OPTIMIZATION OF THE LARVICULTURE OF
THE TROPICAL FISH COBIA *Rachycentron canadum* IN VIETNAM

NHU VAN CAN
Promoter: **Prof. dr. Patrick Sorgeloos**  
Department of Animal Production, Faculty of Bioscience Engineering, Ghent University.  
*Patrick.Sorgeloos@UGent.be*

Local promoters:  
**Dr. Tran Mai Thien**  
**Dr. Le Thanh Luu**  
Research Institute for Aquaculture No.1  
*luuria1@yahoo.com*

**Members of the Examination and Reading Committee (*)**:

**Prof. dr. Herman Van Langenhove**, Chairman  
Department of Organic Chemistry, Faculty of Bioscience Engineering, Ghent University  
*(Herman.VanLangenhove@UGent.be)*

**Prof. dr. ir. Guy Smagghe**, Secretary  
Department of Crop Protection, Faculty of Bioscience Engineering, Ghent University  
*(Guy.Smagghe@UGent.be)*

**Prof. dr. ir Peter Bossier**  
Department of Animal Production, Faculty of Bioscience Engineering, Ghent University  
*(Peter.Bossier@UGent.be)*

**Prof. dr. Patrick Sorgeloos**  
Department of Animal Production, Faculty of Bioscience Engineering, Ghent University  
*(Patrick.Sorgeloos@UGent.be)*

*Prof. dr. Dominique Adriaens*  
Department of Biology, Faculty of Sciences, Ghent University  
*(Dominique.Adriaens@UGent.be)*

*Dr. Alireza Shiri Harzevili*  
*Agency for Nature and Forest, Ministry of Flemish Government, Belgium*  
*(alireza.shiriharzevili@lne.vlaanderen.be)*

*Prof. dr. Yngvar Olsen*  
Department of Biology, Norwegian University of Science and Technology NTNU, Trondheim, Norway  
*(yngvar.olsen@bio.ntnu.no)*

Dean: **Prof. dr. ir. Guido Van Huylenbroeck**

Rector: **Prof. dr. Paul Van Cauwenberge**
Nhu Van Can

OPTIMIZATION OF THE LARVICULTURE OF THE TROPICAL FISH COBIA *Rachycentron canadum* IN VIETNAM

Thesis submitted in fulfilment of the requirements for the degree of Doctor (PhD) in Applied Biological Sciences
Dutch translation of the title:

Optimalisatie van de larvale kweek van de tropische vis cobia *Rachycentron canadum* in Vietnam.

To cite this work:


The author and the promoters give the authorisation to consult and to copy parts of this work for personal use only. Every other use is subject to the copyright laws. Permission to reproduce any material contained in this work should be obtained from the author.


Cover picture: Cobia larvae 3 dph and umbrella-stage of *Artemia franciscana*.

This study was funded by the Belgian Technical Cooperation (BTC/CTB) under a mixed PhD program between the Laboratory of Aquaculture & Artemia Reference Center, Ghent University, Belgium and the Research Institute for Aquaculture No1, Vietnam.
Table of content and index

LIST OF FIGURES ........................................................................................................................................ III

LIST OF TABLES ........................................................................................................................................... V

LIST OF ABBREVIATIONS AND UNITS ...................................................................................................... VII

CHAPTER 1. INTRODUCTION ..................................................................................................................... 1

CHAPTER 2. LITERATURE REVIEW ............................................................................................................ 9

PART I: GENERAL REVIEW OF COBIA *Rachycentron canadum* IN AQUACULTURE ......................... 11

PART II: COBIA *Rachycentron canadum* AQUACULTURE IN VIETNAM: RECENT DEVELOPMENTS AND PROSPECTS ............................................................................................................. 23

CHAPTER 3. HUSBANDRY OF JUVENILES ............................................................................................. 45

EFFECTS OF DIFFERENT REARING DENSITIES, FEEDING FREQUENCIES AND DIETS ON GROWTH AND SURVIVAL OF COBIA (*Rachycentron canadum*) DURING WEANING ............................................................................................................. 47

ABSTRACT ..................................................................................................................................................... 49

1. INTRODUCTION ...................................................................................................................................... 51

2. MATERIALS AND METHODS .................................................................................................................. 53

3. RESULTS ................................................................................................................................................. 58

4. DISCUSSION ............................................................................................................................................ 63

ACKNOWLEDGEMENTS ............................................................................................................................. 66

CHAPTER 4. LIVE FOOD IMPROVEMENT ............................................................................................... 69

CAN UMBRELLA-STAGE *Artemia franciscana* SUBSTITUTE ENRICHED ROTIFERS FOR COBIA (*Rachycentron canadum*) FISH LARVAE? ........................................................................................................... 71

ABSTRACT ..................................................................................................................................................... 73

1. INTRODUCTION ...................................................................................................................................... 74

2. MATERIALS AND METHODS .................................................................................................................. 76

3. RESULTS ................................................................................................................................................. 82

4. DISCUSSION ............................................................................................................................................ 88

ACKNOWLEDGMENTS ................................................................................................................................. 92

CHAPTER 5. EARLY CO-FEEDING AND WEANING ............................................................................. 93
List of figures

Figure 1. 1. Global aquaculture production of marine fish and total marine products (FAO, 2009). .................................................................3

Figure 1. 2. Production and percentage of marine aquaculture products of difference continents in the world in 2007 (FAO, 2009). ..............................................4

Figure 1. 3. Percentage of production and value of marine aquaculture products from Asia in 2007 (FAO, 2009). .................................................................4

Figure 2. 1. 1. Cobia *Rachycentron canadum* Linnaeus, 1766 (Photo: Dao, M.S. collections) .................................................................13

Figure 2. 1. 2. Global captured production of cobia in 2007 (FAO, 2009). .................16

Figure 2. 2. 1. Global production and value of cobia (FAO, 2009). ..........................26

Figure 2. 2. 2. Comparison of the spawning quality (mean±SD) of the natural spawning (*n*=4) and the hormonal induction (*n*=6). .........................31

Figure 2. 2. 3. Growth pattern (mean±SD, *n*=30) and feeding regime of cobia larvae cultured in the intensive system. ........................................33

Figure 2. 2. 4. Flowchart of semi-intensive fingerling production of cobia. ...............34

Figure 2. 2. 5. Grow pattern (mean±SD, *n*=30) of cobia cultured in sea cages, in relation with seawater temperature. .................................................40

Figure 3. 1. Growth (body weight, g) of cobia fry fed different weaning diets ..........62

Figure 4. 1. Development of an *Artemia* cyst into umbrella-stage and instar I nauplius stage (Lavens and Sorgeloos, 1996). ...............................75

Figure 4. 2. Evidence of AF umbrellas in digestive tracts of cobia larvae. ...............82

Figure 4. 3. Feeding incidence of cobia larvae in first feeding stage .......................83

Figure 4. 4. Evidence of digested AF umbrella in the digestive tract of cobia larvae at day 3 post hatching. .................................................................83

Figure 4. 5. Evidence of undigested nauplii leaving the digestive tract of cobia larvae at day 4 post hatching. .................................................................84

Figure 5. 1. Daily mortality of cobia larvae in experiment 1 (A) and experiment 2 (B). .................................................................109

Figure 5. 2. Survival and mortality of cobia juveniles fed three different weaning diets. .................................................................112

Figure 5. 3. Daily mortality of cobia juveniles fed three different weaning diets. .......113

Figure 6. 1. Daily mortality of cobia juveniles (mean±SD, *n*=3) of different treatments in relationship with feeding regime. .................................134

Figure 6. 2. Relationship between DHA contents in formulated diets and survivals and cannibalisms of cobia juveniles. *n*=3. .................................135

Figure 6. 3. Survivals of cobia in the transportation test. ........................................140
## List of Tables

Table 2.2.1. The countries involved in cobia production in 2007 (FAO, 2009) .......27
Table 2.2.2. Fingerling production of cobia produced at RIA-1 facilities in Vietnam during 2003-2008. ..................................................................................................................37
Table 2.2.3. Production (in metric ton) of cobia produced in Vietnam during the recent years. ........................................................................................................................................39
Table 3.1. Growth, survival of cobia rearing at different rearing densities ........59
Table 3.2. Growth, survival of cobia cultured at different feeding frequencies. .......60
Table 3.3. Growth, survival and FCR of cobia fed different diets in the two rearing stages. .................................................................................................................................61
Table 4.1. Experimental feeding regime. .................................................................77
Table 4.2. Highly unsaturated fatty acids (% total fatty acids) of experimental live foods. .........................................................................................................................................85
Table 4.3. Growth and survival of cobia larvae at 8 dph and 18 dph. .........................86
Table 4.4. Salinity stress resistance (at 60 g L⁻¹) and deformity rates of cobia larvae at 8 dph and 18 dph. ......................................................................................................87
Table 5.1. Nutritional profile of the formulated weaning diets ................................102
Table 5.2. Environmental parameters in larval rearing tanks of the three experiments. ........................................................................................................................................105
Table 5.3. Growth performances and survivals of cobia larvae in two co-feeding experiments. .................................................................................................................................107
Table 5.4. Vitality of cobia juveniles (23 dph) in salinity stress test (60 g L⁻¹, 1 h). 110
Table 5.5. Growth performance and quality of cobia juveniles ................................111
Table 6.1. Fatty acid profile (mg g⁻¹ DW) and total lipids (% DW) of the diets for cobia juveniles in the experiment. ......................................................................................................131
Table 6.2. Growth performance of 30-dph cobia juveniles fed different diets. ........133
Table 6.3. Survival and mortality pattern of 30-dph cobia juveniles fed different diets. ........................................................................................................................................133
Table 6.4. Fatty acid profile (mg g⁻¹ DW) and total lipids (% DW) of cobia larvae at different development stages .................................................................136
Table 6.5. Fatty acid profile (mg g⁻¹ DW) and total lipids (% DW) of cobia juveniles in different treatments. ......................................................................................................................137
Table 6.6. Cumulative Stress Index (CSI) and survival of cobia juveniles in the vitality tests. .................................................................................................................................139
List of Abbreviations and Units

µg  Microgram
µm  Micrometer
cm  Centimetre
g   Gram
h   Hour
IU  International unit
L   Liter
min Minute
mm  Millimeter
mL  Milliliter
mt  Metric ton
ºC  Degree celcius
µL  Microliter
AF  *Artemia franciscana*
ANOVA Analysis of variance
ARA  Arachidonic acid
ARC  Artemia Reference Center
BTC/CTB Belgian Technical Cooperation
CSI  Cumulative Stress Index
CV  Coefficient of variance
DHA Docosahexaenoic acid
dph Day post hatching
DW  Dry weight
EFA  Essential fatty acid
EPA Eicosapentaenoic acid
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
</tr>
</thead>
<tbody>
<tr>
<td>FAME</td>
<td>Fatty acid methyl ester</td>
</tr>
<tr>
<td>FAO</td>
<td>Food and Agriculture Organization</td>
</tr>
<tr>
<td>FL</td>
<td>Fork length</td>
</tr>
<tr>
<td>GnRHₐ</td>
<td>Gonadotropin releasing hormone</td>
</tr>
<tr>
<td>HCG</td>
<td>Human chorionic gonadotropin</td>
</tr>
<tr>
<td>HDPE</td>
<td>High density poly-ethylene</td>
</tr>
<tr>
<td>HUFA</td>
<td>Highly unsaturated fatty acids</td>
</tr>
<tr>
<td>ind</td>
<td>Individual</td>
</tr>
<tr>
<td>LSD</td>
<td>Least significant difference</td>
</tr>
<tr>
<td>LC₅₀</td>
<td>Lethal Concentration for 50% of the population</td>
</tr>
<tr>
<td>LH-RHₐ</td>
<td>Luteinizing hormone-releasing hormone</td>
</tr>
<tr>
<td>Min</td>
<td>Minute</td>
</tr>
<tr>
<td>M</td>
<td>Mortality</td>
</tr>
<tr>
<td>NORAD</td>
<td>Norwegian agency for international development</td>
</tr>
<tr>
<td>NTNU</td>
<td>Norwegian University of Science and Technology</td>
</tr>
<tr>
<td>P</td>
<td>Statistical p-value</td>
</tr>
<tr>
<td>PL</td>
<td>Phospholipids</td>
</tr>
<tr>
<td>PUFA</td>
<td>Polyunsaturated fatty acids</td>
</tr>
<tr>
<td>RIA-1</td>
<td>Research Institute for Aquaculture No.1</td>
</tr>
<tr>
<td>SD</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>SGRₜ</td>
<td>Specific growth rate (calculated by body weight)</td>
</tr>
<tr>
<td>SE</td>
<td>Standard error</td>
</tr>
<tr>
<td>SPSS</td>
<td>Statistical Package for the Social Sciences</td>
</tr>
<tr>
<td>UAF</td>
<td>Umbrella-stage of <em>Artemia franciscana</em></td>
</tr>
<tr>
<td>USA</td>
<td>United States of America</td>
</tr>
<tr>
<td>VNN</td>
<td>Viral Nervous Necrosis</td>
</tr>
</tbody>
</table>
Chapter

Introduction
Introduction

During the last two decades, global marine aquaculture production has been increasing rapidly, reaching more than 34 million metric tons in 2007 (Fig. 1.1). Meanwhile, the aquaculture production of marine fish has been increasing relatively slow, compared to other marine products (Fig. 1.1).

![Figure 1.1. Global aquaculture production of marine fish and total marine products (FAO, 2009).](image)

The marine aquaculture production from Asian countries occupied more than 30 million tons, equivalent to 89% of global production in year 2007, followed by Europe (6%) and America (5%) (FAO, 2009) (Fig. 1.2). Although Asia is the leading continent in producing marine aquaculture products, marine fish production made up only 4.5%, while the value was 14.6% (Fig. 1.3). Therefore, marine finfish aquaculture needs more attention to develop to the full extent of the continent’s potential.
Figure 1.2. Production and percentage of marine aquaculture products of different continents in the world in 2007 (FAO, 2009).

Figure 1.3. Percentage of production and value of marine aquaculture products from Asia in 2007 (FAO, 2009).
The low aquaculture production of marine fish in Asia can be explained by the difficulty of their production technology. Marine fish is highly valuable seafood, containing high levels of HUFA and other nutrients. However, farming of marine fish needs high investment such as marine cages and feeds with high protein content. Furthermore, during the hatchery phase, live food production as well as enrichment technology or special diets are necessary for most of marine fish species. Marine fish larvae need live food for first feeding due to their digestive system that is not fully functional (Kolkovski, 2001). Moreover, they need supplementation of essential fatty acids such as DHA, since marine fish larvae cannot biosynthesise de novo nor from the precursor 18:3n-3 (Sargent et al., 1999b). Appropriate feed and feeding for marine fish larvae is species specific and remains major constraint in the present knowledge.

Cobia is an emerging candidate for marine aquaculture, since it gathers a number of advantages. For human consumption, cobia is favorable seafood due to its succulent flesh, high accumulation of fat that is suitable to prepare sashimi, frozen and smoked products (Shiau, 2007). In nature, cobia is a coastal pelagic species, widely distribute over tropical and subtropical waters, which facilitates farming propagation. This species also can reach more than 6 kg after the first year (Liao et al., 2004) and can be easily adapt to formulated diets for industrial-scale production. In addition, cobia has a high fecundity (Brown-Peterson et al., 2001) and can artificially-induced or naturally spawn in captive conditions (Arnold et al., 2002). Although research and development of cobia aquaculture has been conducted in a number of countries at commercial scale, the production was still relatively low (29,859 tons in 2007), occupying only 0.9 % of global production of marine fish (FAO, 2009). As a new marine aquaculture species, there are many technical issues on cobia aquaculture that still need further investigations.
Chapter 1

Fingerling production is an important part in the production of cobia and better fingerling quality is essential to improve profits (Miao et al., 2009). To date, shortage of high quality cobia fingerlings remained a bottle neck for further propagation (Schwarz et al., 2007a). Low survival has been reported during hatchery phase whether using extensive (Benetti et al., 2007; Liao et al., 2004) or intensive systems (Faulk et al., 2007b; Hitzfelder et al., 2006). On the other hand, cobia larvae are different from most marine species as they grow very fast, indicated by elevated oxygen uptake and high rates of nitrogen excretion (Feeley et al., 2007). Thus, optimization of feed, feeding and rearing conditions for larvae and early juveniles become the most important factors for the critical period when larvae grow and pass metamorphosis. In this particular case, rotifers as start diet may be too small and might not contain enough nutrients for the larval development, which may need to be substituted by better diets. Early co-feeding with appropriate formulated diets can be a solution to improve and balance the larval nutrition and to shorten the live prey feeding period. Meanwhile, the appropriate nutritional composition, especially fatty acid contents of the formulated diets for co-feeding and weaning will be crucial for development of cobia larvae and juveniles. In addition, the husbandry factors such as rearing density and feeding frequency during the weaning period might be important for growth, survival and for benefit of hatchery operation. Better growth, survival and quality of cobia during the co-feeding and weaning period will improve fingerling production and thus, satisfy requirements for grow out practices.

The goal of this PhD thesis was to improve growth, survival and quality of cobia during the hatchery phase. The main objective of the thesis was to evaluate the effect of compound feeds, feeding frequency and rearing density on the performance of cobia larvae and early juveniles, with focus on the co-feeding and weaning stage. The
thesis is organized into 7 chapters, covering a literature review, research on the substitution of enriched rotifers by umbrella-stage of *Artemia franciscana*, the possibility of early co-feeding, the effect of dietary DHA contents and DHA/EPA ratios, the effects of feeding frequency and rearing density in order to simplify the rearing protocol, optimize rearing conditions for better growth, survival and quality of cobia from hatching until 38 days post hatching (dph).

In chapter 1, a global overview of marine fish aquaculture in comparison with other marine aquaculture products and a general introduction of cobia are presented to explain the need and potential of cobia aquaculture development. The gaps in development and technology of larviculture are mentioned to determine the researches topics for improvement.

Chapter 2 is a literature study containing two parts. The first part reviews the researches and development of cobia aquaculture in the world, including background information of the species. The second part describes the present situation of research, development and prospects of cobia aquaculture in Vietnam.

Chapter 3 describes the research on the effect of different rearing densities and feeding frequencies on growth and survival of early cobia juveniles during weaning in order to investigate their optimal rearing density and feeding frequency in a recirculation system. In addition, a comparison of cobia fed minced trash fish, locally home-made moist diets or commercial weaning formulated diet is made. Proposals for better practices are suggested.

Chapter 4 mentions an approach in live food improvement for cobia larviculture using umbrella-stage of a small local strain of *Artemia franciscana* for substitution of enriched rotifers during the first feeding stage. Success of this research opens a
possibility to simplify the rearing protocol for cobia larvae, especially through a reduction of labour costs of live food production.

Chapter 5 examines the possibility of earlier co-feeding for cobia larvae from 8 dph and 13 dph compared to the control that normally starts at 18 dph, using two different formulated diets. In addition, the use of three different weaning diets was tested in order to select the appropriate diet for cobia weaning.

Chapter 6 focuses on evaluating the effect of diets with DHA contents and DHA/EPA ratios on performance and quality of early cobia juveniles during weaning. Detection of better growth, survival and quality of the juveniles fed high dietary DHA level and DHA/EPA ratio is discussed for further improvement of fingerling quality and quantity through dietary manipulation.

Chapter 7 sums up the results and conclusions of the researches conducted in chapter 3 to 6. Besides general discussions and the main conclusions from the PhD study, suggestions for future researches are also addressed.
Literature review
Part I:

General review of cobra *Rachycentron canadum* in aquaculture
1. Biology of cobia

*Taxonomy and morphologic characteristics*

Cobia, *Rachycentron canadum* (Linnaeus, 1766), is the only member of the family Rachycentridae. Adult cobia are characterized by an elongated, cylindrical body with a broad flat head. Their caudal fin is paddle shaped during the juvenile stage and becomes lunate and crescent in shape in the adult stage with a longer dorsal lobe compared to the ventral lobe (Fig. 2.1.1). Cobia scales are small and embedded in the skin. The coloration of the cobia is highly variable: uniform chocolate brown to bronze on the dorsal side and white to cream color ventro-laterally. The two lateral stripes from head to tail, clearly visible during the juvenile stage, may be present in the adult stage. The largest recorded was 62.2 kg and had a fork length (FL, measured from the tip of the snout to the end of the middle caudal fin rays) of 1,610 mm (Franks et al., 1999).

![Figure 2.1.1. Cobia Rachycentron canadum Linnaeus, 1766 (Photo: Dao, M.S. collections)](image)

*Figure 2.1.1. Cobia Rachycentron canadum Linnaeus, 1766 (Photo: Dao, M.S. collections)*
Natural distribution, habitat and nutrition:

According to Shaffer and Nakamura (1989), cobia has a worldwide distribution in tropical and subtropical oceans except the eastern Pacific. They are most abundant along the south Atlantic coast and the northern Gulf of Mexico. Cobia is a pelagic migratory species moving in both directions between the Gulf and the Carolinas.

In nature, cobia is normally found solitary or in small groups and they commonly associate with any structure in the water or large animals. They are opportunistic feeders targeting crabs, shrimp, squid and benthic fish (Holt et al., 2007b).

Life history in the nature

Most of cobia mature in the second year, when they reach more than 800 mm or more than 650 mm FL for female or male, respectively. The differences in size and age of both sexes at their first maturation may be related to geographical differences (Franks and Brown-Peterson, 2002). The sex ratio of the natural population was 1/2.7 (male/female) (Franks et al., 1999). The spawning season of cobia occurs from April through September and has a peak between May and July (Brown-Peterson et al., 2001; Franks and Brown-Peterson, 2002). The spawning location is 25-50 km offshore (Hassler and Rainville, 1975). Cobia has a high fecundity and is a multiple spawning species. In the south-east of the United States and the north-central of the Gulf of Mexico, cobia has an estimated spawning frequency of 5 days, while the ones from western Gulf of Mexico can spawn every 9-12 days (Brown-Peterson et al., 2001).

Cobia eggs are pelagic, contain a single, yellow oil globule and vary from 1.1-1.3 mm in diameter; the diameter may increase up to 1.3-1.5 mm after 12 h incubation (Franks
et al., 2001; Hassler and Rainville, 1975). The fertilized eggs are buoyant and the embryos are melamine pigmented and hatch after 23-27 h at 27-24 °C. The newly hatched larvae (3.5 mm) are planktonic and their yolk-sac is absorbed by 3 dph, when the larvae reach around 4.3 mm (Franks et al., 2001). The larvae metamorphose from mainly cutaneous mode of respiration to gill respiration as early as 11-15 dph depending on the water temperature (Benetti et al., 2007), changing their color from reddish brown to black, fins develop and become colorful and stripes appear on their dorsal side (Liao et al., 2001). The juvenile stage may start from 20 dph when larvae reach 16.4 mm standard length and their stomach is fully differentiated (Holt et al., 2007b). In nature, cobia juveniles have been found in estuaries, where salinity ranges from 12.0-19.1 g L⁻¹ and water temperature ranges from 27.5-32.2 °C (Hammond, 2001). According to Franks et al. (1999), cobia has a fast growth in length through age 2⁺ for both sexes and after which it becomes gradually slower. Females grow faster than males. The oldest cobia collected from nature was 9 and 11 years for male and female, respectively. The adults can be caught at a water depth from 2 to 200 m. Tagging studies indicated that cobia have strong homing instincts using estuarine areas for their migration activities (Hammond, 2001).

2. Natural population and fishing production

The age and size structure of the natural cobia population varied from 0-9 and 0-11 years for male and female and their average size was 952 and 1,050 mm FL, respectively (Franks et al., 1999).

Before 1962, only very small amount of captured cobia has been recorded in the United States. The production has been increasing, but is still occupying a very tiny portion of the total captured marine fish. Data from FAO (2009) indicated that 20
countries and territories have been involved in cobia capture with a stable production around 10,000 metric tons. In 2007, the captured production mainly came from 4 countries: Iran, Malaysia, The Philippines and Pakistan. They account for 73.8 % of the total captured production (Fig. 2.1.2). In the United States, the fishing production is less than 183 tons and cobia is well-known for recreational fishery (Hammond, 2001).

Figure 2.1.2. Global captured production of cobia in 2007 (FAO, 2009).

3. Researches on artificial reproduction of cobia

Hassler and Rainville (1975) were the first to propose the potential of cobia for aquaculture by describing hatching, rearing techniques and development of cobia until 131 days in laboratory conditions. Liao et al. (2001) reported the first success of cobia reproduction in Taiwan in 1994 and the technology for mass fry production was developed since 1997 (Liao et al., 2004). Since then, cobia has been considered as a
potential candidate for marine aquaculture. During the period 1998 – 2008, essential researches on larviculture and nursing of cobia have been intensively conducted. To date, artificial reproduction and grow out of cobia on commercial scale has been performed in a number of countries/ territories such as China, Taiwan Province of China (Liao et al., 2004), Vietnam (Nhu, 2005), Japan (Nakamura, 2007), the United States (Benetti et al., 2007), Mexico and Brazil (Benetti et al., 2008a), contributing to the global farmed production of nearly 30,000 metric tons in 2007 (FAO, 2009). In addition, researches and trials on reproduction and grow out have been started in France (Gaumet et al., 2007) and Indonesia (Wahjudi and Michel, 2007).

Breeding induction

Caylor et al. (1994) were the first using human chorionic gonadotropin (HCG) at 275 IU kg⁻¹ of body weight for successful ovulation of ripe, wild-caught cobia females held in a recirculation system. However, fertilization by using cryopreserved sperms did not occur. Franks et al. (2001) were successful in induced spawning with HCG injection for the wild-caught matured females. Hormonal breeding induction has been successfully conducted in Vietnam using 20 µg luteinizing hormone-releasing hormone (LH-RHa) kg⁻¹ female (Nhu, 2005) and in the United States using hormonal spawning aid GnRHₐ implantation at a dose of 100-300 µg female⁻¹ (Weirich et al., 2007). However, this technique might not be necessary since cobia broodstock can mature and spawn spontaneously in captive conditions such as in land-based spawning ponds with flow-through water (Liao et al., 2004; Weirich et al., 2006), outdoor tanks (Nhu, 2005; Weirich et al., 2006) or in recirculation systems (Arnold et al., 2002). The second generation has been maturing and spawning in recirculating systems with a tank volume of 25-42 m³ with photo-thermal conditioning. This
broodstock is more productive than the wild caught, as they can spawn every two weeks and extend the spawning period from March till November (Holt et al., 2007a,b) suggesting the possibility to extend and to control the breeding season for all year around egg production.

