most salmon species are anadromous, similar to some trout species (see section 1.2).

Figure 1.5. Salmo salar *or Atlantic salmon (photo credit © Fjeld H.P. 2006, Wikimedia/CC BY-SA 2.5)*

Some species belonging to the genus *Oncorhynchus* may be known by more than one common name. This is particularly true of *Oncorhynchus keta*, which is known by four different common names (see Table 1.2).

Salmon is a carnivorous fish that feeds on small aquatic insects and mayflies in freshwater. In the marine environment, their diet is based on the predation of various crustaceans, including shrimps rich in astaxanthin, the pigment that gives salmon its red flesh. They also feed on fry or small fish of different species. Independently of this aspect, cannibalism is also known to exist in the *Salmo salar* species (Klemetsen et al. 2003).