**General techniques of larval rearing and nursing**

At present, protocols have been developed and applied: intensive rearing in recirculation systems and extensive rearing in outdoor ponds. Green water with utilization of live food during first feeding stage followed by dry formulated diets is applied in both culture methods.

Extensive rearing in outdoor ponds was first conducted in Taiwan, based on production of natural plankton (Liao et al., 2004). In this method, the fertilized eggs were released directly into 5,000 m² ponds with green water and copepods. The rotifers and copepod nauplii collected from other ponds were provided as supplementation. This larviculture period terminated after 20 days, when larvae reached 0.2 g and had a survival of 5-10 %. The following nursing stage can be divided into three phases: (1) weaning and frequently grading (20-45 dph); (2) nursing in large ponds (>300 m³) with less grading (45-75 dph) and; (3) raising in outdoor ponds or inshore cages before transferring to the offshore cages for grow out (75-150 dph). Similar larval rearing trials in outdoor ponds have been described in Carolina (Weirich et al., 2004), the Americas and the Caribbean (Benetti et al., 2007). In these trials, 2-3 dph larvae were stocked in the ponds instead of fertilized eggs. Cobia larvae have a relatively high specific growth rate (12.5-19.2 % day⁻¹), but very low survival (0-8.5 % and 3 % after 5 weeks and 28 dph, respectively). The authors suggested the
weather conditions in Carolina, avian predation and cannibalism were the main reasons for the poor survival.

The primary intensive rearing method, firstly described by Faulk and Holt (2005), has been improved by Holt et al. (2007a). This rearing protocol based on artificial enriched live foods for first feeding and the use of recirculation systems. The enriched L-type rotifers can be introduced to the larvae from 2-8 dph followed by newly hatched *Artemia franciscana* nauplii from 6-10 dph. The unenriched EG *Artemia* can be fed from 8-12 dph, followed by enriched EG *Artemia* from 10-25 dph. Co-feeding with formulated diets from 18 dph and weaning from 22 dph will allow complete substitution of live foods by 25 dph. The presence of micro algae (*Nannochloropsis oculata* or *Isochrysis galbana* at a density of 100,000-120,000 cells mL$^{-1}$) during the live prey feeding period effectively improves growth and survival (Faulk and Holt, 2005). Use of algae paste has resulted in similar growth and survival compared to the live algae (Holt et al., 2007a; Schwarz et al., 2008). However, the algae effects need further investigation (Faulk and Holt, 2005). This intensive rearing method resulted in 25.7 % survival at 27 dph (Benetti et al., 2008a,b) and 10.4 % or 13.2 % at 43 dph or 29 dph, respectively (Faulk et al., 2007b).

*Cobia larvae nutrition and weaning*

Nutritional requirements are very important, as cobia larvae grow fast. Examination of the fatty acid and biochemical composition of eggs, yolk-sac larvae as well as ovaries from wild caught and captive females contained high levels of PUFAs with DHA, EPA and ARA accounting for approximately 80 % of total fatty acids (Faulk and Holt, 2003). This information provided initial guidelines for the nutritional requirements. In addition, a correlation of HUFA between the enriched live food and
in the body tissues of cobia larvae by 16 dph has been detected (Faulk and Holt, 2005). In the later developmental stage, growth and survival of early juvenile 25-67 dph can be effectively improved by increasing their dietary phospholipids (Niu et al., 2008). Higher dietary phospholipids resulted in remarkable changes in the plasma lipids and lipoprotein profile suggesting that this could modify plasma lipoprotein metabolism and lipids profile and that the optimal dietary phospholipids level may exceed 80 mg g⁻¹ dry matter.

To determine the digestive capacity of cobia larvae, a research on the ontogeny of the digestive system and selected enzymes from hatching up to 22 dph has been conducted by Faulk et al. (2007a). The digestive tract of cobia larvae differentiates into five histological distinct regions during 1-4 dph. Meanwhile, their mouth and anus opened at late 2 dph and most of their yolk had been absorbed by 3 dph indicating that they were ready for first feeding. The stomach is completely developed and differentiated into the cardiac, fundic and pyloric regions forming the characteristic Y-shape stomach by 20 dph (16.4 mm). The digestive enzymes (amylase, chymotrypsin, trypsin and lipase) are present after hatching and steadily increase after 8 dph, when the gastric glands start to be differentiated. The highest trypsin concentration was measured at 18 dph, while chymotrypsin and lipase continued to increase. Based on these results, Faulk et al. (2007a) suggested that it is possible to minimize the use of live food through early co-feeding and weaning to formulated feed at three weeks post-hatching. Presently, a combination of the morphological and physiological techniques with application of microarray technology allows examining thousands of genes at a specific moment in time to study the larval development in toto for better dietary strategies (Schwarz et al., 2007a).
Chapter 2 - Part I

Effects of biotic and abiotic factors in cobia larviculture

Rearing density is an important factor for commercial grow-out facilities. The optimal larval density for an experimental recirculation system for the first 21 days of larviculture was 5-10 larvae L\(^{-1}\) (Hitzfelder et al., 2006). A density of 2.5 weanling L\(^{-1}\) resulted in the best survival and production (Craig and McLean, 2005). From 76 dph, cobia juveniles can be grown in a recirculation system without effects on growth and survival at initial densities of 0.04 to 0.44 g L\(^{-1}\) (Webb et al., 2007).

Water temperature effect is a consequence of growth and energy budget of cobia. The species has the median-lethal low temperature at 12.1±0.36 °C, ceases feeding at 16-17 °C (Atwood et al., 2004), and has an optimal growth rate at 29-32 °C (Schwarz et al., 2007a). The juveniles (22 g) increase ingestion when the water temperature increases from 23 °C to 31 °C, but decrease at temperatures higher than 35 °C; the growth and feed conversion ratio are maximized at temperatures ranging from 27 to 29 °C (Sun et al., 2006a). Cobia showed their growth compensation when moved to 29 °C after being kept at 18 or 23 °C for 10 weeks. However, their specific growth rate did not exceed that of cobia held at 29 °C (Schwarz et al., 2007b).

The tolerance of cobia larvae to low salinity is age dependent and increases with their ontogeny. The 3 dph larvae can survive up to 90 % to an abrupt change of salinity in a range of 20.1-35.6 g L\(^{-1}\), but at 7-9 dph, they can tolerate a wider range of 7.5-32.8 g L\(^{-1}\), while the ones older than 13 dph can be reared in salinity as low as 15 g L\(^{-1}\) (Faulk and Holt, 2006). Cobia juveniles (120 dph) can be exposed to low salinity for a short period of time without mortality, but moderate to high salinity are required to sustain growth and health (Denson et al., 2003). Rearing in 5 g L\(^{-1}\) resulted in lower survival and growth, poor health with skin lesions, fin erosions and discoloration.
compared to the ones reared in 15 or 30 g L\(^{-1}\) (Denson et al., 2003). However, Resley et al. (2006) proved that the same size juveniles can be grown in these salinities without effect on growth and survival when supplemented chelated minerals and complete vitamin premix.

Ammonia can be problematic at relatively low levels for juvenile cobia, while nitrite can affect the juveniles at higher concentrations. Cobia juveniles (1.74 g) cease to eat at 0.62 mg L\(^{-1}\), start erratic swimming at 0.80 mg L\(^{-1}\) and have LC\(_{50}\)-96 h at 1.13 mg L\(^{-1}\) of NH\(_3\)-N. Similar effects occur at NO\(_2\)-N concentrations of 76.1, 88.8 and higher than 210 mg L\(^{-1}\), respectively (Rodrigues et al., 2007).

**Nutrition for on growing cobia**

Research on grow-out of cobia mainly focused on the effect of nutrition on their growth and quality. Craig et al. (2006) concluded that juvenile cobia can utilize a wide range of protein and lipid levels (6-18 % of lipids and 40-50 % protein in dry matter) without impacts on production characteristics, while Chou et al. (2001) calculated the optimum protein and lipid level for cobia should be 44.5 % and 5.76 %, respectively. Optimal dietary methionine, an indispensable amino acid for maximum growth and feed utilization of cobia juveniles was 1.19 % of dry diet in the presence of 2.64 % of dietary protein on a dry weight basis (Zhou et al., 2006). It is important that up to 400 g kg\(^{-1}\) fish meal protein can be replaced by defatted soybean meal and the optimum of this replacement for maximum growth should be 189.2 g kg\(^{-1}\) (Zhou et al., 2005). Taurine supplementation at 0.5 g per 100 g dry diet has been investigated for effectively replacement of 50-75 % fish meal protein by yeast-based protein (Lunger et al., 2007).
Part II:  

Cobia *Rachycentron canadum* aquaculture in Vietnam: Recent developments and prospects

Van Can Nhu $^{a,b}$, Huy Quang Nguyen $^{a,c}$, Thanh Luu Le $^a$, Mai Thien Tran $^a$, Patrick Sorgeloos $^b$, Kristof Dierckens $^b$, Helge Reinertsen $^{(c)}$, Elin Kjørsvik $^c$ and Niels Svennevig $^d$

$^a$ Research Institute for Aquaculture No1, Dinh-bang, Tu-son, Bac-ninh, Vietnam

$^b$ Laboratory of Aquaculture & Artemia Reference Center, Ghent University, 9000 Gent, Belgium

$^c$ Norwegian University of Science and Technology (NTNU), 7491 Trondheim, Norway

$^d$ Tropical Center, SINTEF Fisheries and Aquaculture, 7465 Trondheim, Norway

Paper presented in the Larvi’09: 5th fish and shellfish symposium
European Aquaculture Society
Belgium, September 2009
Abstract

The paper presents a review of the recent developments in research and production of cobia in Vietnam in hatching and cage farming, which have made Vietnam the 3rd largest producer of farmed cobia in the world. Conservative estimations for the 2007 production for the Asian-Pacific region exceed 35,000 metric tons, with remaining global production adding an additional 2,000 metric tons, while official farm production registered by FAO is considerably lower. Estimated 2008 production in Vietnam was 1,500 metric tons, following the major production of PR China and Taiwan Province of China. This review reports on various aspects of hatchery technology such as broodstock management, intensive and semi-intensive larval rearing, fry transportation as well as small-scale grow-out in wooden raft cages and large-scale in Norwegian style circular HDPE cages. Some of the prospects for accelerating future development of this species in aquaculture and challenges to be solved are also identified.

Keywords: Cobia, larviculture, nursing, fingerling production, grow-out
Introduction and background

Cobia has gained popularity as a good candidate for mariculture due to its rapid growth and white meat of versatile use (Shiau, 2007). Research on natural cobia populations commenced in the 1960’s in the United States (Franks et al., 1999; Joseph et al., 1964) and the potential of cobia aquaculture was first proposed by Hassler and Rainville (1975). However, already during the 1970’s cobia farming had started on a small-scale in Taiwan. During 1993-1995 annual aquaculture production was stagnant around 200 tons based on wild-captured fingerlings (Svennevig, Pers. Comm.). The first cobia reproduction took place in Taiwan in 1994 (Liao et al., 2001) and mass reproduction was achieved since 1997 (Liao et al., 2004).

Figure 2.2.1. Global production and value of cobia (FAO, 2009).
Table 2.2.1. The countries involved in cobia production in 2007 (FAO, 2009).

<table>
<thead>
<tr>
<th>Countries/Territories</th>
<th>Captured production</th>
<th>Aquaculture production</th>
<th>Total production per country</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bahrain</td>
<td>4</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Brazil</td>
<td>635</td>
<td>635</td>
<td></td>
</tr>
<tr>
<td>China</td>
<td></td>
<td>25,855</td>
<td>25,855</td>
</tr>
<tr>
<td>Eritrea</td>
<td>19</td>
<td>19</td>
<td></td>
</tr>
<tr>
<td>Iran (Islamic Rep. of)</td>
<td>1,528</td>
<td>1,528</td>
<td></td>
</tr>
<tr>
<td>Kuwait</td>
<td>38</td>
<td>38</td>
<td>38</td>
</tr>
<tr>
<td>Malaysia</td>
<td>1,719</td>
<td>1,719</td>
<td></td>
</tr>
<tr>
<td>Reunion/ Mayotte</td>
<td>6</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Mexico</td>
<td>150</td>
<td>150</td>
<td></td>
</tr>
<tr>
<td>Oman</td>
<td>109</td>
<td>109</td>
<td></td>
</tr>
<tr>
<td>Pakistan</td>
<td>2,253</td>
<td>2,253</td>
<td></td>
</tr>
<tr>
<td>Philippines</td>
<td>2,236</td>
<td>2,236</td>
<td></td>
</tr>
<tr>
<td>Qatar</td>
<td>224</td>
<td>224</td>
<td></td>
</tr>
<tr>
<td>Saudi Arabia</td>
<td>280</td>
<td>280</td>
<td></td>
</tr>
<tr>
<td>Senegal</td>
<td>163</td>
<td>163</td>
<td></td>
</tr>
<tr>
<td>Taiwan Province of China</td>
<td>546</td>
<td>3,998</td>
<td>4,544</td>
</tr>
<tr>
<td>United Arab Emirates</td>
<td>500</td>
<td>500</td>
<td></td>
</tr>
<tr>
<td>United States</td>
<td>80</td>
<td>80</td>
<td></td>
</tr>
<tr>
<td><strong>GLOBAL TOTAL</strong></td>
<td><strong>10,484</strong></td>
<td><strong>29,859</strong></td>
<td><strong>40,343</strong></td>
</tr>
</tbody>
</table>

Due to the rapid growth of cobia and its suitability for commercial volume productions, cobia aquaculture has become more and more popular. To date, research and development of cobia aquaculture has been initiated in over 23 countries and
territories. Half of them in the Asian-Pacific region, including Taiwan Province of China (Liao et al., 2001), Japan (Nakamura, 2007), The Philippines, PR China (Holt et al., 2007a), Indonesia (Wahjudi and Michel, 2007), Malaysia, Thailand, Singapore, Australia, India and Vietnam. The others include the United States (Benetti et al., 2007), Mexico, Brazil (Benetti et al., 2008a), France (Gaumet et al., 2007), Panama (Holt et al., 2007a), Belize, Guatemala, Cuba, Reunion/ Mayotte, Oman, United Arab Emirates and Iran. Statistics of FAO (2009) show that the global aquaculture production of cobia has been increasing rapidly from only 9 tons in 1997 to nearly 30,000 tons in 2007. Meanwhile, the volume from capture fisheries has remained stable, around 10,000 tons annually (Fig. 2. 2. 1). While cobia was produced in a number of countries in 2007, only figures from 3 countries/ territories including China, Taiwan Province of China and Reunion/ Mayotte were specified in the 2007 - statistics (Table 2. 2. 1). It is estimated by the authors that in 2008, Vietnam has produced 1,500 tons thus, being the third largest cobia producer in the world.

History of cobia aquaculture in Vietnam dates back to 1997 when research on cobia reproduction started, leading to the first successful production of about 12,000 fingerlings in 1999 at a marine hatchery located in Cat-ba Island, Hai-phong province. In 2002, the first commercial batches of more than 20,000 cobia fingerlings were produced under a project, co-funded by the Government of Vietnam and the Government of Norway. Since then, research on improvement of larviculture technology of cobia has resulted in better growth, survival and production. During 2008, more than 400,000 fingerlings were produced at a RIA-1 hatchery at Cat-ba Island in addition to a smaller number produced at a private hatchery at Khanh-hoa province.
Earlier farming of cobia – mainly in the southern Vung-tau region depended on imported fingerlings, but the more stable fingerling availability has now led to several larger fish farms to grow cobia – the Norwegian financed Marine Farms Vietnam being the largest. The production during 2009 from the latter company could reach 1,000 tons.

1. Hatchery technology

Hatchery technology of cobia generally involves broodstock conditioning and management, induced breeding, egg collection and incubation, larval rearing and nursing until fingerling stage.

1.1 Broodstock management spawning season

Details of cobia reproduction at marine hatchery scale in Vietnam were firstly described by Nhu (2005). Cobia used for broodstock are produced from hatchery juveniles and reared in sea cages. The breeders are electronically tagged for better management i.e. to keep breeding history and to eliminate inbreeding problems.

Cobia broodstock is fed raw fish at a daily feeding rate of 3-5 % of body weight. About 3 months prior to onset of spawning, a supplement of squid liver oil, vitamin and mineral-premix are given daily till the end of the spawning season. The first research on formulated diets for cobia broodstock was conducted by Nguyen et al. (accepted) by studying the effect of dietary essential fatty acid (EFA) levels on spawning performance and egg fatty acid composition. This study showed that the cobia broodstock fed formulated diets with similar proximate composition, but different n-3 HUFA levels (0.94-1.72 %, in DW), matured, spawned and had comparable eggs sizes, batch fecundity and batch larval production to those of
broodstock fed raw fish (RF) with n-3 HUFA level of 1.86 % DW. The fatty acid composition in broodstock diets influenced directly the fatty acid composition of the eggs (Nguyen et al., accepted).

Under captive conditions in sea cages in the North of Vietnam, cobia spawn naturally from late of April to July with a peak in May. However, in south central Vietnam, where the water temperature is high all year around, the spawning can be induced except during the monsoon (November – January). The short breeding season of cobia in cages in the North of Vietnam created difficulties in the production of fingerlings during the first development stage (1998-2005). Exceptionally, some matured breeders have been detected during the autumn (October - November) in 2004 and some spawns occurred in late 2006 resulting in a production of around 15 million larvae at the marine hatchery in Hai-phong (RIA-1). This investigation opened the possibility to expand the availability of cobia fingerlings.

1.2 Induced breeding and larvae production

In Vietnam, spawning of cobia can be obtained either naturally or by hormonal injection of matured breeders. For the natural spawning, the matured breeders ready for spawning are selected and transferred immediately from sea cages to the breeding tanks in a hatchery nearby for spawning. For the hormonal induction, the breeders need to be inspected periodically for gonad development by cannulation. Females with oocytes larger than 700 µm in diameter and males with condensed milt are selected and transferred to the hatchery for spawning. The selected broodstock are injected with LH-RHa at a dose of 20 µg kg⁻¹ female and 10 µg kg⁻¹ male. Generally, 2 females are paired with 3 males in a spawning tank of 72 m³ for one spawning batch. The fish normally spawn within 12-36 h after hormone injection.
A comparison of the two spawning methods was conducted based on the results of 10 spawns (6 spawns from hormonal induction and 4 natural spawns) in 2005. The natural and hormonal induction methods were implemented in normal conditions i.e. water temperature of 28.8±1.4 °C and 29.0±1.1 °C and salinity of 34.0±0.0 g L⁻¹ and 34.8±0.5 g L⁻¹, respectively. The results indicated that although the hormonal induction method had a lower spawning success, it resulted in similar fecundity and better spawning quality in terms of fertilization rate and hatching rate (Fig. 2.2.2). The lower fertilization rate and hatching rate of the natural spawning was probably caused by handling stress (when catching and transferring) during the ovulation. Thus, the hormonal induction method is more advantageous for hatchery planning and is at present widely applied.

![Parameters](image)

**Figure 2.2.2. Comparison of the spawning quality (mean±SD) of the natural spawning (n=4) and the hormonal induction (n=6) (Nhu et al., 2005).**

After spawning, the breeders are transferred back to the sea cages while their eggs are collected on a 500-µm mesh net, manually or automatically using airlift-collectors.
Incubation is conducted in 500-L cylindro-conical tanks at a density of 1,000-2,000 egg L$^{-1}$, using filtered sea water with fine aeration. The bad eggs accumulating on the bottom are discharged every 5-7 h in order to maintain good water quality. The incubation time depends on water temperature i.e. it takes 23-27 h to incubate at water temperature of 27-24 °C. The newly-hatched larvae should be collected and transferred to the larval rearing tanks as soon as they have hatched.

### 1.3 Larviculture and fingerling production of cobia

In Vietnam, there are two rearing technologies: intensive and semi-intensive have been developed for cobia larviculture. The intensive technology is conducted in the recirculating aquaculture systems using intensive live food production. This technology is mainly performed in a marine hatchery located in Nghe-an province (RIA-1 facility), which however has limited production capacity. The semi-intensive technology is implemented in outdoor ponds using partially natural zooplankton production and mainly performs in the larger facilities of RIA-1 located in Hai-phong province and in the private hatcheries located in Khanh-hoa province.

The protocol for intensive larviculture technology of cobia was established in 2002 and is mainly based on larviculture techniques of other marine finfish species i.e. using microalgae as green water technique (2-28 dph) and use of enriched rotifers (3-11 dph) for the first feeding followed by freshly-hatched *Artemia franciscana* (8-13 dph) and enriched *Artemia* nauplii (11-28 dph) (Fig. 2.2.3). The newly-hatched larvae are stocked at an initial density of 30-50 larvae L$^{-1}$ in order to maximize the live food use. The larval density will be reduced in the second week, when they are actively catching live preys. From 18 dph, formulated micro diets such as NRD®, Proton® (INVE Aquaculture NV, Belgium), Bio-Optima® (Denmark), or Otohime®
(Marubeni Nisshin Feed, Japan) are introduced. The micro diets can be solely used after co-feeding along with *Artemia* nauplii for more than 10 days. When the larvae reach more than 3 cm, they need to be graded every 5 or 7 days to avoid cannibalism. After 45-50 days of rearing, the juveniles reach more than 10 cm and should be transferred to the bigger tanks or hapas in sea cages for further nursing. Survival of cobia juveniles (8-10 cm) reared using this protocol varies from 5-17.5 % though some batches may even reach 30 % survival, compared to the semi-intensive rearing method, which have a survival of less than 3 % for the same size.

![Figure 2.2.3](image)

Figure 2.2.3. Growth pattern (mean±SD, *n*=30) and feeding regime of cobia larvae cultured in the intensive system (Nhu et al., 2005).

The semi-intensive rearing method in outdoor ponds has been developed since 2005 due to the high demand of cobia fingerlings and the limited facilities for the intensive production. The main steps of this rearing method are described in Fig. 2.2.4 including a combined use of indoor tanks and outdoor ponds to rear the larvae before
they are being weaned to formulated diets. Firstly, cobia larvae are pre-nursed in the indoor tanks, fed on rotifers for the first feeding stage. The next rearing phase in outdoor ponds with abundant natural zooplankton, mainly copepods, allows reducing live food production cost. Before introducing the larvae, the outdoor ponds, typically 500 m$^3$ in rectangular or hexagonal shape with sandy bottom, are filled up with filtered sea water and fertilized for natural zooplankton production. When most of the live foods in the ponds have been consumed by the larvae, zooplankton collected from other ponds, using automatic collectors or *Artemia* nauplii will be supplemented. As soon as the larvae reach more than 3 cm, they are transferred to tanks again for weaning and frequent grading to avoid cannibalism. When the juveniles reach 10-12 cm, they can be transferred to the bigger tanks or hapas in sea cages for further nursing for grow-out.

**Figure 2.2.4. Flowchart of semi-intensive fingerling production of cobia.**
The critical periods of cobia larviculture are mainly the first feeding stage, when the larvae start exogenous feeding, and the weaning stage, when they switch from live prey to dry diets. Research on improvement of the first feeding, co-feeding and weaning period was considered as the priority to improve larval growth, survival and quality.

As for the first feeding stage, shortening and replacement of the rotifer feeding period was targeted to simplify the rearing protocol. Although cobia larvae cannot be fed *Artemia* nauplii as a starter feed (Faulk and Holt, 2003), they were able to ingest and digest umbrella-stage of *Artemia franciscana* (UAF) as their first feeding (Chapter 4). Use of UAF resulted in lower growth and quality by 8 dph, but no significant differences were detected by 18 dph compared to cobia larvae fed enriched rotifers (Chapter 4). This finding is important for intensive larviculture of cobia as *Artemia* cysts are readily available and easy to store, making the use of UAF more convenient and more cost-effective than using enriched rotifers.

Early co-feeding of Proton® (INVE Technologies NV) from 8 dph revealed a little improvement of larval growth, but did not improve their survival (Chapter 5). Success of the trials on use of UAF and early co-feeding of Proton® opens a possibility to improve knowledge on cobia larvae nutrition, shortens the live prey feeding period and simplifies the rearing protocol. However, micro-diet digestibility and/or nutritional requirements of cobia are age dependent: the same experimental diet (formulated by INVE technologies NV) with higher levels of dietary protein (62 % DW), n-3 HUFA (35 mg g⁻¹ DW) and DHA/EPA ratio (2.5), but lower lipid content (10 % DW), did not result in any significant effect for the period of 8-23 dph; on the other hand, it effectively improved growth, survival and quality of the juveniles.
during period of 20-38 dph compared to the use of Proton® (Chapter 5). Therefore, development of the appropriate diets for early co-feeding and weaning needs further investigations.

In Vietnam, minced trash fish and then home-made moist diet (prepared from a mixture of minced trash fish, fish meal, rice meal, wheat flour, squid liver oil, mineral and vitamin premix) were earlier used as weaning diets. In 2005, a comparison of those diets and the commercial formulated diet (NRD®, INVE Aquaculture SA) clearly indicated the advantages of the commercial diet in terms of use, daily husbandry management, larval growth and survival improvement (Chapter 3). Then, the commercial micro-diets then were used instead of the local-made diets.

Cobia juveniles require high levels of dietary DHA and DHA/EPA ratio during weaning. The DHA content in larval tissues decreases from 41.29 to 10.33 mg g⁻¹ DW with their age from 0 to 12 dph, but the DHA/EPA ratio increases from 4.6 to 6.6, respectively (Chapter 6). High dietary DHA contents (ranging from 21.12 to 53.31 mg g⁻¹ DW) and DHA/EPA ratios (ranging from 3.6 to 6.0) for the juveniles in the period 12-30 dph resulted in a better specific growth rate (22.60 to 23.77 % day⁻¹), higher survival rate (53.11 to 69.22 %) and it effectively improved survival (66.7-100.0 %) in the transportation test for 36 h (Chapter 6). The results indicated that improvement of growth, survival and production through nutritional manipulation was feasible.

Besides the nutritional aspect, rearing density and feeding frequency during weaning were also considered as the important factors for hatchery-scale production. The experiments on different rearing densities (1, 2 or 4 weanling L⁻¹) and different feeding frequencies (continuous or every 2 h or 4 h) revealed that growth and survival of cobia juveniles are affected by the rearing density, but not by the tested feeding
frequencies; the lower density of 1 or 2 weanling L\(^{-1}\) resulted in better growth and lower cannibalism rate compared to the ones reared at 4 weanling L\(^{-1}\) (Chapter 3). A medium density of around 2 weanling L\(^{-1}\) is suggested for the present practice.

Table 2.2.2. Fingerling production of cobia produced at RIA-1 facilities in Vietnam during 2003-2008.

<table>
<thead>
<tr>
<th>Year</th>
<th>Semi-intensive production</th>
<th>Intensive production (^{(1)})</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>2003</td>
<td>0</td>
<td>22,500</td>
<td>22,500</td>
</tr>
<tr>
<td>2004</td>
<td>0</td>
<td>21,000</td>
<td>21,000</td>
</tr>
<tr>
<td>2005</td>
<td>120,000</td>
<td>25,400</td>
<td>145,400</td>
</tr>
<tr>
<td>2006</td>
<td>62,000</td>
<td>4,000</td>
<td>64,000</td>
</tr>
<tr>
<td>2007</td>
<td>365,000</td>
<td>35,000</td>
<td>400,000</td>
</tr>
<tr>
<td>2008</td>
<td>900,000</td>
<td>0</td>
<td>900,000</td>
</tr>
</tbody>
</table>

\(^{(1)}\) Production obtained from the limited facilities of the marine hatchery located in Nghe-an province (RIA-1).

Comparing the two rearing methods, the semi-intensive rearing method is simpler, low-cost and easy-copied and can lead to fast expansion of the production. Most of cobia fingerlings have been produced in the semi-intensive systems during period 2005-2008 (Table 2.2.2). However, this rearing method also has a latent risk such as pathogens due to most of the rearing conditions are not fully controlled. High mortality (up to 80 %) of cobia fingerlings produced in the semi-intensive production has been experienced at Quy-kim station in 2005 (Le and Svennevig, 2005). Attempts in reducing the risk have been conducted, i.e. pre-treatment of rearing water or collected zooplankton or pre-treatment of juveniles before re-stocking in concrete
tanks using formalin, but with limited success. Therefore, sooner or later, the intensive production should replace the semi-intensive production to avoid those risks.

Development of transportation techniques for cobia fingerlings was one of the priority researches during the period 2005-2008. At that time, the operation of large-scale cobia farms in south-central Vietnam required large amounts of fingerlings, while the hatchery technology in that area was extensive and haunted by parasite and disease history. In the closed-transportation system using 50-L nylon bags containing 20 L seawater, 30 L oxygen and maintaining temperature at 25 °C, the densities of 20, 40 and 60 juveniles (5 cm) L⁻¹ had survivals of 100, 80 and 60 %, respectively after 12 h (Le and Svennevig, 2006). Meanwhile, the open system using 1000-L tanks equipped with aeration to transport the juveniles (6-7 cm) at a density of 3 and 5 juveniles L⁻¹ for duration of 35 h at temperature of 23-24 °C resulted in survivals of 95 and 71 %, respectively (Le and Svennevig, 2006). The latter method, which in fact exists on trucks for live transport of spiny lobsters to China, has since then been used commercially, at a lower density (2-3 juvenile L⁻¹), and has successfully transferred 300,000 juveniles in 2007 and 460,000 juveniles in 2008 from the North to the Central-south.

2. Grow-out of cobia in sea cages

Cobia farming in Vietnam was initially conducted in simple, small-scale wooden raft cages installed in closed bays, using wild-captured fingerlings as seed and trash fish as feed. The hatchery-fingerlings then were imported from Taiwan or China before the locally produced production was available since 2002. In the North of Vietnam the wooden cages with typical dimension of 3x3x3 m are assembled in a raft of 4 cages or more and each family normally operates 1 to 4 rafts. Production yield from each raft
may vary from 1-1.5 tons per rearing cycle. The medium-scale farms (50-100 tons per cycle) are predominant in Vung-tau and Kien-giang provinces and also use wooden cages, which therefore has to be installed in closed bays or the lee side of islands. In a 2005 survey, there was a total of 16,319 marine cages producing approximately 3,510 tons of marine aquaculture products (Ministry of Fisheries and The World Bank, 2006). It is estimated that the annual production of cobia from family-scale farms is around 300-400 tons, which mainly is sold for local consumption. Meanwhile, the medium-scale farms contributed between 300-450 tons year\(^{-1}\) a large part of which was exported, making the total estimated production from wooden cages of around 900 tons in 2008 (Table 2.2.3).

**Table 2.2.3. Production (in metric ton) of cobia produced in Vietnam during the recent years.**

<table>
<thead>
<tr>
<th></th>
<th>2007</th>
<th>2008</th>
<th>2009 (^{(3)})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Production in wooden cages (^{(1)})</td>
<td>750</td>
<td>900</td>
<td>1,000</td>
</tr>
<tr>
<td>Production in HDPE cages (^{(2)})</td>
<td>250</td>
<td>550</td>
<td>1,600</td>
</tr>
<tr>
<td>Total</td>
<td>1,000</td>
<td>1,500</td>
<td>2,600</td>
</tr>
</tbody>
</table>

\(^{(1)}\) Calculated from small-scale and medium-scale (wooden cages) in Hai-phong, Quang-ninh, Thanh-hoa, Vung-tau and Kien-giang provinces.

\(^{(2)}\) Calculated from Marine Farm Vietnam and An-hai Ltd. Company

\(^{(3)}\) Prediction based on fingerling production

Success of the introduction of HDPE circular cages in 1999 by the project SRV 0330, co-funded by the Government of Norway and the Government of Vietnam opened the possibility to expand cage volumes and move culture areas to more exposed areas. In Phu-yen and Khanh-hoa provinces in the southern central, Nha Trang Pearl
(Taiwanese) started industrial level cobia production in 2003 followed by An-hai Ltd. (Russian) in 2005 and Marine Farms Vietnam (Norwegian) in 2006. In 2008, cobia production produced by those companies was about 550 tons (Table 2.2.3). Prediction of cobia production produced by Marine Farms Vietnam in 2009 is 700-900 tons of their full designed capacity of 4,000 tons (Wray, 2007). Thus, the production from HDPE-cages may reach 1,600 ton in 2009.

Figure 2.2.5. Grow pattern (mean±SD, n=30) of cobia cultured in sea cages, in relation with seawater temperature (Nhu et al., 2002).

Cobia grown in 300 m³ HDPE cages and fed trash fish can reach more than 5 kg in a year although growth ceases when water temperature is below 22 °C during winter (Fig. 2.2.5). However cobia fed extruded pellet feed (EWOS Ltd, Canada) gained double size (6.84 kg) compared to those fed trash fish which only reached 3.5 kg for a similar culture period of 13 months and culture conditions (Nguyen et al., 2008).
The FCR of the extruded feed was increasing from 1.2 to 1.8 and to 2.0, respectively to the cobia sizes of 0.16-1.2 kg; 1.2-3.7 kg and 3.7-6.8 kg; for the same size-stage (1.2-3.5 kg), trash fish had a higher FCR (2.4) on dry weight basis compared to that of the extruded feed (Nguyen et al., 2008). Extruded feed is currently used as sole feed in industrial-scale production of cobia in Vietnam.

3. **Prospects, major challenges and solutions for cobia aquaculture in Vietnam**

Cobia becomes more and more popular for marine aquaculture due to its rapid growth and suitability for industrial-scale farming. Vietnam has a potential for cobia farming and has had a very good start. The coastal line of 3,000 km and more than 4,000 islands creates huge potential areas for marine aquaculture (Ministry of Fisheries, 1994). The establishment of cobia larviculture and grow-out technologies is an essential foundation for the marine fish farming development. Cobia is one of the important aquaculture candidates in the recent priority for mariculture that the Government of Vietnam has issued in the overall strategy for coastal-economic development. However, there are some challenges that need to be solved for the sustainable cobia aquaculture development.

The main constraint of cobia farming in Vietnam is market development. In addition insecurity in supply of high quality juveniles and then some geographical or climatic constraints such as low temperature during winter in the North, and tropical typhoons occurring especially in autumn in northern central Vietnam. The main grow-out constraints would be parasites, bacteria and virus and feed quality and management to keep the FCR low.

Quality and quantity of cobia fingerlings affect the profit of cobia farming (Miao et al., 2009). At present, cobia fingerlings in Vietnam are produced mainly in the semi-
intensive systems. Although this rearing method is relatively simple, low-cost and easy-copied, there are some uncontrolled factors and it has been experienced to result in relatively low survivals (Benetti et al., 2007; Liao et al., 2004; Weirich et al., 2004) and the cobia fingerlings obtained from these systems have been reported to be of unstable quality. Thus, the intensive production needs to be developed at appropriate proportion to reduce risk and to ensure sustainable development. However, the present intensive rearing method is relatively expensive and sophisticated and there is a need to simplify the protocol to reduce the production costs. In this regards, to shorten the live prey feeding period, improve nutritional condition and hatchery zoo-techniques will be elements to improve growth, survival and quality of cobia fingerlings.

Low temperature during winter is one difficulty facing cobia farming in the North of Vietnam. Cobia ceases eating at water temperatures below 18 °C. During the abnormal weather experienced in January - February 2008, the low water temperature of 15 °C last for more than 5 weeks causing mass mortality of cobia in sea cages, including broodstock. Low temperature is normally associated with rough sea conditions, which influence feeding and farm management. In case these conditions last more than two weeks, problems of fungal and bacterial infections can be detected with occurrence of ulcerated spots and haemorrhages on the skin. It is suggested that maintaining high biomass in sea cages during winter in the North of Vietnam should be avoided.

Disease outbreak is another challenge for sustainable development of cobia aquaculture in Vietnam. During larval rearing, infections of protozoa such as Vorticella sp., Epistylis sp., Pseudorhabdosynochus epinepheli, Benedenia and Trichodina have been detected. Samples collected from sudden crashes of cobia
larviculture in RIA-1’s hatcheries revealed Viral Nervous Necrosis (VNN) infection of 20-30 % (Le and Svennevig, 2005). The vertical transmission (from cobra breeders) of VNN has been confirmed. The use of iodine and peroxide did not effectively eliminate VNN from fertilized eggs (Le and Svennevig, 2006). Therefore, quarantine and screening of the broodstock before the reproduction cycle is very essential to prevent the VNN vertical transmission. In addition, high mortality caused by *Amyloodinium ocellatum* attaching to gills and skin of cobra juveniles has been detected in RIA-1’s hatcheries in 2005 and 2006. High density of *A. ocellatum* in gills of cobra juveniles might inhibit breathing, lead to slow movement and cause high mortality as consequence. The formalin treatment at a concentration of 30-100 mL L\(^{-1}\) for 1 h with strong aeration or fresh water treatment can be effective in case the first symptom is detected in time.

Another constraint in cobra aquaculture in Vietnam is the lack of locally extruded feeds. At the moment, the large-scale farms still rely on imported extruded feeds, while the small-scale farms are mainly based on trash fish diet. High FCR and the dependency of imported feed supply are obstacles for cobra aquaculture development.

It is also important to mention the challenge of tropical typhoons in the areas. Vietnam belongs to south-east Asian region, situated in the western Pacific rim where is exposed to the tropical typhoons from the Pacific Ocean during autumn. Large-scale farms need to be situated in fairly open sea areas to maximize the production, but can also be exposed to harsh weather conditions. The failure of some cobra farms in the Northern central region during a typhoon in 2005 showed to be caused by insufficient dimensioning of the mooring system. At the moment, the Government of Vietnam are supporting development of new semi-submersible cages (National
project KC07/03-06/10), which can be controlled to sink temporarily to avoid surface damages during stormy conditions. Alternative grow-out systems such as land-based recirculation systems should also be considered to provide more options for the industry in regions haunted by typhoons.

Acknowledgments

The first author acknowledges the Belgian Technical Cooperation (BTC/CTB) for providing a scholarship under a mixed PhD program between the Laboratory of Aquaculture & Artemia Reference Center, Ghent University, Belgium and the Research Institute for Aquaculture No1, Vietnam. We acknowledge also Mr. Vu Van In (RIA-1 Cat-ba station), Mr. Cao Van Hanh (RIA-1 Quy-kim station), Mr. Huynh Van Buu (Kien-giang province) and local farmers for providing information.
Chapter 3

Husbandry of juveniles
Effects of different rearing densities, feeding frequencies and diets on growth and survival of cobia (Rachycentron canadum) during weaning

Van Can Nhu a,b, Kristof Dierckens a, Mai Thien Tran b, and Patrick Sorgeloos a

a Laboratory of Aquaculture & Artemia Reference Center, Ghent University, 9000 Gent, Belgium.

b Research Institute for Aquaculture No1, Dinh-bang, Tu-son, Bac-ninh, Vietnam.

Paper presented in the Aquaculture Conference
Asian-Pacific Chapter of the World Aquaculture Society
Hanoi – Vietnam, August 2007
Abstract

Cobia is a potential fish species for marine aquaculture due to its rapid growth rate and succulent flesh. In Vietnam, although the primary protocol for artificial reproduction has been established, the nursing stage in which the larvae can be weaned from live food into inert diets still remains as a critical period and needs more research to improve growth and survival.

Three experiments (each with three replicates) were conducted in 500-L tanks connected to a recirculation system to evaluate the effects of different rearing densities (EXP1: 1, 2 and 4 weanling L⁻¹); different feeding frequencies (EXP2: continuous, 7 times day⁻¹ and 4 times day⁻¹ feeding) and different weaning diets (EXP3: minced trash fish, moist home-made pellets and commercial diet (NRD®, INVE Aquaculture SA) on growth and survival of cobia larvae. The standard length of initial stocking size (mean±SD, mm) of cobia larvae in the EXP1, EXP2 and EXP3 were 13.0±0.9, 15.2±2.4 and 46.0±4.1 respectively. The trials were terminated when all cobia larvae were fully weaned with the inert diets (i.e. after 15 to 20 days).

Growth and survival of cobia larvae fed the NRD® diet in EXP3 were significantly higher than that of the cobia larvae fed the other diets (P<0.01), revealing an advantage of using a commercial diet during the weaning stage. The results also indicated that growth and survival of cobia larvae during this stage were strongly influenced by the different rearing densities but not by the different feeding frequencies. Growth and survival of cobia larvae in EXP1 were reduced by increasing the rearing density (P<0.05) while no significant difference (P>0.05) was found in the trial with different feeding frequencies. In addition, the effects of rearing density and feeding frequency on cannibalistic mortality and size variation were also
evaluated and these results are discussed as a potential method to further improve survival.

**Key words:** Cobia, weaning, density, feeding frequency, formulated diets
1. Introduction

Cobia is a potential candidate for mariculture and is seen as a potential competitor for salmon on the international markets. In Vietnam, the research on artificial seed production of cobia started in 1998 and has concentrated on the intensive production since 2002. The primary protocol for artificial seed production of cobia has been established, covering aspects of broodstock management, live food production as well as breeding induction and larval rearing. However, practice in recent years has also revealed a number of issues that need to be addressed for further research. The weaning period remains a critical stage in which, stocking density, feeding frequency as well as weaning diets are the main factors affecting growth and survival of the larvae.

In the intensive system, the larval rearing density is increased to maximize production. However, high density affects growth, mortality (Holm et al., 1990), digestive enzyme activity and cortisol level and thus affects feeding and stress (Bolasina et al., 2006). Besides, social interactions also was recorded to have an effect on cannibalism of carnivore species (Dou et al., 2000). The appropriate density is species specific and depends on its development stage as well.

Feeding frequency affects growth and survival of various marine finfish larvae such as Australian snapper (Tucker et al., 2006), Atlantic cod (Rosenlund et al., 2004), sea bream (Guinea and Fernandez, 1997), yellowtail flounder (Dwyer et al., 2002). On the other hand, feeding frequency has no significant effect on growth and survival of gilthead sea bream (Goldan et al., 1997), rohu and mrigal (Biswas et al., 2006b), walking catfish (Petkam and Moodie, 2001). Optimizing feeding frequency has been demonstrated to control size variation and thus reduce stress and labor cost due to
grading or hand sorting and reduce cannibalistic mortality (Dou et al., 2000; Goldan et al., 1997).

Weaning diets play an important role in growth and survival of marine larvae (Curnow et al., 2006; Kolkovski et al., 1997b,c; Rosenlund et al., 1997; Takeuchi, 2001; Yufera et al., 1999). Live food might not satisfy the nutritional requirements in terms of amount, size and nutritional value as larvae grow and the evidence of commercial weaning diets to support growth and development has been proven (Chang et al., 2006; Yufera et al., 2000). Weaning diets were also found to affect size variation within the rearing tank, which influenced rates of cannibalism (Curnow et al., 2006). However, weaning diets must be size-suitable, digestible especially in the beginning of weaning stage as digestive system of the larvae might not be fully functional to the change of live food to the formulated diet.

Cobia is a very fast growing carnivorous species and can utilize feeds with a wide range of protein and lipid levels without any impacts on production characteristics (Craig et al., 2006). The sibling cannibalistic behavior starts as early as metamorphosis and can be partly managed by optimizing rearing density, feeding frequency as well as nutritional composition of food (Liao et al., 2001). Information on effects of stocking density and feeding frequency will be very important for growth and survival improvement. In addition, in Vietnam, the weaning diet has been developed firstly from minced trash fish and the home-made moist diet was produced based on trash fish before using commercial micro diets. Although minced trash fish and moist home-made diets are simple, easy to produce and relative cheap, it is very essential to establish an effective rearing protocol for intensive production. The objective of this research therefore, was to compare the effects of different rearing
densities and different feeding frequencies as well as different diets on growth and survival of cobia in the weaning period under rearing conditions in Vietnam.

2. Materials and methods

2.1 Experiment design and cultivation

Three experiments were separately conducted in 500-L cylinder-conical bottom tanks connected to a biofilter system at Aquaculture Research Sub-Institute for North Central (ARSINC, RIA-1), Vietnam. Each treatment was conducted in three replicates, randomly distributed over nine tanks.

Experiment 1 (EXP1): Three rearing densities of 1 weanling L\(^{-1}\), 2 weanling L\(^{-1}\) and 4 weanling L\(^{-1}\) were tested using commercial micro diet NRD\(^\circledR\) (INVE Aquaculture SA) as feed with feeding frequency of 5 times day\(^{-1}\) (at 6 AM – 9 AM – 12 AM – 3 PM and 6 PM). The initial stocking size of cobia, expressed as standard length (mean\(\pm\)SD), was 13.0\(\pm\)0.9 mm, equivalent to a body weight of 0.021\(\pm\)0.004 g. The experiment was terminated after 15 days rearing.

Experiment 2 (EXP2): The feeding frequency was tested at three levels: continuous (from 6 AM till 18 PM), 7 times day\(^{-1}\) (at 6 AM – 8 AM – 10 AM – 12 AM – 14 PM – 16 PM and 18 PM) and 4 times day\(^{-1}\) (at 6 AM – 10 AM – 14 PM and 18 PM). The initial density was set at of 564 larvae tank\(^{-1}\) (1.13 weanling L\(^{-1}\)) and the initial standard length (mean\(\pm\)SD) was 15.2\(\pm\)2.4 mm equivalent to the body weight of 0.041\(\pm\)0.015 g. The experiment was terminated after 15 days rearing using NRD\(^\circledR\) as weaning diet.

Experiment 3 (EXP3): Three different weaning diets of minced trash fish, home-made moist diet and NRD\(^\circledR\) (a commercial weaning diet formulated by INVE Aquaculture
SA), were tested at a feeding frequency of 5 times day\(^{-1}\) (at 6 AM – 9 AM – 12 AM – 3 PM and 6 PM). Cobia larvae were stocked at an initial density of 200 larvae tank\(^{-1}\) (equivalent to 0.4 larvae L\(^{-1}\)). The initial standard length (mean±SD) was 46.0±4.2 mm equivalent to the body weight of 0.88±0.27 g. After first 10 days, cobia were counted and sorted. Only the average size groups were restocked at a similar density for all treatments and kept for another 10 days.

The larvae in all treatments were fed during day time for the whole rearing period. The supplementation of *Artemia* nauplii as co-feeding was gradually reduced and lasted for the first 5 days. In the beginning of the weaning period, the diets were manually introduced to the tanks 30 min prior of live food feeding. When the juveniles concentrated on the feeding, they were fed until satiation. Redundant feed, dead fish and other organic matters accumulated on the bottom were removed by siphoning daily. Environment parameters such as DO, pH, water temperature (Oxyguard), salinity (Refractometer) and ammonia (N-NH\(_3\) test kit) were monitored and registered daily.

### 2.2 Cobia larvae production and food preparation

Cobia larvae obtained from captive broodstock were fed rotifers enriched with Super-Selco from 3 days post hatching (dph) till 11 dph. *Artemia* nauplii enriched with DHA Selco were introduced from 9 dph onwards. After 18-20 days rearing, cobia larvae were graded for size uniformity before being counted and randomly allocated in the tanks for each experiment.

In the experiment 3, trash fish was minced after carefully washing in anolyte liquid (a positively charged oxidizing agent for disinfection) to prevent parasites from the wild.
The minced trash fish was fed directly using 500-µm plastic screen to create small particles and for equal distribution in the rearing tanks.

The home-made moist diet was prepared from a mixture of minced trash fish, fish meal, rice meal, wheat flour, squid liver oil, mineral and vitamin premix. The composition of the ingredients was adjusted to a certain level of crude protein and crude lipid. The moist diet was fed using the same method for minced trash fish diet as described above.

The commercial diets NRD® 2/3, NRD® 3/5 and NRD® 5/8 (INVE Aquaculture SA) equivalent to the particle sizes of 200/300 µm, 300/500 µm and 500/800 µm, respectively were used according to the fish growth and the product user’s manual.

2.3 Evaluation and calculation

Samples of the diets were preserved at -20 °C before analysis of the nutritional value. Proximate compositions of NRD®, minced trash fish and moist diet expressed in crude protein and crude lipid content were analyzed in the laboratory of RIA-1. The total protein content was calculated from total nitrogen content multiplied by 6.25 (Hamre and Mangor-Jensen, 2006), while total lipid was extracted in ether using a Soxlet device. The proximate water content of minced trash fish, moist home-made diet and NRD® 3/5 was 74.75, 45.07 and 7.75 %, respectively. The proximate crude protein of minced trash fish, moist home-made diet and NRD® expressed in percentage of dry weight basis were 85.99, 48.08 and 63.08 %, respectively and the proximate crude lipid of those diets above were 7.91, 6.60 and 10.17 %, respectively.

Growth of cobia, expressed in mean of standard length (mm) and mean of body weight (g), was determined by random sampling (n=30) and measure/ weight every 5
days. The daily specific growth rate (SGRw) was calculated using the following formula:

$$SGR_w (\% \text{ day}^{-1}) = 100 \times \frac{\ln W_f - \ln W_i}{t}$$

Where: $W_i$ and $W_f$ are mean of initial wet weight and final wet weight, respectively and $t$ is number of experiment days.

Size variation was evaluated according to Wang et al. (1998) in which the mean of three replicates of the coefficient of variation (CV) was used to examine the inter-individual length variation among the fish in each treatment:

$$CV (\%) = 100 \times \frac{\text{SD}}{\text{MSL}}$$

Where, MSL is the mean of standard length and SD is standard deviation of the fish in each treatment.

The condition factor (K) was calculated as formula:

$$K = 100 \times \frac{W}{L^3}$$

Where, $W$ is the body weight and $L$ is the standard length of the fish.

The natural mortality was determined from number of dead fish observed and removed daily from the tanks while cannibalism was calculated as described by Curnow et al. (2006). That is the percentage of the initial number of larvae that could not be accounted for mortalities as a result of sampling and natural mortalities during the trial and were calculated according to the following equation:

$$\text{Cannibalism} (\%) = 100 \times \frac{I - S - F - M}{I}$$
Where, $I$ is the initial larvae numbers stocked, $S$ is the number of larvae sampled for observation during the trial, $F$ is the final number of larvae in each tank and $M$ is the number of natural mortalities during the trial.

The gained biomass was calculated based on descriptions of Masser and Jensen, (1991) using the formula:

$$\text{Gained biomass (g L}^{-1}) = \frac{F \times W_g}{V}$$

Where, $F$ is the final number of larvae, $W_g$ is the average weight gain (g) and $V$ is the tank volume (L)

The feed conversion ratio (FCR) of the diet treatments were evaluated by the formula:

$$\text{FCR} = \frac{C}{B}$$

Where, $C$ is total feed consumption (g, converted to dry weight) and $B$ is total gained biomass (g).

2.4 Statistical analysis

All data of the treatments were tested for significant differences ($P<0.05$ or $P<0.01$) using one-way ANOVA followed by Duncan test for multiple comparisons of means. The data analysis was based on normality assumptions of ANOVA. The data are expressed as average ± SD and statistical analyzed was performed using SPSS version 13.0 and Microsoft Office EXCEL for Window.
3. Results

3.1 Effects of rearing densities on growth and survival of cobia

Effect of different rearing densities on growth of cobia is shown in Table 3.1. The mean of the final standard length of the larvae at three rearing densities were significantly \((P<0.05)\) different and the higher density culture resulted in lower growth (Table 3.1). Specific growth rate (SGR\(_{w}\)) expressed in percent of weight increase per day was significantly lower at high density compared to the low density indicating the trend of better growth performance when reducing rearing density. However, the gained biomass was significantly \((P<0.05)\) higher in the high density treatment compared to the medium and the low density ones. The condition factors were significantly different \((P<0.05)\) in all treatments and were positively correlated to the rearing densities. That means the fish were fatter in the high density or cobia, at this stage, seemed to develop length rather than weight in the lower density.

The coefficient of variation (CV) of the larvae in different rearing densities was relatively high and varied from 19.97 to 23.58 % (Table 3.1), but was not significantly different in all treatments \((P>0.05)\) showing that size variation of the larvae was not be effected by different rearing densities.

Survival of cobia larvae related to the natural and cannibalistic mortality rate. Survival of cobia juveniles in the low density treatment was significantly higher \((P<0.05)\) than that of the high density treatment. Survival of the juveniles at the medium rearing density was not significantly different from the others. The cannibalistic rate at the low density treatment (1 weanling L\(^{-1}\)) was the lowest compared to the treatments with the densities of 2 or 4 weanling L\(^{-1}\). The medium and high density cultures resulted in high cannibalistic mortality and were not significantly different from each.
Chapter 3

other \((P>0.05)\). The natural mortality rate was equal in all treatments \((P>0.05)\). Therefore, the mortality of cobia larvae in this trial was mainly based on cannibalism.

**Table 3.1. Growth, survival of cobia rearing at different rearing densities**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Low density (1 weanling L(^{-1}))</th>
<th>Medium density (2 weanling L(^{-1}))</th>
<th>High density (4 weanling L(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Growth pattern</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Final length (SL, mm)</td>
<td>44.0±2.3(^{a})</td>
<td>40.5±0.4(^{b})</td>
<td>37.1±1.2(^{c})</td>
</tr>
<tr>
<td>SGR(_{w}) (% day(^{-1}))</td>
<td>24.61±0.94(^{a})</td>
<td>23.47±0.23(^{ab})</td>
<td>22.15±0.68(^{b})</td>
</tr>
<tr>
<td>Coefficient of variation (CV, %)</td>
<td>19.97±1.35(^{a})</td>
<td>21.55±2.85(^{a})</td>
<td>23.58±1.58(^{a})</td>
</tr>
<tr>
<td>Gained biomass (g L(^{-1}))</td>
<td>0.31±0.08(^{b})</td>
<td>0.37±0.02(^{b})</td>
<td>0.56±0.12(^{a})</td>
</tr>
<tr>
<td>K-factor</td>
<td>0.74±0.13(^{c})</td>
<td>0.79±0.10(^{b})</td>
<td>0.84±0.11(^{a})</td>
</tr>
<tr>
<td>Survival and mortality pattern</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Survival (%)</td>
<td>59.67±10.34(^{a})</td>
<td>42.29±3.22(^{ab})</td>
<td>8.42±10.54(^{b})</td>
</tr>
<tr>
<td>Cannibalistic mortality (%)</td>
<td>17.67±4.04(^{b})</td>
<td>35.00±7.81(^{a})</td>
<td>35.67±3.51(^{a})</td>
</tr>
<tr>
<td>Natural mortality (%)</td>
<td>23.33±13.65(^{a})</td>
<td>23.33±6.66(^{a})</td>
<td>25.00±6.08(^{a})</td>
</tr>
</tbody>
</table>

Values (mean±SD, \(n=3\)) followed by different superscript letters within a row are significantly different \((P<0.05)\).

### 3.2 Effect of different feeding frequencies on growth and survival of cobia larvae

The different feeding frequencies did not have any effect on growth, survival and natural mortality of cobia juveniles, except for the cannibalistic mortality rate (Table 3. 2). The growth pattern of cobia juveniles in terms of final length, gained biomass, SGR\(_{w}\), coefficient of variance as well as condition factor, survival and mortality were not significantly different \((P>0.05)\) in all treatments. The cannibalism was relatively low in the continuous feeding treatments compared to the ones with the feeding
frequency of 7 times day$^{-1}$ or 4 times day$^{-1}$ ($P<0.05$). No significant difference of cannibalistic mortality between the feeding frequency treatments of 7 times day$^{-1}$ or 4 times day$^{-1}$ was detected.

Table 3.2. Growth, survival of cobia cultured at different feeding frequencies.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Continuous</th>
<th>7 times day$^{-1}$</th>
<th>4 times day$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Growth pattern</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Final length (SL, mm)</td>
<td>44.2±0.7$^a$</td>
<td>45.5±0.1$^a$</td>
<td>46.9±0.8$^a$</td>
</tr>
<tr>
<td>$\text{SGR}_w$ (% day$^{-1}$)</td>
<td>17.63±0.31$^a$</td>
<td>18.47±0.37$^a$</td>
<td>18.73±0.43$^a$</td>
</tr>
<tr>
<td>Coefficient of variation (CV, %)</td>
<td>17.60±5.60$^a$</td>
<td>17.76±4.53$^a$</td>
<td>25.27±2.42$^a$</td>
</tr>
<tr>
<td>Gained biomass (g L$^{-1}$)</td>
<td>0.39±0.04$^a$</td>
<td>0.43±0.04$^a$</td>
<td>0.36±0.15$^a$</td>
</tr>
<tr>
<td>K-factor</td>
<td>0.71±0.12$^a$</td>
<td>0.73±0.12$^a$</td>
<td>0.73±0.11$^a$</td>
</tr>
<tr>
<td><strong>Survival and mortality pattern</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Survival (%)</td>
<td>56.50±3.94$^a$</td>
<td>54.08±1.75$^a$</td>
<td>51.36±1.88$^a$</td>
</tr>
<tr>
<td>Cannibalistic mortality (%)</td>
<td>22.67±2.52$^b$</td>
<td>31.33±5.51$^a$</td>
<td>34.33±0.58$^a$</td>
</tr>
<tr>
<td>Natural mortality (%)</td>
<td>21.00±5.29$^a$</td>
<td>14.33±4.73$^a$</td>
<td>14.33±1.53$^a$</td>
</tr>
</tbody>
</table>

Values (mean±SD, $n=3$) followed by different superscript letters within a row are significantly different ($P<0.05$).

### 3.3 Effect of different diets on growth and survival of cobia

The experiment was divided into two rearing stages. To reduce variation of size, fish was sorted by hand reducing cannibalism effectively. The cannibalism and coefficient of variation was therefore, not included in this experiment evaluation.

The mean of N-NH$_3$ was below 0.012 mg L$^{-1}$ and was not significantly different in all treatments. This explains the effective operation of the biofilter system although the use of minced trash fish and moist diet resulted in deterioration of the water quality.
Table 3.3. Growth, survival and FCR of cobia fed different diets in the two rearing stages.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Stage 1 (first 10 days)</th>
<th>Stage 2 (last 10 days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NRD</td>
<td>MOIST</td>
</tr>
<tr>
<td>Final length (SL, mm)</td>
<td>79.5±0.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>62.8±1.6&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Gained biomass (g L&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>0.93±0.12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.30±0.03&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>SGR&lt;sub&gt;W&lt;/sub&gt; (% day&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>14.91±0.22&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.21±0.68&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>K-factor</td>
<td>0.78±0.15&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.79±0.13&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Survival (%)</td>
<td>76.83±9.07&lt;sup&gt;a&lt;/sup&gt;</td>
<td>67.83±5.50&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>FCR (dry weight basis)</td>
<td>0.53±0.08&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.90±0.15&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values (mean±SD, n=3) followed by different superscript letters within a row in each stage are significantly different (P<0.01). NRD, MOIST and Minced TF = Treatments fed NRD<sup>®</sup> (INVE Aquaculture SA), moist home-made and minced trash fish diets, respectively.
Figure 3.1. Growth (body weight, g) of cobia fry fed different weaning diets.

Values = mean±SD (n=3). NRD, MOIST and Minced TF = Treatments fed NRD® (INVE Aquaculture SA), moist home-made and minced trash fish diets, respectively.

The growth performance of cobia juveniles fed moist home-made diet and minced trash fish was similar (Fig. 3.1), no significant difference (P>0.01) in terms of final length, biomass gain as well as SGRw was detected for the whole experimental period. Cobia fed NRD® diet had a better growth performance after 10 days rearing: their final length, gained biomass and SGRw (P<0.01) were significantly higher compared to the ones fed minced trash fish and moist diet. After 20 days rearing, the juveniles fed NRD® reached 10.73 g, more than double of the ones fed minced trash fish (4.40 g) and moist home-made diet (3.89 g) (Fig. 3.1). The gained biomass in the treatment fed NRD® was three times the one fed minced trash fish or moist home-made diet (Table 3.3). The condition factor of the juveniles in the home-made moist-fed
treatment in the second stage was lower than the others ($P<0.01$) meaning the juveniles in this treatment tended to increase weight rather than length.

After the first 10 days rearing, the survival of the juveniles fed minced trash fish was significantly lower than that of the ones fed moist and NRD® diets ($P<0.01$). In the second stage, survival rates were higher compared to the first stage and were not significantly different ($P>0.01$) in all treatments (Table 3.3). As the juveniles have been fully weaned with inert feed and have been well adapted to the feeds and the culture conditions, high survival was obtained in the second stage.

The different weaning diets resulted in different feed conversion ratios (FCR). The home-made moist diet resulted in the highest FCR followed by the minced trash fish and NRD® diet. For the same diet, the younger juvenile stage had the lower FCR than the older stage (Table 3.3).

4. Discussion

4.1 Growth

The growth of cobia juveniles was strongly affected by rearing density and weaning diets, but was not affect by feeding frequency. High density of fish larval rearing has been discovered to increase cortisol level due to stress and reduces the growth rate (Bolasina et al., 2006). Cobia is not very resistant to stress and requires high DO as they are pelagic and active species (Liao et al., 2004). In fact, observation after feeding showed acute deterioration of water quality in the tank fed minced trash fish and moist home-made diet. However, this problem was solved effectively by recirculating water over a biological filter and by daily siphoning tank sediments.
Growth and survival of marine fish larvae post metamorphosis can be promoted by formulated diets (Cahu and Zambonino Infante, 2001; Curnow et al., 2006). The formulated weaning diets (NRD®) resulted in improved growth of cobia juveniles compared to the minced trash fish and moist home-made diet. At a size of more than 30 mm SL, the digestive system of cobia was functional with digestive enzymes present (Craig and McLean, 2005). As cobia is a rapid growing species, appropriate nutritional supplementation should be a key factor for growth (Craig and McLean, 2005). In fact, in Taiwan, minced Sergestidae shrimp has been successfully used for cobia after metamorphosis (Liao et al., 2001) and natural sardine fish has been illustrated to be a preferential diet for cobia juveniles at a size of more than 28 g (Sun et al., 2006b). In our trial with fixed feeding frequency of 5 times day⁻¹, although trash fish and moist home-made diet had a natural composition, the crude protein and lipid content (in wet form) were lower than those of the formulated diet (in dry form) and they may not satisfy the requirements of cobia at this stage. Moreover, size and palatability of dietary particles and the method of feeding may also affect the feed uptake and thus impact the growth (Curnow et al., 2006; Petkam and Moodie, 2001). NRD® prevailed, not only on nutritional quality, but also in digestibility and attractiveness compared to the two other diets. In addition, NRD® has the advantage of storability, size suitability, feeding management, reduction of the risk of introducing pathogens into the rearing tanks and maintenance of a high water quality. At satiation feeding, the FCR of NRD® was the lowest compared to the local-made diets (in dry weight basis). This result may not be only derived from its nutritional value. The moist pellets and minced trash fish based diet had a particle size that was too big for some juveniles at the start of the experiment. The bigger FCR in the early juvenile stage (the first 10 days rearing) compared to the late juvenile stage (the
second 10 day stage) was in agreement with a calculation from (Glencross, 2006) for the barramundi *Lates calcarifer*: at the size of 10 g, they may have FCR of 0.59, but at the size of 50 or 500 g, the FCR may increase up to 0.74 or 1.07, respectively.

The positive relation of the K-factor and the rearing density indicated that cobia larvae at low density rather increase in length than in weight at this stage. The cause of this observation should be addressed in future research.

### 4.2 Survival, size variation and cannibalism

Survivals in all treatments were mainly related to cannibalism, because the natural mortalities were small compared to the cannibalistic mortality and were not significantly different in all the experiments. Although the low density treatment (1 weanling L$^{-1}$) resulted in higher survival compared to the high density one (4 weanling L$^{-1}$), survival of the medium density treatment (2 weanling L$^{-1}$) was not significant different from the others. It also appeared that the biomass gained in the high density was significantly higher compared to that of the low and the medium densities. This creates possibilities to manage the rearing density in regard of maximizing survival and biomass production. The result is in agreement with Craig and McLean (2005) describing that the best production was obtained in recirculation systems at a density of 2.5 weanling L$^{-1}$ and can be applied for the present rearing conditions to maximize the production. Low survival in the treatment fed minced trash fish was probably due to the inappropriate size of the feed particles. It is more difficult to make small and similar particles of minced trash fish for equal distribution in the tank.

Size variation is an important factor as it relates to the cannibalistic rate (Dou et al., 2000). Increasing feeding frequency resulted in a more uniform size as reported by
Goldan et al., (1997) and Wang et al. (1998). In our treatments, although size variation was not significantly affected by feeding frequency or rearing density, the coefficient of variation was higher in the cultures with low feeding frequency and high rearing density. These results were consistent with those of Tucker et al. (2006) who studied the effect of feeding frequency on performance of newly weaned Australian snapper.

The cannibalistic mortality was affected by the rearing density and the feeding frequency. Continuous feeding and low rearing density resulted in reduced cannibalistic rate. Cannibalism is promoted by size differences (Baras et al., 2000a) and can be affected by exogenous factors such as stocking density and feed availability (Goldan et al., 1997; Wang et al., 1998). The cannibalistic rate of cobia during the weaning stage was relatively high (35.00±7.81 % after only 15 days at a density higher than 2 weanling L⁻¹), but it can be reduced by reducing rearing density (cannibalistic rate of 17.67±4.04 % at a density of 1 weanling L⁻¹) or by continuous feeding (cannibalistic rate of 22.67±2.52 %). The results suggested that for an intensive rearing system where high density cultures are applied, grading should be the most effective method to reduce the cannibalistic mortality.

Acknowledgements

The research was supported by the Norwegian Agency for International Development (NORAD) under the project SRV 0330 “Building Advanced Research, Education and Extension Capacity of the Research Institute for Aquaculture No1 (RIA-1)” and the Belgian Technical Cooperation (BTC/CTB) under a mixed PhD scholarship program between the Laboratory of Aquaculture & Artemia Reference Center (ARC), Ghent University, Belgium and RIA-1, Vietnam. The first author thanks Mr. Nicolas Mace
(University of Science of Montpellier, France) and Ms. Le Thi Thanh Thuy (RIA-1) for their assistance during the trial.
Live food improvement
Can Umbrella-stage *Artemia franciscana* Substitute Enriched Rotifers for Cobia (*Rachycentron canadum*) Fish Larvae?

Van Can Nhu a,b, Kristof Dierckens a, Thu Huong Nguyen b, Mai Thien Tran b, Patrick Sorgeloos a

a Laboratory of Aquaculture & Artemia Reference Center, Ghent University, 9000 Gent, Belgium

b Research Institute for Aquaculture No1, Dinh-bang, Tu-son, Bac-ninh, Vietnam

*Aquaculture 289 (2009) 64-69*
Abstract

Appropriate food of suitable nutritional value is crucial for first-feeding stages of the larvae of cobia *Rachycentron canadum*, a very fast growing marine fish species. Best survival and growth results in cobia larviculture have been reported with a starter diet of HUFA-enriched rotifers and – as mouth size permits – followed by freshly-hatched and eventually HUFA-enriched *Artemia* nauplii. Using the smaller-sized Vietnam *Artemia franciscana* (AF) strain instead of the Great Salt Lake *Artemia franciscana* strain, it has been shown that the rotifer-feeding period could be shortened with 3 days, resulting in significant improvements in larval survival and growth. This study verified the possibility to use umbrella-stage *Artemia* as food for further shortening and eventually completely substituting rotifer start feeding.

The experiment was conducted in 200-L tanks and lasted 18 days. AF-umbrella *Artemia* was used as sole feed during the whole rotifer feeding period (UAF), compared to the use of enriched rotifers for the first 2 days followed by AF-umbrella (ER+UAF) and the use of enriched rotifers as control (ER). The feeding incidence of UAF treatments was significantly lower \( (P<0.05) \) in the 1st feeding day, however, the ingestion and digestion of AF was evident. Growth and survival as well as deformities at day 18 post hatching were not significantly different for all treatments \( (P>0.05) \). The viability of cobia larvae after exposure to high salinity stress was lower in the ER treatment at day 8 post hatching, but higher at day 18 post hatching \( (P<0.05) \). The ability of cobia larvae to ingest and digest AF umbrella at first feeding as well as the nutritional suitability of AF umbrella are discussed. The possibility to use umbrella-stage *Artemia* opens an opportunity to simplify the rearing protocol and to reduce production costs of cobia larviculture.
Keywords: cobia, umbrella-stage, *Artemia*, larvae culture

1. Introduction

The tropical marine fish cobia (*Rachycentron canadum* L., 1766) has a broad distribution, a high flesh quality and is a rapid growing species, making it an excellent candidate for mariculture (Chou et al., 2001; Liao et al., 2004). Although artificial reproduction of cobia has been successful since 1994 in Taiwan (Liao et al., 2001), the seed production has mainly been based on extensive larval rearing in ponds (Liao et al., 2004), often resulting in unpredictable quality and quantity. Intensive production in closed recirculating system on a diet of rotifers and *Artemia* followed by weaning onto formulated feeds has resulted in an average larval survival of 13.2 % at day 29 post hatching (Faulk et al., 2007b).

Nutrition at first feeding is a key factor affecting growth and survival in most marine fish species (Koven et al., 1999; Rainuzzo et al., 1997; Rønnestad et al., 1999, 2003). Rotifers, freshly-hatched *Artemia* nauplii followed by nauplii enriched with specific lipids and vitamins are a very classic feeding regime for many fish species (Dhert et al., 2001; Sorgeloos et al., 2001). In very fast growing species, such as cobia, the larval diet must suit both energetic as well as nutritional requirements. Faulk and Holt (2003) and Benetti et al. (2007) have shown that freshly-hatched *Artemia* nauplii of the smallest *Artemia franciscana* (AF) strain should be offered at an earlier larval development stage of cobia i.e. from day 5 after onset of exogenous feeding. In this study, we want to explore if further improvements can be expected by starting *Artemia* feeding still earlier through the use of pre-hatched, so-called umbrella-stage *Artemia*, eventually allowing the complete replacement of rotifers, a diet which is
cumbersome to produce and prone to bacterial contamination (Battaglene et al., 2006; Øie et al., 1994; Vanhaecke et al., 1990).

Umbrella-stage Artemia can be collected during the hatching process of Artemia cysts, i.e. upon breaking of the cyst shell or chorion, the pre-nauplius larva, still surrounded by its hatching membrane protrudes from the shell and as a result of its specific buoyancy characteristics hangs underneath the empty shell, hence the name umbrella-stage. This cyst “breaking” process ends when the hatching membrane breaks and the free-swimming instar-I nauplius larva emerges (Fig. 4.1).

Figure 4.1. Development of an Artemia cyst into umbrella-stage and instar I nauplius stage (Lavens and Sorgeloos, 1996).

Among the Artemia strains recently used in aquaculture, Artemia franciscana produced in Vietnam (Clegg et al., 2000) has a small size and high eicosapentaenoic acid (EPA) content and was therefore selected to produce umbrella for use as feed for
the first feeding trial of cobia larvae. The idea of using AF umbrella, if successful, can open several opportunities: (1) simplify the larvae rearing protocol by eliminating the use of enriched rotifers; (2) alternative substitution feed in case of sudden crashes of rotifer production or shortage of rotifer production; (3) provide a more energy-rich food in comparison with enriched rotifers for the larvae during the development and (4) benefit from extra energy present in cysts that is lost during the hatching process and/or swimming activities of nauplii. The objectives of this research therefore, were to test the possibility of total or partial replacement of rotifers by using AF umbrella and evaluate its effects on growth and survival of cobia larvae at an early development stage.

2. Materials and methods

2.1 Experimental design

Three treatments were randomly allocated in nine 200-L fibre glass tanks (three replicates for each treatment) under indoor conditions at the Aquaculture Research Sub-Institute for North Central, Nghe-An Province, Vietnam. AF umbrella were used as sole food during the whole period (treatment 3 = UAF), compared to the use of enriched rotifers for the first two days followed by AF umbrella (treatment 2 = ER+UAF) and the use of enriched rotifers as control (treatment 1 = ER) (table I).
<table>
<thead>
<tr>
<th>Day after hatching</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
<th>13</th>
<th>14</th>
<th>15</th>
<th>16</th>
<th>17</th>
<th>18</th>
</tr>
</thead>
<tbody>
<tr>
<td>General rearing protocol (as applied for all treatments)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>N. oculata</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nauplii of AF <em>Artemia</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Enriched EG <em>Artemia</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dry feed NRD 2/3 (*)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment 1 (ER)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Enriched rotifers</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment 2 (ER+UAF)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Enriched rotifers</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AF umbrella</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment 3 (UAF)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AF umbrella</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(*) INVE Aquaculture SA, Belgium
2.2 Live food preparation

*Nannochloropsis oculata* and *Isochrysis galbana* were cultured in 15-L plastic bags following standard protocols (Lavens and Sorgeloos, 1996) in natural seawater at 22 °C. The *N. oculata* were added to the larvae tanks, while *I. galbana* was used as food for the rotifers after enrichment.

Rotifers (*Brachionus plicatilis* sensu strictu) were cultured in 500-L tanks using Culture Selco Plus and enriched in separate tanks using Protein Selco Plus (INVE Aquaculture SA, Belgium) for about 11 to 15 h according to the manufacturer’s protocol. After careful rinsing of the enriched rotifers with sea water on a 55-μm mesh, some were transferred to the larval rearing tanks, while the rest was stored for maximum 8h in 30-L fibre glass tanks at 10-12 °C with *I. galbana* at a density of 1x10⁷ cells mL⁻¹ in order to maintain optimal nutritional quality.

AF-brand *Artemia* cysts (INVE Aquaculture SA, Belgium) were disinfected and incubated at 28-30 °C in 33 g L⁻¹ seawater under continuous light and strong aeration (Lavens and Sorgeloos, 1996). After 10-12 h incubation, samples were taken to check the breaking stage of the cysts and the presence of umbrella. In case more than 50 % of the cysts had reached umbrella stage, they were collected on 70-μm mesh, washed with seawater and stirred for 15 minutes in a 2-L beaker using a magnetic stirrer. The umbrellas were separated by sedimentation in a cylindro-conical bottle, while unhatched cysts and empty shells, floating at the surface, were collected and re-incubated. This procedure was repeated every hour and lasted for 2-3 h (depending on the cyst quality and water temperature). The collected umbrellas were either directly used or stored at 5-7 °C for maximum 5 days. Since *Artemia* umbrella sediment
quickly, they were kept in suspension in aerated plastic bottles installed above the larval fish tank from where they were added via a valve-operated dripping system.

EG-brand *Artemia* cysts (Great Salt Lake USA, INVE Aquaculture SA, Belgium) were disinfected and incubated as described above (Lavens and Sorgeloos, 1996). The nauplii were enriched with A1 DHA Selco (INVE Aquaculture SA, Belgium) for 24 h following the manufacturer’s protocol.

### 2.3 Larval rearing

Newly-hatched cobia larvae obtained from captive broodstock fed experimental pellets (EWOS Ltd, Canada, containing 1.8 % HUFA), were stocked at a density of 50 ind. L⁻¹ in 200-L cylindro-conical tanks equipped with aeration and light in an air-conditioned room. The photoperiod was set at 10h : 14 h (dark : light). All tanks were connected to a bio-filter (modified from (Menasveta et al., 2001)), a protein skimmer and UV treatment system to maintain stable water quality, i.e. salinity 33.0±0.5 g L⁻¹, DO 6.37±0.20 mg L⁻¹, water temperature 28.0±0.1 °C and pH 8.0-8.2. After 4 days post hatching (dph), water was exchanged at an initial flow rate of 1 L.min⁻¹ and gradually increased up to 4 L min⁻¹ by 12 dph. A 500-μm filter cylinder ensured removal of uneaten rotifers and *Artemia* umbrellas. The effluent water was drained over a 55-μm mesh followed by a 22-μm mesh filter to remove rotifers and *Artemia* before the inlet of the bio-filter. The removal of uneaten rotifers and *Artemia* and regularly replacement by the new ones, ensured high quality live food in the rearing tanks.

From 2 dph, *N. oculata* were added to the larval tanks at an algal density of 1x10⁵ cells mL⁻¹. Enriched rotifers or AF umbrella (according to the different treatments) were added from 3 dph at a starting density of 5-7 ind. mL⁻¹ or 0.1 ind. mL⁻¹,
respectively and remained at a density of 10-12 ind. mL⁻¹ and 0.5-2 ind. mL⁻¹, respectively, during the following days. Live food (algae, rotifers, *Artemia*) was added four times a day to keep the density constant. Environmental factors such as DO, pH, water temperature (OxyGuard Hundy Gama) and salinity (refractometer model FG-211) were inspected twice a day (7 am and 2 pm). Every two days, the bottom was siphoned to remove accumulated organic matter. The experiment was terminated after 18 days rearing.

2.4 Sampling and fatty acid analysis

The enriched rotifers and enriched nauplii of EG-brand *Artemia* were collected in the interval of feeding times (two hours after harvest from enrichment tanks) on a sieve of 55 μm (for rotifers) and 110 μm (for *Artemia* nauplii) and rinsed carefully with sea water, followed by extensive washing with distilled water. Excess water was removed by putting the net on tissue paper. Subsequently, the samples were put in 2-mL plastic tubes, submerged in Liquid Nitrogen Biological Container (YDS-3, -196 °C) before being stored in a freezer at -80 °C for fatty acids analysis.

The fatty acids were analyzed according to the standard protocol described in Coutteau and Sorgeloos (1995) and were expressed in mg g⁻¹ dry weight.

2.5 Evaluation of feeding, growth, survival and viability

The size of rotifer loricae (Fu et al., 1991), AF umbrella, as well as fish larval mouth opening (jaw length) at first feeding were measured under a microscope to compare and check for ingestion at first feeding of the cobia larvae. Feeding incidence was evaluated by examining 20 cobia larvae collected randomly 30 minutes after the last feeding daily. The sampling continued until all larvae had fed.
Larval length (using a stereo microscope at a magnification of 1.8x10x; for the size bigger than 5 mm using a Mitutoyo ruler No. 8355) and larval density (volumetrically counted at night time using 250 or 500-mL beakers) were randomly sampled (n= 20) and measured at 3 dph (first feeding), 8 dph (completion of different feeding regimes) and 18 dph (start weaning period) to evaluate growth and survival in all treatments. The samples were also visually inspected under the microscope for deformities.

The quality of the cobia larvae was estimated by the Cumulative Stress Index (CSI) and mortality rate (MR) upon exposure of the larvae to a high salinity shock (modified from (Dhert et al., 1992; Dhert et al., 1990) at 8 dph and 18 dph. The right salinity concentration was determined in a range-finding test of 40, 50, 60, 70, 80, 90 and 100 g L\(^{-1}\) in which at least 50 % of the larvae died within one hour. Water was taken from the rearing tank to avoid any changes in water temperature and NaCl was added to adjust the salinity to the desired concentration. For evaluation, 10 larvae were randomly sampled from each cultured tank and put in a 500-mL beaker using 3 replicates for each tank. The number of dead larvae in each beaker was counted every 3 minutes for 60 minutes. The average of CSI and MR of each treatment was calculated from the replicates of each tank, the higher CSI and MR, the lower the quality of the larvae.

2.6 Statistical analysis

Feeding incidence, growth, survival and viability of cobia among the treatments were tested for significant differences (\(P<0.05\)) using one-way ANOVA followed by a Fisher LSD test for pair comparisons of means using SPSS version 13.0 and Microsoft Office EXCEL for Windows. The data were expressed as mean ± S.E.M
3. Results

3.1 Size suitability and digestibility of AF umbrella Artemia at first feeding of cobia larvae

At first feeding, the cobia larvae were 4.9±0.2 mm with a mouth opening of 551.0±9.7 μm. AF umbrellas were 219.4±2.0 μm in width and 378.4±3.0 μm in length, while the rotifer loricae were 100.8±3.1 μm, respectively 148.6±4.2 μm. Although AF umbrellas were bigger than rotifers, they were more uniform in size and early detection of AF umbrellas in the digestive tract (Fig. 4. 2) confirmed their ingestion.

Figure 4. 2. Evidence of AF umbrellas in digestive tracts of cobia larvae.
Figure 4.3. Feeding incidence of cobia larvae in first feeding stage.

Values (average±S.E.M) followed by different superscript letters at the same age are significantly different ($P<0.05$). ER = Enriched rotifers; ER+UAF = Enriched rotifer followed by AF umbrella; UAF = AF umbrella.

Figure 4.4. Evidence of digested AF umbrella in the digestive tract of cobia larvae at day 3 post hatching.
On the first day of exogenous feeding, the feeding incidence in the UAF treatments (0.08±0.02) was significantly ($P<0.05$) lower than in the ER (0.31±0.03) and ER+UAF (0.29±0.04) treatments. As of the next days, feeding incidence in all treatments were not significantly different ($P>0.05$) although at 4 dph, feeding incidence of UAF treatments was still lower (0.38±0.06) than in ER (0.50±0.09) and ER+UAF (0.55±0.00) treatments. As of 5 dph, about 70 % of the larvae in all treatments were found with full stomachs (Fig. 4. 3). However, in all treatments some larvae had empty stomachs till 6 dph. Digestion of AF umbrella *Artemia* was evident (Fig. 4. 4), while some of the newly-hatched *Artemia* nauplii (developed from uneaten AF umbrella) were found passing through the digestive tract without being digested (Fig. 4. 5).

Figure 4. 5. Evidence of undigested nauplii leaving the digestive tract of cobia larvae at day 4 post hatching.
3.2 Fatty acid profile of the live feed

The percentage of selected essential fatty acids of AF umbrella, enriched rotifers, newly-hatched (Instar I) and enriched meta-nauplii of *Artemia* are presented in Table 4.2. The essential fatty acids, including eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) and other highly unsaturated fatty acids of the enriched rotifers, reflect the lipid composition of the enrichment products. AF umbrellas, AF nauplii and enriched EG meta-nauplii were very low in DHA and arachidonic acid (ARA). AF umbrella contained mainly EPA at 8.78 % of total fatty acids (Table 4.2) equivalent to 17.33 mg g⁻¹ dry weight. The EPA content, however, decreased as umbrella developed into nauplius. Although the DHA content of enriched EG meta-nauplii was as high as 6.67 %, the percentage of total HUFA was not different from the levels found in AF nauplii. The total HUFA content in AF umbrella was lower than that of the AF nauplii, enriched EG nauplii and much lower than that of the enriched rotifers.

**Table 4.2. Highly unsaturated fatty acids (% total fatty acids) of experimental live food.**

<table>
<thead>
<tr>
<th></th>
<th>Enriched rotifers</th>
<th>AF umbrella</th>
<th>AF nauplii</th>
<th>Enriched EG nauplii</th>
</tr>
</thead>
<tbody>
<tr>
<td>20:4(n-6) (ARA)</td>
<td>1.62</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>20:5(n-3) (EPA)</td>
<td>7.97</td>
<td>8.78</td>
<td>5.22</td>
<td>2.51</td>
</tr>
<tr>
<td>22:6(n-3) (DHA)</td>
<td>16.54</td>
<td>-</td>
<td>-</td>
<td>6.67</td>
</tr>
<tr>
<td>n-3 HUFA</td>
<td>25.91</td>
<td>8.86</td>
<td>9.18</td>
<td>9.18</td>
</tr>
<tr>
<td>n-6 HUFA</td>
<td>1.62</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Sum HUFA</td>
<td>27.53</td>
<td>8.86</td>
<td>9.18</td>
<td>9.18</td>
</tr>
</tbody>
</table>

ARA = Arachidonic acid; EPA = Eicosapentaenoic acids; DHA = Docosahexaenoic acid; HUFA = Highly Unsaturated Fatty Acids defined as fatty acids with at least 20 carbon atoms and more than two double bonds.
3.3 Growth and survival of cobia larvae

The cobia larvae (SL in mm) of the UAF treatment were a little smaller than the ones of the ER treatments ($P<0.05$) on 8 dph, but no significant difference ($P>0.05$) was detected on 18 dph (Table 4.3). Larval survival decreased in all treatments from 8 dph onwards. The differences in survival were not significant ($P>0.05$).

Table 4.3. Growth and survival of cobia larvae at 8 dph and 18 dph.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Standard Length (mm)</th>
<th>Survival (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>8 dph</td>
<td>18 dph</td>
</tr>
<tr>
<td>ER</td>
<td>6.4±0.1$^a$</td>
<td>11.4±0.6$^a$</td>
</tr>
<tr>
<td>ER+UAF</td>
<td>6.2±0.1$^{ab}$</td>
<td>11.6±0.6$^a$</td>
</tr>
<tr>
<td>UAF</td>
<td>6.2±0.1$^b$</td>
<td>11.3±0.7$^a$</td>
</tr>
</tbody>
</table>

Values (average±S.E.M) followed by different superscript letters within a column are significantly different ($P<0.05$). ER = Enriched rotifers; ER+UAF = Enriched rotifer followed by AF umbrella; UAF = AF umbrella.

3.4 Quality of and deformity in cobia larvae

Cumulative stress index and mortality rate of cobia larvae at 8 dph exposed to 60 g L$^{-1}$ salinity for 60 minutes were relatively low in ER treatments and significantly different ($P<0.05$) with the UAF and ER+UAF treatments. At 18 dph, the opposite trend was observed although mortality rates were not significantly different ($P>0.05$). Furthermore, no difference in larval deformities could be detected (Table 4.4).
Table 4.4. Salinity stress resistance (at 60 g L\(^{-1}\)) and deformity of cobia larvae at 8 dph and 18 dph.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>8 dph</th>
<th>18 dph</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CSI</td>
<td>M</td>
</tr>
<tr>
<td>ER</td>
<td>46.61±9.63( ^a )</td>
<td>64.6±9.3( ^a )</td>
</tr>
<tr>
<td>ER+UAF</td>
<td>81.42±14.33( ^b )</td>
<td>93.9±3.8( ^b )</td>
</tr>
<tr>
<td>UAF</td>
<td>74.54±15.76( ^b )</td>
<td>92.0±8.0( ^b )</td>
</tr>
</tbody>
</table>

Values (average±S.E.M) followed by different superscript letters within a column are significantly different (\(P<0.05\)). CSI = Cumulative Stress Index; M = Mortality; ER = Enriched rotifers; ER+UAF = Enriched rotifer followed by AF umbrella; UAF = AF umbrella.
4. Discussion

4.1 AF umbrella ingestion

In first-feeding, fish larvae prey ingestion is function of prey size (Pena-Aguado et al., 2007; Pepin and Penney, 1997; Planas and Cunha, 1999; Van der Meeren, 1991a). According to Munk (1997), the preferred prey size in cod larvae is about 5% of its larval length. For gilthead sea bream larvae, Fernandez-Diaz et al. (1994) reported that the preferred live and inert prey size was a function of mouth size showing the preference for prey in the range of 0.1-0.8 times mouth width. Although there is a positive correlation between mouth opening size and prey size, marine larval fish switch to bigger prey slower than their physical capacity to ingest larger prey (Pepin and Penney, 1997). According to Faulk et al. (2007a), cobia larvae open mouth and anus by late 2 dph with consequent differentiation of digestive organs in buccopharynx, oesophagus, stomach and intestine and are ready for first feeding by 3 dph (Faulk and Holt, 2003; 2005; Faulk et al., 2007a; Liao et al., 2001). As the jaw opening of first-feeding cobia larvae in our experiments averaged 551.0±9.7 μm, larvae were able to ingest the AF umbrella Artemia of 378.4±3.0 μm. However, the low feeding incidence in the UAF treatment recorded at initial exogenous feeding might have several reasons: as the umbrellas do not move, they might be less attractive and even when delivering the umbrellas via a dripping device, they might not create a homogeneous distribution of AF umbrella as with live rotifers. The increase of feeding incidence in all treatments (including the ER treatments) in the following days might be related to eye development resulting in more effective prey detection and consequent ingestion.
4.2 Digestion of AF umbrella

It is well documented that at start feeding, the digestive system of fish larvae is not fully functional yet (Kolkovski, 2001). Microalgae are actively ingested at early larval stages e.g. cod *Gadus morhua* (Van der Meeren, 1991b) and appear, in some marine species to function as a trigger for further maturation of the digestive system (Reitan et al., 1998, 1997). It is believed that supplementation of digestive enzymes from live prey is important to further support digestion in early developmental stages of most marine fish larvae (Kolkovski, 2001; Kolkovski et al., 1997a; Munilla-Moran et al., 1990). As documented by Faulk et al. (2007a), the digestive system in cobia larvae is functional as of mouth opening, i.e. enzymes and zymogen granules are present in the pancreas. Moreover, the majority of morphological changes observed in the gastrointestinal tract occurred over the first 1-4 dph (3.6-4.4 mm) and the enzymatic activity for trypsin, lipase and especially amylase increased by 3-4 dph to return to relatively low levels by 8-12 dph (5.7-8.1 mm), at which point a general increase was observed for all enzymes examined (Faulk et al., 2007a). As AF umbrellas are actively metabolizing embryos, they might already contain free amino acids and fatty acids resulting from protein and lipid hydrolysis during embryonic development (Garcia-Ortega et al., 1998).

In our experiment, the oil globule of cobia larvae was depleted by 8 dph at 28.0±0.1 °C, similar to the description of Faulk et al. (2007a) that the yolk sac was completely absorbed by 3-4 dph, followed by a rapid decrease in oil globule size, disappearing entirely by 9-10 dph at a rearing water temperature of 25.9±0.7 °C. If suitable food is not ingested in time, 8-9 dph is a critical period for survival. The high survival in the
UAF-fed treatments at 8 dph revealed that at least 60% of the cobia larvae could rely on AF umbrella for this rearing period.

4.3 Nutritional value of AF umbrella

It is well documented that *Artemia* nauplii are a suitable prey for the larvae of many fish species (Sorgeloos et al., 2001). Although cobia larvae have a high DHA requirement (Faulk and Holt, 2005), DHA-deficient umbrella *Artemia* appear to be a suitable starter diet in comparison to DHA-enriched rotifers. Fast-growing cobia larvae have high energetic requirements and the amount of calories ingested per prey catching effort could be critical, especially as the larvae are growing, i.e. a significantly smaller net energy gain per gram DW food uptake (calories ingested minus calories spent to catch more prey) is expected when feeding too long on a diet of small rotifers. This may not be critical at start feeding, but it certainly becomes critical within the following days as was documented by N. King (in Fish Farming International, January 2006) and by Holt et al. (2007a): each day of advancing *Artemia* feeding (by switching to a strain producing smaller nauplii) resulted in improved survival and growth.

The higher stress sensitivity at 8 dph in the umbrella-fed fish might result from the dietary DHA deficiency. According to Rainuzzo et al. (1997) the inappropriate HUFA ratios, such as DHA/EPA ratio, may create an imbalance of the structural composition of the phospholipids which could affect the normal growth and the quality of the larvae. The DHA level was very high in yolk sac larvae (Faulk and Holt, 2003; Holt et al., 2007a). The DHA level in the larval tissue at 16 dph and in live prey was positive correlated, except EPA and ARA (Faulk and Holt, 2005). A correlation between low stress tolerance and larvae fed diets low in DHA has been documented in many
marine fish species (Brinkmeyer and Holt, 1998; Kanazawa, 1997; Om et al., 2001). Although the DHA and the total n-3 HUFA levels were higher in enriched rotifers as compared to AF umbrella, no significant difference in growth and survival was detected in the larvae fed those diets on 18 dph. This confirms the finding of Faulk and Holt (2005) and Benetti et al. (2007) that in terms of prey enrichment, rotifers may be less important than *Artemia* for cobia larvae due to the short rotifer-feeding period. The requirement for essential fatty acids of cobia larvae during the rotifer and *Artemia* feeding period is still not resolved.

It is also important to remind that growth and survival of marine larval fish not only depends on exogenous nutrition and digestive and metabolic enzyme capacity of newly-hatched larvae (Lamarre et al., 2004), but also on egg quality and consequently broodstock nutrition (Navas et al., 1997; Watanabe and Vassallo-Agius, 2003). In this experiment, cobia larvae were obtained from broodstock fed a diet with a high level of HUFA, which is reflected in high DHA levels in the newly-hatched larvae.

In summary, it is possible for cobia larvae to ingest and digest AF umbrella since first feeding. Replacing enriched rotifers by AF umbrella-stage *Artemia* as starter food for cobia larvae has very little effect on growth by 8 dph and appears to have no significant negative effect on larval quality, growth nor on survival by 18 dph. The successful use of AF umbrella opens a possibility to simplify the rearing protocol for cobia larvae, especially through a reduction of labour costs for live food production. Research on the use of AF umbrella including production and improvement of the availability in larval rearing tanks should be addressed. It is also important to consider research on co-feeding of AF umbrella with formulated feeds to adjust the nutritional
balance and thus compensate for the suboptimal nutritional value of AF umbrella for early larval growth and survival in cobia.

Acknowledgments

VCN acknowledges the Belgian Technical Cooperation (BTC/CTB) for a mixed PhD program between the Laboratory of Aquaculture & Artemia Reference Center, Ghent University, Belgium and the Research Institute for Aquaculture No 1 (RIA1), Hanoi, Vietnam. We also wish to thank NORAD for infrastructural support to RIA1 and INVE Technologies, Belgium for providing experimental test diets and training of VCN at their marine fish hatchery center Maricultura Rosignano Solvay, Italy.
Chapter 5

Early co-feeding and weaning
Effect of early co-feeding and different weaning diets on performance of cobia (*Rachycentron canadum*) larvae and juveniles

Van Can Nhu\textsuperscript{a,b}, Kristof Dierckens \textsuperscript{a}, Hong T. Nguyen \textsuperscript{b}, Tuyet Minh T. Hoang \textsuperscript{c}, Thanh Luu Le \textsuperscript{b}, Mai Thien Tran \textsuperscript{b}, Christel Nys \textsuperscript{d} and Patrick Sorgeloos \textsuperscript{a}.

\textsuperscript{(a)} Laboratory of Aquaculture & Artemia Reference Center, Ghent University 9000 Gent, Belgium

\textsuperscript{(b)} Research Institute for Aquaculture No1, Dinh-bang, Tu-son, Bac-ninh, Vietnam

\textsuperscript{(c)} Faculty of Agriculture-Forestry-Fisheries, Vinh University, Nghe-an, Vietnam

\textsuperscript{(d)} INVE Technologies, 9200 Dendermonde, Belgium

Abstract

Cobia is a very fast growing species. This can only be achieved if sufficient amounts of feed are provided from early larval development onwards. In this study, we tested the effect of early co-feeding and different co-feeding formulated diets on growth, survival and vitality of cobia larvae and juveniles.

During the larval stage, two formulated diets: Proton® and an experimental diet (INVE, Belgium) were used along with live food from 8 day post hatch (dph) and 13 dph compared to 18 dph as the control. Results from the study indicated that early co-feeding of Proton® from 8 dph had a significantly positive effect on growth ($P<0.05$), but not on survival and stress resistance ($P>0.05$) of cobia larvae. For the experiment with the experimental diets, no significant difference ($P>0.05$) was detected between all treatments in terms of growth, vitality and survival. However, high mortality occurred in the treatment with the experimental diet as of 12 dph. The study suggested that early co-feeding of Proton® to cobia larvae from 8 dph is possible and research on the appropriate nutritional composition of weaning diets needs to be addressed.

In the juvenile stage, three formulated diets, i.e. the experimental diet, Proton® and NRD® (INVE Aquaculture NV) were evaluated for growth performance and survival of early cobia juveniles (20-38 dph). The diets were manually introduced from 22 dph at a feeding frequency of every 2 hours until satiation, while feeding of enriched EG *Artemia* was maintained until 30 dph. Average length and weight of the 38-dph juveniles fed the experimental diet were significantly higher ($P<0.05$) compared to the ones fed Proton® and NRD®. However, the coefficient of size variation as well as the cumulative stress index in a salinity challenge test were not significantly different ($P>0.05$). Survival in the Proton® treatment was the lowest, while no significant
difference was evident between the experimental diet and NRD® treatments. The mortality of all 3 treatments had 2 peaks: one at the beginning of the experiment and one when live food feeding was discontinued. This result indicates that the nutritional requirements of cobia are age dependent and prolongation of live food co-feeding during weaning may be necessary. The higher DHA/EPA ratio in the experimental diet can be a clue for the improvement of growth and survival of cobia during the weaning stage.

**Keywords:** Cobia juvenile, co-feeding, weaning, formulated diet
1. Introduction

Cobia has potential for marine aquaculture due to its fast growing and good characteristics as seafood in local and international markets. The main bottle-neck for industrial expansion of cobia farming is the limitation of fingerling production (Holt et al., 2007a; Schwarz et al., 2007c). Although much progress has recently been made in identifying better nutritional, environmental and zootechnical parameters resulting in improved larval growth and survival, the weaning from live food to formulated feed remains a critical stage that needs more investigation.

In intensive production of most marine finfish species, weaning is a very critical period in which there is a gradual change from live prey to formulated diets (Rosenlund et al., 1997). An overlapping co-feeding period during which live food is gradually replaced by increasing quantities of formulated feed has shown to improve growth and survival of marine fish larvae compared to the use of live food only. Formulated diets can balance the nutritional composition of the live food especially with regard to amino acids which are not easy to modify in live food (Rønnestad et al., 1999). Moreover, the formulated diet was found to affect larval size variation within the rearing tank, which influenced rates of cannibalism (Curnow et al., 2006). Live food may influence ingestion, digestion and assimilation of formulated diets (Kolkovski et al., 1997a; Koven et al., 2001). Live food also influences digestion by stimulating endocrine responses (Koven et al., 2001) and providing exogenous digestive enzymes (Kolkovski, 2001). In addition, co-feeding is expected to improve the nutritional condition of the larvae and might thus facilitate earlier transition onto dry feeds only (Chang et al., 2006; Rosenlund et al., 1997).
The success of weaning depends on the appropriate time to start co-feeding and the appropriate artificial diets both in terms of their nutritional composition, palatability and digestibility (Chu and Ozkizilcik, 1999; Faulk et al., 2007a). Kestemont et al. (2007) indicated that the best weight gain of pike perch larvae was found when co-feeding was started from 19 dph compared to 12 dph or 26 dph. Early co-feeding is more beneficial, since it will reduce the use of live food, which are cumbersome to produce and difficult to manipulate nutritionally (Dhert et al., 1999). Success of early co-feeding has been widely documented in various species such as tongue sole (Chang et al., 2006), striped bass (Chu and Ozkizilcik, 1999), barramundi (Curnow et al., 2006), turbot (Dhert et al., 1999; Rosenlund et al., 1997), Atlantic halibut (Hamre et al., 2001; Rosenlund et al., 1997), red drum (Lazo et al., 2000), gilthead sea bream (Rosenlund et al., 1997; Yufera et al., 2000), seabass (Rosenlund et al., 1997) and winter flounder (Khemis et al., 2003). However, the starting time for co-feeding is species-specific according to the maturity of the digestive system (Cahu and Zambonino Infante, 2001).

Cobia larvae and juveniles require very high dietary levels of DHA (Faulk and Holt, 2003; Holt et al., 2007a). In intensive production, enriched *Artemia* are the main food during live prey feeding. However, it is very difficult to reach optimal enrichment levels as ingested DHA is catabolised and used as energy source by *Artemia* (Bell et al., 2003; Evjemo et al., 1997; Olsen et al., 2000; Takeuchi, 2001). Therefore, the supplementation of formulated diets could be a solution to satisfy the larval nutritional requirements. In Vietnam, cobia larvae are fed live prey from 3 to 18 dph at which moment weaning onto artificial diets is started. During this process, enriched live food is partially replaced by artificial diets until the juveniles can be fed solely on dry diets. In addition, successful use of *Artemia franciscana* in umbrella stage as an option for
earlier substitution of enriched rotifers, allows simplifying the rearing protocol for cobia larvae, but may at the same time result in too limited provision of DHA (Nhu et al., 2009a). Thus, research on earlier weaning with formulated diets is very essential to determine and adjust optimal nutritional balances. Co-feeding may be started much earlier (8-12 dph) since Faulk et al. (2007a) have documented secretion of pancreatic enzymes in the animals less than 18 dph. In this study, we want to investigate if larvae can be weaned at earlier developmental stages, starting at 8 dph or 13 dph instead of 18 dph. In order to select the appropriate diet for cobia weaning, we studied growth and survival of cobia juveniles (20 dph) fed three different weaning diets.

2. Materials and methods

2.1 Experimental design

Three experiments were designed to test the possibility of early co-feeding of formulated diets for cobia larvae (period 8-18 dph) and to compare the effect of 3 formulated weaning diets for cobia juveniles (period 20-38 dph) in terms of growth performance, quality and survival. Each treatment was set up in 3 replicates randomly allocated in 200-L tanks which were connected to a bio-filter. The tanks were equipped with a central filter (200-500 µm mesh), aeration lines and dripping bottles for *Artemia* umbrella feeding as described in Nhu et al. (2009a). All experiments were conducted in the marine hatchery of the Research Institute for Aquaculture No1 located in Nghe-an province, Vietnam.

Three formulated feeds: Proton® and NRD® and the experimental diet were used from INVE Aquaculture SA (Dendermonde, Belgium). Different sizes of the diets were used according to the larval size and the manufacture’s manuals such as Proton® 2 (150-300 µm), Proton® 3 (200-400 µm), Proton® 4 (300-500 µm), NRD 2/3 (200-300 µm), and the experimental diet (200-500 µm).
µm), NRD 2/4 (200-400 µm) and NRD 3/5 (300-500 µm). The formulated experimental diet was extruded, grinded and sieved into 2 size classes of 150-250 µm and 250-500 µm. Proton® and the experimental feed were used along with live food. From 8 dph and 13 dph, Proton® and the experimental feed were tested in comparison to 18 dph as control. The treatments were named P1-D8, P2-D13 and P3-D18 for Proton® (experiment 1) and Ex1-D8, Ex2-D13 and Ex3-D18 for the experimental diet (experiment 2), indicating the diet and the day when it was first fed to the larvae. The experiments were terminated after 23 days rearing. For the juvenile stage, three treatments were named: Ex, PROTON and NRD according to the formulated diet that was fed (experiment 3). The objective of this experiment was to test the effect of those weaning diets on growth, survival and quality of cobia juvenile for the period 20-38 dph. The experiments were terminated after 18 days rearing in the same culture conditions.

Table 5.1. Nutritional profile of the formulated weaning diets

<table>
<thead>
<tr>
<th></th>
<th>Proton®</th>
<th>NRD®</th>
<th>Experimental diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude protein (%)</td>
<td>54</td>
<td>59</td>
<td>62</td>
</tr>
<tr>
<td>Crude lipid (%)</td>
<td>15</td>
<td>16</td>
<td>10</td>
</tr>
<tr>
<td>Moisture (%)</td>
<td>7</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>Vitamin E (mg kg⁻¹)</td>
<td>700</td>
<td>400</td>
<td>400</td>
</tr>
<tr>
<td>Vitamin C (mg kg⁻¹)</td>
<td>2000</td>
<td>2000</td>
<td>2000</td>
</tr>
<tr>
<td>n-3 HUFA (1) (mg g⁻¹ DW)</td>
<td>30</td>
<td>28</td>
<td>35</td>
</tr>
<tr>
<td>DHA/EPA</td>
<td>2.0</td>
<td>2.0</td>
<td>2.5</td>
</tr>
</tbody>
</table>

(1) HUFA = Highly unsaturated fatty acids defined as fatty acids with at least 20 carbon atoms and two or more double bonds.
Chapter 5

The nutritional composition of the three formulated diets is shown in table 5.1. Proton® is a commercial micro-particulated extruded diet that was designed for weaning marine fish larvae, substituting gradually the *Artemia* nauplii. This product characterized by sinks slowly. From a nutritional point of view, Proton® is highly attractive which is established by free amino acids, high quality marine proteins, high level of vitamin E, adequate level of n-3 HUFA (30 mg g⁻¹ dry weight) and DHA/EPA ratio (2). Meanwhile, NRD® is also a commercial micro-particulated extruded diet, but designed for post-weaning and nursing phase. This product has a lower n-3 HUFA level, but a higher protein and lipid content compared to Proton®. The experimental diet was designed especially for cobia larvae with a higher level of crude protein (62 %), n-3 HUFA (35 mg g⁻¹ dry weight) content and DHA/EPA ratio (2.5), except for crude lipids (10 %) compared to the two commercial diets.

2.2 Larval rearing and feeding

The newly-hatched cobia larvae obtained from captive broodstock were volumetrically counted and randomly allocated to the tanks at a density of 50 larvae L⁻¹. Live food preparation and larval rearing was implemented according to Faulk and Holt (2005) and Nhu et al. (2009a), i.e. from 2 dph *Nannochloropsis oculata* was added and maintained at a density of 1 x 10⁵ cell mL⁻¹, enriched rotifers (*Brachionus plicatilis*) were introduced from 3-6 dph at a density of 10-12 rotifer mL⁻¹, and umbrella-stage of *Artemia franciscana* (UAF) were introduced from 5-8 dph at a density of 2-3 umbrella mL⁻¹; enriched *Artemia* nauplii were added from 8 dph at a density of 0.4-0.5 nauplii mL⁻¹. Every 4 hours, live food density in the larvae tanks was checked and eventually topped up. Dead larvae and other deposited waste were removed by siphoning during daily water exchange as of 8 dph.
In experiment 1 and 2, Proton® or the experimental diet was manually distributed at a feeding frequency of 4 times day\(^{-1}\). To encourage the ingestion of the formulated diet by the larvae, live food was only distributed 30 min following addition of the dry diet. The sizes of the formulated diets were adjusted according to the larvae size and user manual of the product.

In experiment 3, cobia larvae were first reared in a production tank (3000 L) according to the procedure described above. At 20 dph, juveniles were manually graded and randomly allocated in 9 experimental tanks at a density of 5 ind. L\(^{-1}\). The standard length and body weight of the cobia juveniles (mean±SD) were 10.9±1.4 mm and 17.5±5.1 mg, respectively. The larvae were conditioned for 2 days with live food to minimize the effect of handling stress before weaning with different formulated diets. The formulated diets were manually introduced at a feeding frequency of 7 times day\(^{-1}\), while the enriched EG metanauplii were fed 4 times day\(^{-1}\). The amount of Artemia nauplii was gradually reduced and replaced by formulated diets. Live food supplementation was terminated 8 days after weaning had started. The experiment was terminated after 18 days rearing.

Environmental factors such as DO, pH, water temperature, and salinity and NH\(_3\) concentration were analyzed twice a day (7 am and 2 pm). Records of the environmental conditions in each experiment are presented in Table 5.2.
Table 5.2. Environmental parameters in larval rearing tanks of the three experiments.

<table>
<thead>
<tr>
<th></th>
<th>DO (mean±SD, mg L⁻¹)</th>
<th>Water temperature (mean±SD, °C)</th>
<th>Day degree</th>
<th>pH (Min-Max)</th>
<th>Salinity (mean±SD, g L⁻¹)</th>
<th>NH₃ (mean±SD, mg L⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experiment 1</td>
<td>5.7±0.3</td>
<td>29.8±0.7</td>
<td>684</td>
<td>tments.</td>
<td>31±1</td>
<td>0.023±0.010</td>
</tr>
<tr>
<td>Experiment 2</td>
<td>5.7±0.2</td>
<td>30.2±0.9</td>
<td>694</td>
<td>7.4-8.1</td>
<td>33±1</td>
<td>0.029±0.038</td>
</tr>
<tr>
<td>Experiment 3</td>
<td>5.8±0.2</td>
<td>30.3±0.3</td>
<td>546</td>
<td>7.6-7.9</td>
<td>32±2</td>
<td>0.001±0.001</td>
</tr>
</tbody>
</table>

2.3 Evaluations of growth, survival and larval quality

Cobia larvae were randomly sampled (n=30) at 18 dph and 23 dph (experiment 1, experiment 2) and at 27 dph and at 38 dph (experiment 3) for growth evaluation. The standard length (mm) was measured using a Mitutoyo ruler No. 8355, while the body weight (mg) was measured using a digital balance (AAA 250L, Adam Equipment Co. LTD, d=0.1 mg). Size variation was evaluated according to Wang et al. (1998) for which the mean of three replicates of the coefficient of variation (CV) was used to examine the inter-individual weight variation among the fish in each treatment:

\[ CV(\%) = 100 \times \frac{SD}{M} \]

Where M is mean of body weight and SD is standard deviation of the fish in each treatment.

Percentage of survival in each treatment was determined by summing up the number of live fish at the end of the experiment and the number of larvae taken for samples in the course of the experiment divided by the number of larvae at the start of the experiment and multiplied by 100. In experiment 3, the natural mortality was determined from the number of dead fish observed and removed on a daily basis.
while cannibalism was calculated following Curnow et al. (2006) i.e. cannibalism = 100*(I-S-F-M)/I, where, I is the initial number of larvae, S is the number of sampled larvae, F is the final number of larvae and M is the number of natural mortalities during the trial.

The quality of the cobia larvae was evaluated following Nhu et al. (2009a) by submitting the larvae to a salinity shock at the end of each experiment. The test was conducted at a salinity of 60 g L$^{-1}$ for 1 h and dead larvae were counted every 3 min, the higher the cumulative stress index (CSI) and mortality (M), the lower the quality of the larvae.

2.4 Data analysis

All data were tested for significant differences ($P<0.05$) using one-way ANOVA followed by Tukey test for multiple comparisons of means. The data are expressed as mean±SD and statistical analyses were performed using GraphPad Prism 4.0 and Microsoft Office EXCEL for Windows.

3. Results

3.1 Effect of early co-feeding of Proton® and the experimental diet on performance of cobia larvae

Growth and survival

In the early co-feeding experiments, ingestion of the formulated diets was clearly observed; however, it was not possible to visually evaluate differences in digestion ability.
Table 5.3. Growth performances and survivals of cobia larvae in two co-feeding experiments.

<table>
<thead>
<tr>
<th></th>
<th>Size of cobia 18 dph</th>
<th>Survival (%) at 23 dph</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Standard length (mm)</td>
<td>Body weight (mg)</td>
</tr>
<tr>
<td></td>
<td>Standard length (mm)</td>
<td>Body weight (mg)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Experiment 1. Early co-feeding of Proton® diet</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P1-D8</td>
<td>12.2±1.3a</td>
<td>19.5±5.0a</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P2-D13</td>
<td>11.0±0.5a</td>
<td>14.8±1.2a</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P3-D18</td>
<td>11.0±0.7a</td>
<td>14.2±2.7a</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Experiment 2. Early co-feeding of the experimental diet</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ex1-D8</td>
<td>11.0±0.6a</td>
<td>13.6±2.4a</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ex2-D13</td>
<td>10.4±0.3a</td>
<td>11.5±0.7a</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ex3-D18</td>
<td>10.7±0.8a</td>
<td>11.1±2.6a</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values (mean±SD, n=3) followed by different superscript letters within a column of each experiment are significantly different (P<0.05). CV = Coefficient of variation; P1-D8, P2-D13 and P3-D18 and Ex1-D8, Ex2-D13 and Ex3-D18 are the treatments co-fed Proton® and the experimental diet from 8, 13 and 18 dph, respectively.
In experiment 1, larval standard length and body weight in treatment P1-D8 (start of co-feeding Proton® as of 8 dph) were significantly higher at 23 dph compared to the other two treatments (Table 5.3). Treatment P2-D13 (start of co-feeding at 13 dph) in contrast, was not significantly different from the control. Early co-feeding at 8 dph and 13 dph resulted in a smaller coefficient of size variation ($P<0.05$), which is regarded as an important factor to minimize cannibalism during later larval stages. Among the three treatments, the size in the control treatment was also more variable as the CV was significantly different from the one co-fed from 8 dph ($P<0.05$). The survival rate of all Proton® co-feeding treatments were variable between replicates and no significant difference between the treatments was detected ($P>0.05$).

All results for experiment 2, in which the experimental diet was tested, were not significantly different. Larval size, weight and survival in this experiment were also smaller as compared to data in experiment 1, although the day degree was higher (Table 5.2). Results of this experiment indicated that early co-feeding of the experimental diet was not as effective as feeding Proton®.

**Mortality**

In experiment 1, mortality was similar in all treatments with a peak at 8 dph and decreased in the next two days. Later, mortalities remained low (Fig. 5.1A). The cumulative mortality (mean±SD) of treatment P1-D8, P2-D13 and P3-D18 during the rearing period of 8-22 dph was 21.0±4.4, 19.6±2.4 and 20.3±8.0 %, respectively. No significant difference in cumulative mortality between the treatments was detected ($P>0.05$).
Figure 5.1. Daily mortality of cobia larvae in experiment 1 (A) and experiment 2 (B).

Data expressed as mean ± SD (n=3). P1-D8, P2-D13 and P3-D18 are the treatments co-fed Proton® from 8, 13 and 18 dph; Ex1-D8, Ex2-D13 and Ex3-D18 are the treatments co-fed the experimental diet from 8, 13 and 18 dph, respectively.

Mortality in experiment 2 at 8 dph was similar as in experiment 1 and had a peak around 12 dph for all treatments including the control (co-fed at 18 dph). Treatment Ex1-D8 had the highest mortality. This explained the low survival of the experimental diet co-feeding treatments as described above (Fig. 5.1B). During period of 8-22 dph, the cumulative mortality (mean ± SD) in treatment Ex1-D8, Ex2-D13 and Ex3-D18...
was 38.2±9.8, 33.6±7.5 and 27.8±2.3 %, respectively and was not significantly
different between the treatments ($P>0.05$).

**Quality of cobia juveniles**

The quality of the cobia larvae was evaluated as the cumulative stress index (CSI) and
mortality (M) in salinity stress tests. There was no significant difference ($P>0.05$) in
CSI and M values among the treatments of experiment 1 or experiment 2. However,
there was a tendency of lower CSI and M values in the earlier co-feeding of Proton®
from 8 dph compared to the treatments those started co-feeding from 13 or 18 dph
(Table 5. 4).

**Table 5. 4. Vitality of cobia juveniles (23 dph) in salinity stress test (60 g L$^{-1}$, 1 h).**

<table>
<thead>
<tr>
<th></th>
<th>CSI</th>
<th>M (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Experiment 1: Early co-feeding of Proton® diet</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P1-D8</td>
<td>34.89±10.22a</td>
<td>55.6±10.2a</td>
</tr>
<tr>
<td>P2-D13</td>
<td>31.44±8.19a</td>
<td>56.7±11.6a</td>
</tr>
<tr>
<td>P3-D18</td>
<td>57.11±20.55a</td>
<td>78.9±22.7a</td>
</tr>
<tr>
<td><strong>Experiment 2: Early co-feeding of the experimental diet</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ex1-D8</td>
<td>36.78±4.07a</td>
<td>67.8±13.5a</td>
</tr>
<tr>
<td>Ex2-D13</td>
<td>41.11±8.51a</td>
<td>75.6±7.7a</td>
</tr>
<tr>
<td>Ex3-D18</td>
<td>35.67±8.67a</td>
<td>63.3±3.3a</td>
</tr>
</tbody>
</table>

Values (mean±SD, $n=3$) followed by different superscript within a column of each
experiment are significantly different ($P<0.05$). P1-D8, P2-D13 and P3-D18 and Ex1-D8,
Ex2-D13 and Ex3-D18 are the treatments co-fed Proton® and the experimental diet from 8, 13
and 18 dph, respectively; CSI = Cumulative Stress Index; M = Mortality.
3.2 Effect of the different weaning diets on the performance of cobia juveniles

**Growth performance of cobia juveniles and their quality**

Length and weight of cobia juveniles at 27 dph was not significantly different \((P>0.05)\) among the three formulated weaning diet treatments. However, at 38 dph, the length and weight of the juveniles fed the experimental diet was the highest \((P<0.05)\). The length of cobia juveniles fed Proton® was the smallest \((P<0.05)\), while body weight of the ones fed NRD® and Proton® were not significantly different \((P>0.05, \text{Table 5.5})\). The different diets did not affect the size variation significantly. In addition, no difference in CSI of cobia juveniles was detected at the end of the experiment.

**Table 5.5. Growth performance and quality of cobia juveniles.**

<table>
<thead>
<tr>
<th></th>
<th>Ex</th>
<th>PROTON</th>
<th>NRD</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Size of cobia juveniles 27 dph</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Standard length (mm)</td>
<td>17.6±2.0(^a)</td>
<td>18.9±1.6(^a)</td>
<td>17.6±1.1(^a)</td>
</tr>
<tr>
<td>Body weight (mg)</td>
<td>50.9±7.5(^a)</td>
<td>52.9±8.9(^a)</td>
<td>46.0±5.8(^a)</td>
</tr>
<tr>
<td><strong>Size of cobia juveniles 38 dph</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Standard length (mm)</td>
<td>37.1±1.6(^a)</td>
<td>32.1±3.1(^c)</td>
<td>34.8±0.4(^b)</td>
</tr>
<tr>
<td>Body weight (mg)</td>
<td>374.3±34.6(^a)</td>
<td>251.4±52.4(^b)</td>
<td>294.7±16.5(^b)</td>
</tr>
<tr>
<td>CV</td>
<td>33.55±0.78(^a)</td>
<td>43.31±10.16(^a)</td>
<td>37.04±9.21(^a)</td>
</tr>
<tr>
<td><strong>Vitality of cobia juveniles 38 dph in salinity stress test (60 g L(^{-1}), 1 h)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CSI</td>
<td>128.90±5.16(^a)</td>
<td>126.40±8.17(^a)</td>
<td>124.60±7.81(^a)</td>
</tr>
</tbody>
</table>

Values (mean±SD, \(n=3\)) followed by different superscript within a row are significantly different \((P<0.05)\); CV = Coefficient of variation; Ex, PROTON and NRD = treatment fed the experimental diet, Proton® and NRD®, respectively as weaning diets.
Survival and mortality of cobia juveniles

The use of Proton® resulted in a significantly lower survival ($P<0.05$) compared to the experimental diet or NRD® (Fig. 5. 2). The survival of cobia fed the experimental diet was not significantly different to the ones fed NRD® ($P>0.05$). Although the cannibalism was the lowest in the Ex treatment, it was, as well as the natural mortality rate, variable among the replicates of each treatment and was not significantly different among the three treatments ($P>0.05$). Cannibalism was related to the size variation as described above.

![Graph showing survival and mortality of cobia juveniles fed three different weaning diets.](image)

**Figure 5.2. Survival and mortality of cobia juveniles fed three different weaning diets.**
Values (mean±SD, $n=3$) followed by different letters within the same legend are significantly different ($P<0.05$). Ex, PROTON and NRD = treatment fed the experimental diet, Proton® and NRD® diets, respectively.

The daily mortality recorded during the rearing period (Fig. 5. 3) showed two periods of high mortality. The first mortality peak happened between the 2nd and the 4th day of
the culture. This mortality may be related to the handling stress before the experiment and to the partial replacement of live food with formulated diets. The second mortality, from day 9 till day 14, might be explained by the progressive reduction (from day 7) until complete stop of live food supplementation on day 10. The highest mortality was recorded on day 11 for all treatments. These mortalities indicated that the supplementation of live food was still very essential for the cobia juveniles and that co-feeding of live food should be extended. Interestingly, the mortality was variable between replicates of the same treatment, but the occurrence and ratios of mortality were similar regardless the treatment.

Figure 5.3. Daily mortality of cobia juveniles fed three different weaning diets.

Value = Mean±SD, n=3. Ex, PROTON and NRD = treatment fed the experimental diet, Proton® and NRD® diets, respectively
4. Discussion

4.1 Acceptance and effect of the formulated diet in early co-feeding

Better growth of the juveniles in P1-D8 treatment demonstrated that early co-feeding of Proton® was effective and cobia larvae were able to ingest and digest the diet. These results confirmed the conclusions of Faulk et al. (2007a), namely that co-feeding of cobia larvae around 8 dph was possible. Acceptance of dry diet in early co-feeding depends on the suitability of the diet and development of the larval digestive system. Normally, levels of pancreatic enzymes are low when marine fish larvae start exogenous feeding, but they increase as metamorphosis approaches and thus introduction of formulated diets can be started (Cahu and Zambonino Infante, 2001). In the case of cobia larvae, the differentiation of the gastric glands begins on 9-10 dph in the incipient fundic stomach with an increase in pancreatic enzyme activity at 8-12 dph suggesting that cobia larvae would be capable of digesting and assimilating compound diets around this time (Faulk et al., 2007a). According to Cahu and Zambonino Infante (2001), the enzyme activity pattern is age-dependent, but can be modulated by the diet. Live food given together with a formulated diet was reported to enhance the efficiency of the formulated diet by promoting the assimilation and deposition of dietary nutrients in the larval body (Kolkovski et al., 1997a). However, the growth improvement of the juveniles in the early co-feeding treatments in experiment 1 was small. This result indicated that the present nutritional composition of Proton® still is not optimal for cobia larvae during the early co-feeding period. Therefore, more research on the larval nutritional requirements needs to be conducted and the appropriate nutritional composition of formulated diets for cobia larvae needs to be developed.
According to Baskerville-Bridges and Kling (2000); Cahu and Zambonino Infante (2001) and Chu and Ozkizilcik (1999), the physical aspects such as particle size, distribution and attractiveness of the formulated diets can affect the larval ingestion. Proton® and the experimental diet both have physical characteristics that are suitable for co-feeding i.e. small particle size (80-500 µm, accordingly with the larval sizes) that is comparable or even smaller than *Artemia* nauplii (190 µm in width and 400-900 µm in length, depending on their development stages (Lavens and Sorgeloos, 1996) and low sinking rate. Proton® is a commercial micro diet and has been used successfully for various marine fish larvae. Therefore, the differences of the two diets in respect to the physical aspects should be minimized. On the other hand, the experimental diet was formulated with a higher content of n-3 HUFA, crude protein and higher DHA/EPA ratio, but lower crude lipids content, compared to Proton® (Table 5.1). However, the use of this experimental diet at early development stages did not affect the growth, survival and quality of cobia larvae compared to the control. In addition, high mortality occurred after the first introduction of the experimental diet in treatment Ex1-D8 suggesting that nutritional composition of the experimental diet might not be appropriate for this larval stage. According to Baskerville-Bridges and Kling (2000) and Morais et al. (2006), feed ingestion and digestion does not appear to be regulated by total lipid content, but, by the lipid source and fatty acid composition. High mortality also happened during the first week of weaning pikeperch larvae (12-18 dph) (Kestemont et al., 2007). The poor survival of cobia in experiment 2 might also have resulted from larval quality variation of different egg batches. Our results indicated that success of early co-feeding of cobia larvae is dependent on the micro diet, which was also concluded by Curnow et al. (2006) when studying the effects of early weaning of Proton® and the other commercial diets for Asian seabass.
4.2 Changing digestive capacity and/or nutritional requirements during development

The results of experiment 1 and 3 showed the expected effects of the two commercial diets in which Proton® was designed for the early co-feeding stage and NRD® for post weaning. Proton®, with a high level of lipids and vitamin E, resulted in better growth of the larvae for the period 8-23 dph, but was less effective compared to the experimental diet and NRD® for the juvenile stage (20-38 dph). Among three weaning diets, the experimental diet with a higher n-3 HUFA content, crude protein and DHA/EPA ratio was not effective for early co-feeding of the larvae 8-23 dph, but it was the best for cobia juveniles in the period 20-38 dph. According to Cahu and Zambonino Infante (2001), marine fish larvae have different specificities in digestion and nutritional requirements compared to juveniles since mechanisms of digestion and absorption change during development. The effect of larval size and age at weaning has been evident (Kestemont et al., 2007). In cobia larvae, the digestive system starts to be functional from 8 dph (5.7 mm), but the development continues until 20 dph when they reach 16.4 mm (Faulk et al., 2007a). This explains the difference in ingestion and digestion capacity between larvae and juveniles of cobia. Our results indicated that nutritional requirements and/or digestive capacity of cobia were size and age-dependent. This also suggested a possibility, that cobia larvae might require higher dietary lipid content, while the juveniles probably need more protein in their diet. In addition, our results revealed that high dietary n-3 HUFA and dietary DHA/EPA ratio become more important for early cobia juveniles as they rely on the formulated diets during gradual reduction of live food. Our conclusion is in agreement with Curnow et al. (2006): the optimal live food weaning protocol is specific to the micro diet.
It has been widely documented that live food plays an important role during early larval development of marine fish. However, during larval development, live food needs to be replaced by an appropriate formulated diet. Among the three tested diets, Proton® was shown to be better suited for a longer *Artemia* inclusion period which has also been demonstrated in the rearing protocol of sea bass (Curnow et al., 2006). However, withdrawal of live food from 27 dph and feeding solely dry diets as of 30 dph in experiment 3 resulted in high mortality in all treatments. In this experiment, the cobia juveniles at 27 dph were still small (17.6-18.9 mm) and smaller than the juveniles at 22 dph (20.1 mm) (Faulk et al., 2007a). Therefore, their digestive system may not be fully developed. This may explain the late weaning success as described by Holt et al. (2007a,b), where the weaning was successfully completed around 25 dph. The high mortality that occurred after stopping live food feeding in this experiment indicated that the development of the digestive system may be related to their size rather than age. The tested formulated diets could not substitute live food completely with the present nutritional composition. Improvement of digestibility and nutritional composition of these diets needs to be obtained.

### 4.3 Effect of environmental factors and husbandry improvement for co-feeding

Co-feeding reduced the water quality. Due to low water exchange and high density of live food, the NH$_3$ concentration in the larval rearing tanks (experiment 1 and experiment 2) was higher than in the juvenile rearing tanks (experiment 3) (Table 5.1). According to Rosenlund et al. (1997) there is a major risk of reduced water quality when using dry feeds which in turn leads to loss of appetite and subsequently to mortality. As a result, problems with tank management often obscure the effects of dietary composition. Husbandry should be an integrated part in future research aiming
to develop more optimal feeds for marine fish larvae. In our study, each experiment was conducted in a recirculation system in which, the adverse environmental factors can be easily circulated to all rearing tanks. Cobia is a very sensitive species and can be easily affected by the rearing conditions. This could be an explanation for the mortality in the treatments of each experiment although the formulated diets were introduced at different times or different diets were introduced at the same time.

5. Conclusions

Early co-feeding of Proton® from 8 dph was possible and resulted in better growth, but not in survival of cobia larvae compared to the treatments started co-feeding from 13 or 18 dph. The experimental diet was not appropriate for early co-feeding, but effectively supported growth and survival of the juveniles during weaning (20-38 dph) compared to Proton®. Results of this study clearly indicated that the nutritional requirements of cobia during early development are age-dependent. When using the present formulated diets (Proton®, NRD® or the experimental diet), a longer co-feeding of live food is needed for growth and survival improvement. The larval nutritional requirements, especially the essential fatty acid requirements, husbandry and development of more optimal feeds for cobia larvae and juveniles need to be addressed in future research.

Acknowledgments

The first author thanks the Belgian Technical Cooperation (BTC/CTB) for his PhD scholarship under a mixed PhD program between the Laboratory of Aquaculture & Artemia Reference Center, Ghent University, Belgium and the Research Institute for Aquaculture No1 (RIA-1), Vietnam. He wishes to thank NORAD for infrastructure provision to RIA-1 and INVE Technologies NV for providing the experimental diets.
Chapter 6

Dietary DHA and DHA/EPA ratio for weaning
Effect of dietary DHA content and DHA/EPA ratio on performance and quality of cobia (*Rachycentron canadum*) juveniles during weaning

Van Can Nhu\textsuperscript{a,b}, Kristof Dierckens\textsuperscript{a}, Thu Thuy T. Nguyen\textsuperscript{c}, Lam Hong T. Pham\textsuperscript{c}, Mai Thien Tran\textsuperscript{b}, Thanh Luu Le\textsuperscript{b}, Christel Nys\textsuperscript{d} and Patrick Sorgeloos\textsuperscript{a}

\textsuperscript{a}Laboratory of Aquaculture & Artemia Reference Center, Ghent University, 9000 Gent, Belgium

\textsuperscript{b}Research Institute for Aquaculture No1, Dinh-bang, Tu-son, Bac-ninh, Vietnam

\textsuperscript{c}Hanoi University of Agriculture, Gia-lam, Hanoi, Vietnam

\textsuperscript{d}INVE Technologies NV, 9200 Dendermonde, Belgium
**Abstract**

Cobia is a fast growing marine fish species. The yolk-sac larvae contain high levels of docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), suggesting that cobia may require high dietary levels of these fatty acids during their larval/juvenile development. This study examined the effect of different dietary DHA levels and DHA/EPA ratios on growth, survival and quality of early juveniles of cobia during the period of 12-30 day post hatching (dph). During early development of cobia larvae, their content of the essential fatty acid DHA decreased from 41.29 to 39.91 (day 0 to 3) and to 10.33 mg g\(^{-1}\) (day 12), but DHA/EPA ratios increased from 4.6 to 5.6 and to 6.6, respectively. Three experimental diets (Ex1, Ex2 and Ex3) with different DHA levels (21.12, 37.57 and 53.31 mg g\(^{-1}\) dry weight, DHA/EPA ratios of 3.6, 3.8 and 6.0) were tested. Results from this experiment indicated that higher DHA levels and DHA/EPA ratios in the formulated diets resulted in better specific growth rate (varying from 22.60-23.77 % day\(^{-1}\)). Cannibalism was the highest in the treatment with low dietary DHA. Levels of DHA and DHA/EPA ratio in juvenile tissues could not be correlated with dietary contents. Although no differences were noticed among treatments in the salinity tests, best survival in the transportation test was recorded in the highest DHA treatment. Growth, survival and quality of cobia juveniles were improved by feeding diets with high DHA contents and with a high DHA/EPA ratio.

**Keywords:** Cobia juvenile, dietary DHA, DHA/EPA ratio, weaning, natural zooplankton, copepods
1. Introduction

The dietary requirement of highly unsaturated fatty acids (HUFA) of most marine fish species has been well documented. Marine fish naturally contain high levels of essential n-3 HUFA such as docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA) and arachidonic acid (ARA) (Sargent et al., 1999a,b; Sargent et al., 1997). Among the essential fatty acids (EFA), DHA plays an important role during early development of marine and freshwater fish (Sargent et al., 1999a). According to Takeuchi (2001), DHA fulfils a key role in the development of the nervous and visual system of marine fish. This fatty acid is normally found particularly in rod cells, outer segment membranes and the membranes of synaptic junctions (Sargent et al., 1999a; Sargent et al., 1997). Marine fish can neither biosynthesise DHA de novo nor convert DHA from the shorter chain precursor 18:3n-3, therefore they need DHA as an essential dietary constituent (Sargent et al., 1999b).

Because of the competitive interaction in the metabolism of HUFA, the requirement of each EFA needs to be considered in their relative as well as absolute amounts, i.e., in terms of DHA/EPA ratio (Sargent et al., 1999a). The requirement of dietary DHA/EPA ratio varies among marine fish species (Sargent et al., 1999a,b; Sargent et al., 1997). A higher content of dietary DHA over EPA enhances growth and survival of striped trumpeter *Latris lineata* larvae (Bransden et al., 2005), Pacific bluefin tuna *Thunnus orientalis* larvae and juveniles (Seoka et al., 2007; Seoka et al., 2008), supports survival of mahimahi *Coryphaena hippurus* through the early larval period (Ostrowski and Divakaran, 1990), increases stress tolerance of red seabream *Pagrus major* larvae and marbled sole *Limada yokohamae* juveniles (Kanazawa, 1997) and supports growth, survival and stress tolerance of various marine species farmed in
Japan (Takeuchi, 2001). A very high DHA/EPA ratio improves survival during larval stage of fast growing marine species such as Pacific bluefin tuna *Thunnus orientalis* (Biswas et al., 2006a; Seoka et al., 2007; 2008) and yellowtail *Seriola quinqueradiata* (Takeuchi, 2001).

Cobia is a very fast growing marine fish species therefore, nutrition is considered as one of the most important factors affecting growth and survival, especially for early development. According to Zambonino Infante and Cahu (2001), marine fish larvae undergo major morphological and cellular changes during the first month of life and their gastrointestinal tract combines these two aspects of the development. Cobia larvae start their metamorphosis around 11-15 dph (Benetti et al., 2007) with differentiated gastric glands and developing digestive system (Faulk et al., 2007a), that result in efficient early co-feeding with formulated diets (Nhu et al., 2009b). During the early life stage, the diet is the primary source of polyunsaturated fatty acids (PUFA) in the body tissues of cobia (Turner and Rooker, 2005). Therefore, supplementation of appropriate nutrients is crucial for larval growth and survival especially during metamorphosis. Cobia juveniles can digest proteins and lipids from both plant and animal sources (Zhou et al., 2004) and they were found to thrive on a wide range of protein and lipid levels without impacts on production characteristics i.e. growth, survival and biomass (Chou et al., 2001; Craig et al., 2006). However, the research recently conducted by Niu et al. (2008) indicated that phospholipids are indispensable nutrients for juvenile cobia as their growth and survival was improved with increasing dietary phospholipids. Moreover, high DHA levels and DHA/EPA ratios in eggs and larvae from both wild caught (Faulk and Holt, 2003) and captive spawning broodstock (Faulk and Holt, 2008) suggest that cobia larvae and juveniles may have high dietary requirements of these EFAs.
We assume that larvae and early juveniles of cobia, like other fast growing marine species, such as Pacific bluefin tuna *Thunnus orientalis* (Brill, 1996; Sawada et al., 2005), mahimahi *Coryphaena hippurus* (Brill, 1996; Lee, 1997) or yellowtail *Seriola quinqueradiata* (Takeuchi, 2001), may also require high dietary DHA content and high DHA/EPA ratio. The objective of this research is to evaluate the effects of different dietary DHA levels and DHA/EPA ratios on growth, survival and quality of cobia juveniles during weaning, the most critical period of the artificial production in a marine hatchery. The result of this study will provide useful data to develop appropriate formulated diets for cobia juveniles and thus will improve quantity and quality of the fingerling production for grow-out.

2. Materials and methods

2.1 Cobia larvae production and experimental design

The experiment was conducted in a marine hatchery of the Research Institute for Aquaculture No.1, located in Quy-kim district, Hai-phong city, Vietnam. The experimental rearing system consisted of nine 200-L cylindro-conical tanks with a recirculation system as described in Nhu et al. (2009a).

Cobia larvae were selected from the production system of the marine hatchery. At 12 days post hatching (dph), the larvae were manually sorted for size uniformity, treated for parasites and transferred to the experimental recirculation system. The parasite treatment was conducted by using formalin bath at a concentration of 30 µm L⁻¹ for 1 h with aeration. A total of 30 larvae were then randomly sampled to determine the initial size and weight. The average standard length (mean±SD) of the larvae was 1.1±0.1 cm equivalent to the body weight of 0.010±0.003 g. After that, 300 larvae
were manually counted and allocated in each tank, equivalent to a density of 1.5 larvae L\(^{-1}\).

Three treatments were set up: T1, T2, and T3 corresponding to the names of the experimental diets Ex1, Ex2, and Ex3 (formulated by INVE Technologies NV), respectively. The three replicates of each treatment were randomly distributed over nine 200-L cylindro-conical tanks connected to a recirculation system. The experiment terminated after 18 days rearing.

### 2.2 Feed and feeding

Three experimental weaning diets (Ex1, Ex2, and Ex3) were formulated to meet the requirements of a fast growing fish species as cobia. These formulations were based on the NRD\(^{®}\) diet (developed and used for different marine fish species (De Wolf et al., 2001)), but with specific lipid ingredients to achieve different DHA contents as well as DHA/EPA ratios. Diets were extruded, grinded and sieved into 2 size classes: 160-300 µm and 300-400 µm. All dry diets as well as the live foods (enriched *Artemia* nauplii) were analyzed to determine their total lipid contents as well as fatty acids composition.

Cobia juveniles were fed enriched *Artemia* nauplii which were gradually replaced by formulated diets. EG-brand *Artemia* cysts (Great Salt Lake USA, INVE Aquaculture NV) were disinfected in fresh water for 1 hour with a hypochlorite solution (20 mL L\(^{-1}\)), washed with tap water on a 125 µm screen and then transferred to seawater for 24 h at 28-30 °C under continuous light and strong aeration (Lavens and Sorgeloos, 1996). After hatching, the nauplii were harvested and enriched with A1 DHA Selco (INVE Aquaculture NV) for 24 h following the manufacturer’s protocol.
During the first two days, larvae were pre-conditioned in the tanks with only enriched *Artemia* nauplii at a density of 0.3-0.5 ind. mL\(^{-1}\). Feeds were added 4 times a day. From day 3 onwards, the formulated feeds in the size range of 160-300 µm were manually introduced in the tanks, 30 minutes prior to *Artemia* feeding. After 2-3 days, the bigger size (300-400 µm) was used. The feeding frequency of formulated diets was gradually increased up to 7 times per day. Feed amounts were adjusted in function of the appetite of the juveniles. From 19 dph, the amount of enriched *Artemia* was gradually reduced and stopped after 4 days. The experiment terminated when the cobia were solely fed formulated feed for 8 days (Fig. 6.1).

Good water quality was maintained in the culture tanks by daily siphoning tank sediments and by recirculating water over a biological filter at an exchange rate of 3-5 L min\(^{-1}\) tank\(^{-1}\). Environmental factors such as DO, pH, water and salinity were measured twice a day (7 am and 2 pm). The results (mean±SD) were 29.0±0.62 °C, 6.4±0.3 mg g\(^{-1}\) and 18.1±1.0 g salts L\(^{-1}\) and pH was ranged of 7.2 to 8.9 over the culture period.

### 2.3 Evaluations

The growth of cobia juveniles was evaluated by measuring size and weight. Size of cobia, expressed as means of standard length (SL, cm) and body weight (g), was determined by random sampling (n>20) and measured before and after the experiment using a graduated ruler (mm) and electronic balance (HF-1200G, d=0.01g), respectively. The daily specific growth rate (SGR\(_w\)) was calculated using the following formula:

\[
SGR_w (% \text{ day}^{-1}) = \frac{100 \times (\text{LnW}_t - \text{LnW}_i)}{t}
\]
Where: \( W_i \) and \( W_f \) are mean of initial wet weight and final wet weight, respectively and \( t \) is number of experimental days.

Size variation was evaluated according to Wang et al. (1998) in which the mean of three replicates of the coefficient of variation (CV) was used to examine the inter-individual weight variation among the fish in each treatment:

\[
CV(\%) = 100 \times \frac{SD}{M}
\]

Where \( M \) is the mean body weight and \( SD \) is the standard deviation of the fish in each treatment.

The natural mortality was determined from the number of dead fish removed daily from the tanks, while cannibalism was calculated following Curnow et al. (2006): the percentage of the initial number of larvae that could not be accounted for mortalities as a result of sampling and natural mortalities during the trial and were calculated according to the following equation:

\[
\text{Cannibalism}(\%) = 100 \times \frac{(I-S-F-M)}{I}
\]

Where, \( I \) is the number of larvae initially stocked, \( S \) is the number of larvae sampled for measurements during the trial, \( F \) is the final number of larvae in each tank and \( M \) is the number of natural mortalities during the trial.

The vitality of the cobia larvae was estimated by the Cumulative Stress Index (CSI) following exposure of the larvae to a high salinity (70 g L\(^{-1}\)) for 1 hour as described in Nhu et al. (2009a). The average of CSI of each treatment was calculated from the replicates of each tank, the higher CSI, the lower the quality of the juveniles.
The transportation test was conducted in plastic bags. In this test, 10 cobia juveniles were randomly collected from each tank, starved for at least 24 h in a separated tank before being packed in a plastic bag containing 1 L seawater. Subsequently, 2 L pure oxygen was added to each bag. All bags then were packed in a polystyrene box. Ice was added between the plastic bags to maintain the water temperature at 22-24 °C. The boxes were gently shaken every 2 hours and fish behavior as well as their mortality was inspected every 6 hours. The test was terminated after 48 hours.

Cobia larvae and juveniles were randomly sampled for fatty acid analysis at 0 dph (newly hatched), 3 dph (start exogenous feeding), 12 dph (beginning of experiment) and 30 dph (end of experiment). The juveniles collected at 12 dph and 30 dph were starved for 24 hours to allow evacuation of feed from their digestive tract. Subsequently, all samples were washed in fresh water, put in 10-mL plastic tubes and submerged in liquid nitrogen before being stored in a freezer at -80°C. The samples were then lyophilized in a freeze dryer (MODULYOD-230, USA, serial P16S-653719-PS) and frozen at -80°C until later analysis.

The total lipid content (% of dry weight) was analyzed by using the method described by Bligh and Dyer (1959). Fatty acids, expressed in mg g⁻¹ dry weight (DW), were analysed by using the method described by Metcalfe et al. (1966). The analytical work was done in SeaLab, Norwegian University of Science and Technology, Trondheim, Norway.

2.4 Data analysis

All data were tested for significant differences ($P<0.05$) of growth, survival, mortality and quality (including survival in the transportation test and CSI salinity test) as well as fatty acids profile of the juveniles between the treatments using one-way ANOVA.
followed by LSD test for multiple comparisons of means. The data are expressed as mean±SD. Linear regression analysis was used to evaluate the relationship between DHA content as well as the DHA/EPA ratio in the formulated diets, on one hand, and performance of cobia juveniles in terms of growth, survival, cannibalism and DHA content as well as DHA/EPA ratio in their body, on the other hand. Statistical analyses were performed using SPSS version 13.0 and Microsoft Office EXCEL for Windows version 2007.

3. Results

3.1 Fatty acids profile and total lipids of the diets for the cobia juveniles.

Table 6. 1. Fatty acid profile (mg g⁻¹ DW) and total lipids (% DW) of the diets for cobia juveniles in the experiment.

<table>
<thead>
<tr>
<th>φEA</th>
<th>EA</th>
<th>Ex1</th>
<th>Ex2</th>
<th>Ex3</th>
</tr>
</thead>
<tbody>
<tr>
<td>14:00</td>
<td>0.50</td>
<td>4.48</td>
<td>4.31</td>
<td>6.14</td>
</tr>
<tr>
<td>14:1(n-5)</td>
<td>0.00</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td>16:00</td>
<td>7.74</td>
<td>18.82</td>
<td>18.15</td>
<td>23.14</td>
</tr>
<tr>
<td>16:1(n-7)</td>
<td>2.52</td>
<td>3.34</td>
<td>3.41</td>
<td>3.57</td>
</tr>
<tr>
<td>18:00</td>
<td>7.14</td>
<td>2.81</td>
<td>2.73</td>
<td>3.11</td>
</tr>
<tr>
<td>18:1(n-9)</td>
<td>18.14</td>
<td>9.76</td>
<td>9.70</td>
<td>9.80</td>
</tr>
<tr>
<td>18:1(n-7)</td>
<td>7.66</td>
<td>2.51</td>
<td>2.44</td>
<td>2.54</td>
</tr>
<tr>
<td>18:2(n-6)</td>
<td>4.31</td>
<td>9.77</td>
<td>9.89</td>
<td>8.74</td>
</tr>
<tr>
<td>18:3(n-6)</td>
<td>0.29</td>
<td>0.13</td>
<td>0.19</td>
<td>0.24</td>
</tr>
<tr>
<td>18:3(n-3)</td>
<td>18.28</td>
<td>1.49</td>
<td>1.59</td>
<td>1.39</td>
</tr>
<tr>
<td>18:4(n-3)</td>
<td>1.66</td>
<td>0.90</td>
<td>1.20</td>
<td>1.19</td>
</tr>
<tr>
<td>20:00</td>
<td>0.19</td>
<td>0.14</td>
<td>0.14</td>
<td>0.16</td>
</tr>
<tr>
<td>20:1(n-9)</td>
<td>0.89</td>
<td>3.82</td>
<td>3.69</td>
<td>3.46</td>
</tr>
<tr>
<td>20:2(n-6)</td>
<td>0.30</td>
<td>0.19</td>
<td>0.23</td>
<td>0.18</td>
</tr>
<tr>
<td>20:3(n-6)</td>
<td>0.08</td>
<td>0.15</td>
<td>0.20</td>
<td>0.24</td>
</tr>
<tr>
<td>Fatty Acid</td>
<td>Ex1</td>
<td>Ex2</td>
<td>Ex3</td>
<td>Ex4</td>
</tr>
<tr>
<td>--------------------</td>
<td>--------</td>
<td>--------</td>
<td>--------</td>
<td>--------</td>
</tr>
<tr>
<td>20:4 (n-6) ARA</td>
<td>0.71</td>
<td>1.07</td>
<td>2.08</td>
<td>1.59</td>
</tr>
<tr>
<td>20:3 (n-3)</td>
<td>0.61</td>
<td>0.17</td>
<td>0.23</td>
<td>0.20</td>
</tr>
<tr>
<td>20:4 (n-3)</td>
<td>0.27</td>
<td>0.52</td>
<td>0.72</td>
<td>0.75</td>
</tr>
<tr>
<td>20:5 (n-3) EPA</td>
<td>4.86</td>
<td>5.79</td>
<td>9.77</td>
<td>8.91</td>
</tr>
<tr>
<td>22:00</td>
<td>0.18</td>
<td>0.14</td>
<td>0.15</td>
<td>0.16</td>
</tr>
<tr>
<td>22:1 (n-9)</td>
<td>0.25</td>
<td>0.55</td>
<td>0.54</td>
<td>0.50</td>
</tr>
<tr>
<td>22:5 (n-6)</td>
<td>0.08</td>
<td>4.45</td>
<td>5.11</td>
<td>8.81</td>
</tr>
<tr>
<td>22:5 (n-3)</td>
<td>0.19</td>
<td>0.74</td>
<td>1.46</td>
<td>2.16</td>
</tr>
<tr>
<td>24:00</td>
<td>0.03</td>
<td>0.13</td>
<td>0.19</td>
<td>0.18</td>
</tr>
<tr>
<td>22:6 (n-3) DHA</td>
<td>1.00</td>
<td>21.12</td>
<td>37.57</td>
<td>53.31</td>
</tr>
<tr>
<td>24:1</td>
<td>0.00</td>
<td>0.35</td>
<td>0.40</td>
<td>0.32</td>
</tr>
<tr>
<td>Total HUFA</td>
<td>8.11</td>
<td>32.87</td>
<td>57.37</td>
<td>76.16</td>
</tr>
<tr>
<td>Total FAME</td>
<td>85.42</td>
<td>101.77</td>
<td>125.43</td>
<td>150.17</td>
</tr>
<tr>
<td>Total lipids</td>
<td>13.30</td>
<td>15.91</td>
<td>17.45</td>
<td>20.27</td>
</tr>
<tr>
<td>DHA/EPA</td>
<td>0.2</td>
<td>3.6</td>
<td>3.8</td>
<td>6.0</td>
</tr>
</tbody>
</table>

HUFA = Highly Unsaturated Fatty Acids defined as fatty acids with at least 20 carbon atoms and more than one double bond; EA = Enriched Artemia nauplii; Ex1, Ex2 and Ex3 = Experimental and commercial diets formulated by INVE Technologies NV.

The DHA/EPA ratios in the diet Ex3 are relatively high compared to those of the other experimental diets (Table 6.1). It was noted that total lipids and contents of other essential fatty acids in the enriched Artemia nauplii were lower than in the formulated feeds. In this experiment, enrichment of Artemia nauplii was not very effective as they had a low fatty acid content compared to the other diets.

### 3.2 Growth performance, survival and mortality

#### 3.2.1 Growth performance of cobia juveniles

The final weight as well as the specific growth rate (SGRw) of cobia juveniles was the lowest in treatment T1 ($P<0.05$). The final weight and SGRw of treatment T2 were not
significantly different from treatment T3 \((P>0.05)\). There was no significant difference \((P<0.05)\) in final length and in size variation among fish fed the experimental diets (Table 6.2). Daily observation indicated that cobia juveniles in treatment T3 were more interested in the diet Ex3 and had schooling behavior during feeding.

**Table 6.2. Growth performance of 30-dph cobia juveniles fed different diets.**

<table>
<thead>
<tr>
<th>Treatments</th>
<th>T1</th>
<th>T2</th>
<th>T3</th>
</tr>
</thead>
<tbody>
<tr>
<td>SGR(_w) (% day(^{-1}))</td>
<td>22.60±0.11(^b)</td>
<td>23.46±0.24(^a)</td>
<td>23.77±0.47(^a)</td>
</tr>
<tr>
<td>Final weight (g)</td>
<td>0.66±0.02(^b)</td>
<td>0.77±0.02(^a)</td>
<td>0.80±0.07(^a)</td>
</tr>
<tr>
<td>Final length (cm)</td>
<td>4.8±0.1(^a)</td>
<td>5.1±0.2(^a)</td>
<td>5.1±0.2(^a)</td>
</tr>
<tr>
<td>Size variation (%)</td>
<td>14.37±0.87(^a)</td>
<td>14.20±3.24(^a)</td>
<td>12.12±0.12(^a)</td>
</tr>
</tbody>
</table>

Values (mean±SD, \(n=3\)) followed by different superscript letters within a row are significantly different \((P<0.05)\). SGR\(_w\) = Specific growth rate. T1, T2 and T3 = Treatments fed the experimental diets Ex1, Ex2 and Ex3, respectively.

3.2.2 Survival and mortality of cobia juveniles

**Table 6.3. Survival and mortality pattern of 30-dph cobia juveniles fed different diets.**

<table>
<thead>
<tr>
<th>Treatments</th>
<th>T1</th>
<th>T2</th>
<th>T3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Survival (%)</td>
<td>53.11±3.34(^b)</td>
<td>62.11±4.67(^ab)</td>
<td>69.22±8.85(^a)</td>
</tr>
<tr>
<td>Natural mortality (%)</td>
<td>25.56±2.17(^a)</td>
<td>22.56±5.70(^a)</td>
<td>21.22±2.34(^a)</td>
</tr>
<tr>
<td>Cannibalism (%)</td>
<td>21.33±2.96(^a)</td>
<td>15.33±6.89(^ab)</td>
<td>9.56±8.00(^b)</td>
</tr>
</tbody>
</table>

Values (mean±SD, \(n=3\)) followed by different superscript letters within a row are significantly different \((P<0.05)\); T1, T2 and T3 = Treatments fed the experimental diets Ex1, Ex2 and Ex3, respectively.
The juveniles fed diet Ex3 had significant higher survival compared to the ones fed diet Ex1 \((P<0.05)\), while the survival of the juveniles fed diet Ex2 was not significantly different from the others. The natural mortality was similarity in all treatments, but the cannibalism rate of the juveniles fed diet Ex3 was significantly lower \((P<0.05)\) than the ones fed diet Ex1 (Table 6. 3). It should also be mentioned here that the natural and cannibalistic mortality was an approximate number: the cannibalism rate was defined as the rate of fish disappearing during the experiment, while the ones that died as a result of biting or not successful swallowing were referred to as natural mortality. Therefore, the real cannibalistic rate could be higher.

![Figure 6.1](image)

**Figure 6.1.** Daily mortality of cobia juveniles (mean±SD, \(n=3\)) of different treatments in relationship with feeding regime.

T1, T2 and T3 = Treatments fed the experimental diets Ex1, Ex2 and Ex3, respectively.
The daily mortality indicated that there were mortality peaks at day 2 and around day 11 of the culture (Fig. 6. 1). These high mortalities happened at the beginning of the experiment and coincide with the time of reduction and stop of live food feeding. Although the number of dead fish fluctuates between the treatments, the trend of mortality was similar for all treatments.

The regression analysis revealed that there were no significant relationships between the DHA/EPA ratios in the diets and growth, survival as well as cannibalism of cobia juveniles. However, the levels of DHA in the diets had a strong-positive correlation only with mean of survival and a negative correlation with mean of cannibalism ($P<0.05$, Fig. 6. 2).

![Figure 6. 2. Relationship between DHA contents in formulated diets and survivals and cannibalisms of cobia juveniles. $n=3$.](image-url)
**3.3 Quality of cobia juveniles**

### 3.3.1 Total lipids and fatty acids profile of cobia juveniles

The newly hatched larvae (0 dph) were analysed together with the first feeding larvae (3 dph) and the ones at the start of the experiment (12 dph) to investigate the change in their fatty acids profile. Those data can also be used to compare with the juveniles fed the experimental diets. In this regard, the content of the selected fatty acids, total fatty acids as well as total lipids had an inverse relationship with the larval age but their DHA/EPA ratios were increasing (Table 6.4).

**Table 6.4. Fatty acid profile (mg g\(^{-1}\) DW) and total lipids (% DW) of cobia larvae at different development stages.**

<table>
<thead>
<tr>
<th>Fatty Acid</th>
<th>Cobia 0 dph</th>
<th>Cobia 3 dph</th>
<th>Cobia 12 dph</th>
</tr>
</thead>
<tbody>
<tr>
<td>14:00</td>
<td>1.75±0.00</td>
<td>1.58±0.00</td>
<td>0.41±0.01</td>
</tr>
<tr>
<td>16:00</td>
<td>34.01±0.12</td>
<td>25.11±0.20</td>
<td>9.81±0.15</td>
</tr>
<tr>
<td>16:1(n-7)</td>
<td>10.24±0.02</td>
<td>9.88±0.13</td>
<td>1.37±0.03</td>
</tr>
<tr>
<td>18:00</td>
<td>12.94±0.35</td>
<td>10.12±0.10</td>
<td>7.90±0.00</td>
</tr>
<tr>
<td>18:1(n-9)</td>
<td>41.06±1.19</td>
<td>35.79±0.55</td>
<td>8.97±0.13</td>
</tr>
<tr>
<td>18:1(n-7)</td>
<td>7.90±0.03</td>
<td>6.56±0.09</td>
<td>3.13±0.03</td>
</tr>
<tr>
<td>18:2(n-6)</td>
<td>2.68±0.03</td>
<td>2.29±0.03</td>
<td>2.01±0.07</td>
</tr>
<tr>
<td>18:3(n-6)</td>
<td>0.65±0.33</td>
<td>0.76±0.03</td>
<td>0.18±0.00</td>
</tr>
<tr>
<td>18:3(n-3)</td>
<td>1.07±0.03</td>
<td>1.09±0.05</td>
<td>8.58±0.13</td>
</tr>
<tr>
<td>18:4(n-3)</td>
<td>0.84±0.02</td>
<td>0.95±0.01</td>
<td>0.82±0.02</td>
</tr>
<tr>
<td>20:00</td>
<td>0.74±0.49</td>
<td>1.22±0.27</td>
<td>0.18±0.00</td>
</tr>
<tr>
<td>20:1(n-9)</td>
<td>0.64±0.48</td>
<td>0.39±0.01</td>
<td>0.24±0.02</td>
</tr>
<tr>
<td>20:2(n-6)</td>
<td>0.23±0.01</td>
<td>0.25±0.02</td>
<td>0.13±0.00</td>
</tr>
<tr>
<td>20:3(n-6)</td>
<td>0.24±0.01</td>
<td>0.22±0.00</td>
<td>0.09±0.00</td>
</tr>
<tr>
<td>Fatty Acid</td>
<td>T1</td>
<td>T2</td>
<td>T3</td>
</tr>
<tr>
<td>-----------</td>
<td>---------------</td>
<td>---------------</td>
<td>---------------</td>
</tr>
<tr>
<td>20:4(n-6) ARA</td>
<td>5.93±0.00</td>
<td>4.32±0.00</td>
<td>1.59±0.00</td>
</tr>
<tr>
<td>20:3(n-3)</td>
<td>0.10±0.03</td>
<td>0.11±0.01</td>
<td>0.42±0.10</td>
</tr>
<tr>
<td>20:4(n-3)</td>
<td>0.49±0.07</td>
<td>0.49±0.01</td>
<td>0.23±0.00</td>
</tr>
<tr>
<td>20:5(n-3) EPA</td>
<td>8.96±0.10</td>
<td>7.11±0.15</td>
<td>1.57±0.02</td>
</tr>
<tr>
<td>22:00</td>
<td>0.35±0.16</td>
<td>0.18±0.00</td>
<td>0.18±0.00</td>
</tr>
<tr>
<td>22:1(n-9)</td>
<td>0.09±0.02</td>
<td>0.07±0.00</td>
<td>0.02±0.02</td>
</tr>
<tr>
<td>22:5(n-6)</td>
<td>2.00±0.03</td>
<td>2.27±0.02</td>
<td>1.28±0.01</td>
</tr>
<tr>
<td>22:5(n-3)</td>
<td>3.01±0.02</td>
<td>3.04±0.04</td>
<td>0.53±0.00</td>
</tr>
<tr>
<td>24:00</td>
<td>0.12±0.00</td>
<td>0.14±0.00</td>
<td>0.15±0.00</td>
</tr>
<tr>
<td>22:6(n-3) DHA</td>
<td>41.29±0.42</td>
<td>39.91±0.77</td>
<td>10.33±0.13</td>
</tr>
<tr>
<td>24:1</td>
<td>0.10±0.14</td>
<td>0.24±0.00</td>
<td>0.08±0.00</td>
</tr>
<tr>
<td>Total HUFA</td>
<td>62.25±0.47</td>
<td>57.72±1.01</td>
<td>16.16±0.05</td>
</tr>
<tr>
<td>Total FAME</td>
<td>205.63±18.01</td>
<td>176.83±9.74</td>
<td>67.19±0.61</td>
</tr>
<tr>
<td>Total lipids</td>
<td>37.13±0.00</td>
<td>31.06±1.17</td>
<td>13.73±0.77</td>
</tr>
<tr>
<td>DHA/EPA</td>
<td>4.6</td>
<td>5.6</td>
<td>6.6</td>
</tr>
</tbody>
</table>

Value = Mean±SD (n=2); HUFA = Highly unsaturated fatty acids defined as fatty acids with at least 20 carbon atoms and more than one double bond.

Table 6.5. Fatty acid profile (mg g⁻¹ DW) and total lipids (% DW) of cobia juveniles in different treatments.
<table>
<thead>
<tr>
<th></th>
<th>T1</th>
<th>T2</th>
<th>T3</th>
</tr>
</thead>
<tbody>
<tr>
<td>18:2(n-6)</td>
<td>9.18±1.67</td>
<td>7.25±0.03</td>
<td>6.05±0.83</td>
</tr>
<tr>
<td>18:3(n-6)</td>
<td>0.13±0.03</td>
<td>0.12±0.01</td>
<td>0.10±0.01</td>
</tr>
<tr>
<td>18:3(n-3)</td>
<td>1.44±0.29</td>
<td>1.04±0.09</td>
<td>0.83±0.21</td>
</tr>
<tr>
<td>18:4(n-3)</td>
<td>0.54±0.15</td>
<td>0.46±0.04</td>
<td>0.36±0.08</td>
</tr>
<tr>
<td>20:00</td>
<td>0.21±0.00</td>
<td>0.18±0.01</td>
<td>0.17±0.01</td>
</tr>
<tr>
<td>20:1(n-9)</td>
<td>2.97±0.51</td>
<td>2.24±0.19</td>
<td>1.73±0.26</td>
</tr>
<tr>
<td>20:2(n-6)</td>
<td>0.30±0.04</td>
<td>0.24±0.01</td>
<td>0.20±0.02</td>
</tr>
<tr>
<td>20:3(n-6)</td>
<td>0.16±0.02</td>
<td>0.12±0.01</td>
<td>0.10±0.01</td>
</tr>
<tr>
<td>20:4(n-6) ARA</td>
<td>1.34±0.13\textsuperscript{a}</td>
<td>1.41±0.15\textsuperscript{b}</td>
<td>1.08±0.02\textsuperscript{b}</td>
</tr>
<tr>
<td>20:3(n-3)</td>
<td>0.18±0.03</td>
<td>0.14±0.01</td>
<td>0.10±0.02</td>
</tr>
<tr>
<td>20:4(n-3)</td>
<td>0.43±0.12</td>
<td>0.30±0.02</td>
<td>0.22±0.05</td>
</tr>
<tr>
<td>20:5(n-3) EPA</td>
<td>3.39±0.95\textsuperscript{b}</td>
<td>3.09±0.41\textsuperscript{bc}</td>
<td>2.49±0.34\textsuperscript{c}</td>
</tr>
<tr>
<td>22:00</td>
<td>0.66±0.83</td>
<td>0.11±0.01</td>
<td>0.11±0.01</td>
</tr>
<tr>
<td>22:1(n-9)</td>
<td>0.26±0.21</td>
<td>0.32±0.03</td>
<td>0.24±0.04</td>
</tr>
<tr>
<td>22:5(n-6)</td>
<td>3.61±0.73</td>
<td>2.22±0.25</td>
<td>2.20±0.66</td>
</tr>
<tr>
<td>22:5(n-3)</td>
<td>0.97±0.26</td>
<td>0.72±0.10</td>
<td>0.73±0.18</td>
</tr>
<tr>
<td>24:00</td>
<td>0.16±0.01</td>
<td>0.16±0.01</td>
<td>0.15±0.01</td>
</tr>
<tr>
<td>22:6(n-3) DHA</td>
<td>16.49±4.03\textsuperscript{a}</td>
<td>13.46±2.28\textsuperscript{a}</td>
<td>14.23±1.19\textsuperscript{a}</td>
</tr>
<tr>
<td>24:1</td>
<td>0.29±0.03</td>
<td>0.26±0.01</td>
<td>0.25±0.08</td>
</tr>
<tr>
<td>Total HUFA</td>
<td>26.86±6.29\textsuperscript{a}</td>
<td>21.71±3.22\textsuperscript{b}</td>
<td>21.36±2.18\textsuperscript{b}</td>
</tr>
<tr>
<td>Total FAME</td>
<td>100.89±14.54\textsuperscript{a}</td>
<td>81.62±3.27\textsuperscript{b}</td>
<td>76.90±7.22\textsuperscript{b}</td>
</tr>
<tr>
<td>Total lipids</td>
<td>15.39±0.69\textsuperscript{b}</td>
<td>17.31±1.15\textsuperscript{a}</td>
<td>15.01±1.08\textsuperscript{b}</td>
</tr>
<tr>
<td>DHA/EPA</td>
<td>4.9</td>
<td>4.4</td>
<td>5.8</td>
</tr>
</tbody>
</table>

Values (mean±SD, \textit{n}=3) followed by different superscript letters within a row are significantly different ($P<0.05$); HUFA = Highly unsaturated fatty acids defined as fatty acids with at least 20 carbon atoms and more than one double bond. T1, T2 and T3 = Treatments fed the experimental diets Ex1, Ex2 and Ex3, respectively.
The DHA content of cobia juveniles fed different formulated diets was not significantly different ($P>0.05$) among the treatments. A positive correlation between the DHA/EPA ratio in the tissues of the juveniles and the DHA/EPA ratio in the diet Ex3 (treatment T3) was found (Table 6.5). However, DHA content and DHA/EPA ratio in the tissue could not be significantly correlated ($P>0.05$) with the dietary content among the treatments.

It is noticed that total HUFA as well as total FAME of the cobia juveniles in treatment T1 were the highest ($P<0.05$), while those of the others were not significantly difference ($P>0.05$). On the other hand, total lipids of cobia juveniles in treatment T2 were the highest. No significant differences in total lipids were detected between treatment T1 and T3.

### 3.3.2 Vitality of cobia juveniles

Table 6.6. Cumulative Stress Index (CSI) and survival of the juveniles in vitality tests.

<table>
<thead>
<tr>
<th></th>
<th>T1</th>
<th>T2</th>
<th>T3</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CSI of cobia juveniles in salinity and freshwater tests</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CSI of salinity test (1 h, $t=27, ^\circ C$, $S=70, g, L^{-1}$)</td>
<td>28.00±14.73&lt;sup&gt;a&lt;/sup&gt;</td>
<td>12.67±5.13&lt;sup&gt;a&lt;/sup&gt;</td>
<td>24.67±14.36&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>CSI of fresh water test (1 h, $t=27, ^\circ C$, $S=0, g, L^{-1}$)</td>
<td>6.33±5.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.33±3.06&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.67±2.89&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Survivals (%) of cobia juveniles in transportation test</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>After 36 h</td>
<td>66.7±11.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>100.0±0.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>100.0±0.0&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>After 48 h</td>
<td>56.7±20.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>80.0±10.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>80.0±10.0&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values (mean±SD, $n=3$) followed by different superscript letters within a row are significantly different ($P<0.05$); S = Salinity; T1, T2 and T3 = Treatments fed the experimental diets Ex1, Ex2 and Ex3, respectively.
In the vitality tests, the CSI of salinity and fresh water tests were variable, but there were no significant differences \((P>0.05)\) between the treatments (Table 6. 6). The survival of treatment T2 and T3 in transportation conditions remained high (100 %), up to 36 and 42 h, respectively, while that of T1 started to decrease just after 30 h. Survivals of the juveniles in treatment T1 were significantly lower \((P<0.05)\) than the others after 36 h (Table 6. 6). After 48 h in transportation conditions, survivals of treatment T2 and T3 decreased, but still remained 80 % and were not significantly difference \((P<0.05)\) between the treatments (Fig. 6. 3).

![Figure 6. 3. Survivals of cobia in the transportation test.](image)

Value = Mean±SD \((n=3)\); T1, T2 and T3 = Treatments fed the experimental diets Ex1, Ex2 and Ex3, respectively.

4. Discussion

The dietary requirement for EFA of most marine fish larvae has been well documented in which DHA was considered as the most important one (Ostrowski and
Divakaran, 1989; 1990; Sargent et al., 1999a,b). Results of this experiment confirmed the importance of dietary DHA for growth, survival and vitality of cobia juveniles during weaning. Marine fish are not able to convert 18:3(n-3) to EPA nor to convert 18:2(n-6) to 20:4(n-6) as they contain very low or negligible Δ-5 fatty acid desaturate activity. In addition, the conversion of EPA to DHA cannot fully meet the high demand of DHA during larval growth since EPA can be converted to 22:5(n-3), but not further to DHA (Sargent et al., 1997). Therefore, DHA is very essential for growth and survival of marine fish larvae. According to Cahu et al. (2009), the ideal diet for fish larvae should have a fatty acid composition close to that of fish eggs. The high amount of EFA in cobia eggs i.e. total DHA, EPA and ARA account for 80% of the total fatty acids (Faulk and Holt, 2003). Moreover, the DHA content of 41.3-42.8 mg g⁻¹ DW in eggs from captive broodstock (Faulk and Holt, 2008) indicated their high dietary requirement for these fatty acids. In our experiment, the DHA content of Ex2 diet (37.57 mg g⁻¹ DW) was comparable to the cobia eggs (41.3-42.8 mg g⁻¹ DW (Faulk and Holt, 2008)). The higher DHA content in Ex3 diet (53.31 mg g⁻¹ DW) did not increase the SGRₜₑ (P>0.05). This result revealed that the diet Ex2 with DHA content of 37.57 mg g⁻¹ DW seemed to meet the DHA requirement. However, higher survival in treatment T3 indicated that the appropriate dietary DHA content and appropriate DHA/EPA ratio need to be clarified in order to optimize growth and survival of cobia juveniles. The low DHA content in diet Ex1 (21.12 mg g⁻¹ DW) resulted in significantly lower growth (P<0.05), confirming the importance of this essential fatty acid. On the other hand, in terms of general nutritional requirements, live food remained important to satisfy the balance for other nutrients and to support the effective digestion by cobia juveniles, such as providing exogenous enzymes, amino acids, vitamins... The higher daily mortality happening during the reduction
and stop of enriched *Artemia* nauplii feeding, regardless of the different dietary DHA contents and DHA/EPA ratios, was an indication that the live food was not effectively enriched to obtain the required levels of the fatty acids.

The content of EFA as well as total lipids in cobia larvae was reduced during their early development. This is in agreement with Holt et al. (2007a) in a study on the early stages of cobia larvae. The DHA content in newly hatched larvae (41.29 mg g$^{-1}$ DW) was similar to the result obtained by Faulk and Holt (2008). The content of other EFA in cobia larvae in our experiment was slightly lower, but on the other hand, the DHA/EPA ratio was higher than the ones reported by Holt et al. (2007a). This is probably due to the broodstock diets, which is reflected in the egg composition (Nguyen et al., accepted). Rainuzzo et al. (1997) reviewed that in fish, lipid depletion occurred quickly during starvation and the fatty acids that play an important structural role in the cell membrane, tend to be preserved. In this regard, the DHA was more strongly conserved than EPA and other fatty acids. The increase of the DHA/EPA ratio in accordance with their age indicated that cobia larvae tend to reserve DHA rather than other fatty acids and confirmed the importance of dietary DHA for their early development. The result also indicated a possibility that nutritional supplementation might not satisfy the larval requirement during period 3-12 dph.

Levels of DHA contents and DHA/EPA ratios in the tissues did not strictly correlate with the dietary contents although the higher DHA/EPA ratio was detected in the treatment fed a diet with an extremely high DHA/EPA ratio (treatment T3). This is in agreement with Turner and Rooker (2005), who investigated that the dietary HUFA can be easily incorporated in cobia larvae and juveniles, but did not mention the correlation of these fatty acid levels between diets and their body. The incorporation
of dietary DHA into the tissue is affected not only by the dietary DHA content, but also by the class of the phospholipids fraction and source (Cahu et al., 2009; Sargent et al., 1997), the presence and content of others fatty acids (Sargent et al., 1997). In this regard, Sargent et al. (1997) assumed that a too low DHA/EPA ratio may be harmful as EPA may inhibit production of eicosanoids from ARA. Therefore, comparison of DHA content and DHA/EPA ratio in the tissues of juveniles with different sizes may be not reasonable. High level of total HUFA, total FAME as well as DHA/EPA ratio in the juveniles fed diet Ex1 with a low DHA content and a low DHA/EPA ratio, might be derived from cannibalism. In this case, the juveniles in treatment T1 had very high cannibalism (21.33 %) and the fatty acid contents in their tissues might be affected not only by the experimental diet but also by the smaller juveniles that were eaten as a result of sibling predation.

Cannibalism in fish larvae affects the yield and has been well documented in various species with causative factors among others: size difference, lack of turbidity, larval density and food composition and abundance (Liao et al., 2001). In our experiment, fish were cultivated in the same conditions and their size variation was not significantly different among dietary treatments. Therefore, the nutritional composition was probably the main reason of their cannibalism. Increasing of dietary DHA level seems to increase the satiation of the exogenous nutritional requirements of cobia juveniles, reducing their cannibalistic behavior. Thus, the increase in dietary DHA levels was positively correlated with the survival.

The mortality in the first two days of the experiment may be caused by stress of handling as cobia is an active species and is not resistant to stressors (Liao et al., 2004). Cobia were showing a response to acute stress i.e. the cortisol increased
dramatically and there was an extended impact on glucose concentration as well as a continuous increase in ceruloplasmin when being exposed to the air for only 1 min (Cnaani and McLean, 2009). Low stress resistance was also the main reason for high mortality during transportation of cobia juveniles (Liao et al., 2004). In our transportation test, better survivals of juveniles from treatments T2 and T3 compared to T1 revealed the positive effect of dietary DHA content and DHA/EPA ratio on cobia juvenile quality. The dietary DHA levels above 37.6 mg g$^{-1}$ DW (diet Ex2 and Ex3) resulted in higher survival (80%) for longer time (48 h) compared to treatment T1. It has been demonstrated that high dietary DHA levels improve stress tolerance of various marine fish species such as red seabream *Pagrus major* larvae, marbled sole *Limanda yokohamae* juveniles (Kanazawa, 1997) and yellowtail *Seriola quinqueradiata* (Takeuchi, 2001). In comparison with other transportation tests, the juveniles in our experiment were stocked at higher biomass (equivalent to 66-80 kg m$^{-3}$) and kept for the longer time (100% survival after 18, 36 and 42 h, for T1, T2 and T3 respectively) compared to the optimal stocking biomass (> 20 kg m$^{-3}$) for optimal duration (24 h) in an open transportation system, suggested by Colburn et al. (2008). However, dietary DHA did not lead to a higher mortality rate in the salinity tests. Cobia showed a good response to the change of salinity (Denson et al., 2003) or the effect of dietary DHA could be only detected after long period (Faulk and Holt, 2006). In addition, the different result of the two vitality tests, probably due to the stress tolerance of the cobia juveniles in the salinity test was affected only by the changing in osmotic regulation. The juveniles in the transportation test, in contract, were affected by a combination of different stressors such as fish interaction, low dissolved oxygen content, high concentration of NH$_3$ and others compounds from fish excretion. Overall, better survival in the transportation is very important as Liao et al.
Chapter 6

(2004) reported mortality during transportation as one of the most serious problems in cobia aquaculture in Taiwan. The result of this test opened the possibility to improve survival of cobia fry for long distance of transportation through nutritional manipulation. This result is important as more than 300,000 fingerlings annually need to be transferred from the North to the South of Vietnam, where a high potential of grow-out exits, but fingerling production is still not properly established. Results of this experiment indicated the possibility to improve quantity and quality of cobia juveniles in intensive rearing systems by the use of appropriate formulated diets.

5. Conclusions

Results of this experiment revealed that the contents of the essential fatty acids of cobia larvae decreased with their age, from 0 to 12 dph, but the DHA/EPA ratios increased. A high dietary DHA level and DHA/EPA ratio in the formulated weaning diets effectively improved growth and survival of cobia juveniles for the period 12-30 dph. Cannibalism of cobia juveniles was negatively correlated to the level of dietary DHA which eventually resulted in higher survival during the weaning stage. A high dietary DHA level and DHA/EPA ratio did not affect the vitality of cobia juveniles in the salinity test, but it effectively improved survival during the transportation test. Better growth and survival of cobia juveniles fed a high dietary DHA level and DHA/EPA ratio during weaning, indicated that the better quantity, quality and management of fingerling production can be achieved by appropriate formulated diets. Due to the important effect of this fatty acid, the optimal DHA content and the optimal ratio of DHA to other EFA needs to be clarified in future research.
Acknowledgments

The first author acknowledges the Belgian Technical Cooperation for his scholarship under a mixed PhD program between the Laboratory of Aquaculture & Artemia Reference Center, Ghent University, Belgium and the Research Institute for Aquaculture No1 (RIA1), Vietnam. The first author thanks Prof. Helge Reinertsen and Prof. Elin Kjørsvik, SeaLab, Norwegian University of Science and Technology (NTNU), Norway for kind support for the analytical works. He thanks also Ms. Kjersty Rennan Dahl and his colleague, Mr. Nguyen Quang Huy, SeaLab, NTNU, Norway for helps during his analytical works. The infrastructure of the experiments and analytical costs were supported by NORAD and the experimental test diets were provided by INVE Technologies NV, Belgium.
Chapter 7

General discussion and conclusions
1. General discussion

**Introduction**

Improvement of growth, survival and quality of juveniles reared in the intensive systems is essential for cobia aquaculture development. Generally, the first month of life is very critical for marine fish due to their morphological and cellular changes (Zambonino Infante and Cahu, 2001). During this period, the first feeding stage and the metamorphosis remain a bottle-neck, especially for fast growing species. High mortality occurs during the first 5-7 days for yellowtail kingfish *Seriola lalandi* (Hilton et al., 2008) or during the first 10 dph and from 14 to 30 dph of Pacific bluefin tuna *Thunnus orientalis* (Sawada et al., 2005) or during the first 30 dph of mahimahi *Coryphaena hippurus* (Lee, 1997). Cobia larvae grow very fast and may reach 0.4-0.8 g at 30 dph, comparable to the Pacific bluefin tuna *Thunnus orientalis* juveniles reared in nearly similar water temperature, reaching an average body weight of 0.6 g at the same age (Seoka et al., 2008). Thus, cobia belongs to the fast growing species and experiences similar development stages. After the first feeding stage, the metamorphosis and post metamorphosis are the most critical periods, when the larvae grow very fast, become more piscivorous and need to be weaned to formulated diets (Benetti et al., 2007; Holt et al., 2007b). Appropriate rearing density and feeding frequency increase benefit, while reduction of live food use will lower production cost of the fingerlings. Early co-feeding with appropriate formulated diets can meet the nutritional requirements of the larvae, resulting in growth and survival improvement. The main critical factors in cobia larviculture are appropriate nutrition, size variation and cannibalism (Schwarz et al., 2007a). Thus, improvement of live food for the first feeding stage, optimizing rearing conditions as well as providing appropriate feed
during metamorphosis and the weaning period are crucial for maximizing growth, survival and vitality of cobia juveniles.

**Optimal rearing density and feeding frequency during weaning**

Rearing density and feeding frequency are important factors to maximize the profit for commercial production of cobia juveniles. In our production system in Vietnam, the initial larval density is relatively high (30-50 larvae L⁻¹) to maximize the effectiveness of live food use during first feeding. As soon as the larvae start actively catching food, their density will be reduced to improve survival. Determining the appropriate rearing density during the weaning period is crucial for growth and survival improvement. At the same feeding frequency (5 times day⁻¹) and saturated feeding, lower density (1 weanling L⁻¹) resulted in better growth and survival compared to the high density (4 weanling L⁻¹), although the highest biomass was obtained in the latter (Chapter 3, experiment 1). This might be explained by the combined effects of fish interaction, nutrition and cannibalism. Our results were in agreement with Craig and McLean (2005) who obtained the best production in recirculation systems during weaning at a density of 2.5 weanling L⁻¹. Low survival in the high density treatment of our experiment was mainly related to high cannibalism.

In this regard, cobia is different from mahimahi *Coryphaena hippurus*, that a stocked juveniles (period 20-30 dph) at a density as high as 3 juveniles L⁻¹ to reduce their aggressive behavior and cannibalism (Kim et al., 1993; Lee, 1997). Therefore, we suggest a density of around 2 weanling L⁻¹ (0.02 g, equivalent to a biomass of 0.04 g L⁻¹), should be applied for the current rearing conditions. In the latter stage, rearing density becomes less important as Webb et al. (2007) demonstrated that the initial
rearing biomass of cobia juveniles (6.7 g) up to 0.44 g L\(^{-1}\) has no effect on their growth and survival compared to the initial stocking biomass of 0.04 or 0.22 g L\(^{-1}\).

The feeding frequency of every 4 or 2 h or continuous feeding did not affect cobia growth, but it significantly reduced cannibalism at continuous feeding (Chapter 3, experiment 2). In fact, more frequent food distribution and food abundance has been successfully applied to reduce cannibalism of species such as mahimahi *Coryphaena hippurus* (Lee, 1997; Lutesky and Szyyper, 1991) or Pacific bluefin tuna *Thunnus orientalis* (Sawada et al., 2005). As cobia juveniles are very sensitive to handling, frequent grading to avoid cannibalism during early development is not always effective in practice. Therefore, manipulation of feeding frequency can be considered.

**Possibility of enriched rotifers substitution by umbrella-stage of Artemia franciscana during first feeding**

Live foods production is cumbersome for up-scaling, enrichment and routine maintenance (Dhert et al., 1999) and special equipment is needed for operation. Therefore, live food production is regarded as a challenge for larviculture and propagation of marine fish. Replacement of live food by formulated diets will contribute to lower production costs and to a more predictable juvenile production. However, dry formulated diets can only be used several weeks after first feeding (Cahu and Zambonino Infante, 2001). Cobia larvae, similar to other fast growing species, require large amounts of high quality nutrition. In fact, mahimahi *Coryphaena hippurus* larvae with total length of 4.2 mm at hatching can rely upon *Artemia* nauplii as sole feed for their first feeding instead of rotifers (Kim et al., 1993). The newly hatched *Artemia* nauplii can be introduced during the first two days of feeding to mahimahi *Coryphaena hippurus* larvae, followed by enriched *Artemia*
metanauplii (Lee, 1997). In contrast, cobia larvae cannot be fed *Artemia* nauplii from the onset of exogenous feeding (Faulk and Holt, 2003). On the other hand, although UAF are inert diet, they are actively metabolizing embryos that might already contain free amino acids and fatty acids resulting from protein and lipid hydrolysis during embryonic development (Garcia-Ortega et al., 1998). The mouth size of cobia larvae at the first feeding was suitable for UAF ingestion. This possibility has been confirmed by observation of UAF in the digestive tract. The ability of cobia larvae to ingest and digest UAF was demonstrated by the similarity in growth, survival and quality of the larvae by 18 dph compared to the control which was fed enriched rotifers (Chapter 4). The digestive enzyme production increases from mouth opening (Faulk et al., 2007a). *Artemia* cysts are readily available and easy for storage, making UAF more convenient and more cost-effective than enriched rotifers. It may also be a potential substitution feed in case of low availability of enriched rotifers and/or as long as the appropriate compound feed for first feeding has not been formulated. As UAF can be successfully ingested and digested since first feeding of cobia larvae, a new live food strategy for cobia larviculture, which may also be applied for other fish larvae, is suggested.

**Feasibility of early co-feeding and the importance of live foods**

Early co-feeding (Chapter 5) was tested following the successful trial on using UAF as substitution of enriched rotifers for cobia larvae. This trial aimed to balance the nutritional requirements since UAF has a low DHA content in respect to the nutritional requirements of cobia larvae. Early co-feeding also targeted to reduce live foods for larviculture, improve the nutritional condition of the larvae and facilitate earlier weaning of the larvae onto a diet of dry feeds only. Among the two tested
diets, Proton® is a commercial diet that was designed for early co-feeding of marine fish larvae. Better growth of cobia larvae in the early co-feeding treatments indicated that Proton® can be ingested and digested from around 8-13 dph. These results confirmed the observations by Faulk et al. (2007a) that cobia larvae are capable of digesting and assimilating compound diets from 8 dph onwards due to the presence of gastric glands, periodic acid-Schiff positive surface mucous cells in the stomach and the appearance of lipid vacuoles together with an increase in pancreatic enzyme activities. Gastric glands of most of marine fish species differentiate at 22 dph (Zambonino Infante and Cahu, 2001), but for the fast growing species, they occur earlier i.e. at 11-14 dph in Pacific bluefin tuna Thunnus thynnus (Kaji et al., 1996) or at 15 dph in yellowtail kingfish Seriola lalandi (Chen et al., 2006). Pacific bluefin tuna Thunnus orientalis larvae can be fed on formulated diet supplemented with salmon roe phospholipids to achieve a survival of 13 % for the first 10 days (Seoka et al., 2008). However, small growth improvement in the treatment co-fed Proton® from 8 dph compared to the control (from 18 dph) indicated that the diet did not fulfil the larval requirements in terms of digestibility and nutritional composition. On the other hand, the experimental diet with a marked higher protein, as well as n-3 HUFA content and DHA/EPA ratio compared to Proton®, did not give any effect on the larvae. Our results suggest that during early development stage, when the larval digestive system is not fully functioning and the larvae still have to rely on live food, the digestibility/ palatability of the diet seems to be more important than the composition.

While mahimahi Coryphaena hippurus juveniles can start weaning from 20-25 dph and end after 5-7 days (Lee, 1997), Faulk et al., (2007a) and Holt et al. (2007b) suggested that it is possible to wean cobia juveniles from three weeks onwards and
weaning can be completed around 25 dph. However, mortality increased after reduction and withdrawal of live food in our weaning experiments (Chapter 5, experiment 3; Chapter 6) indicating that the digestive system of the juveniles was not fully functional to thrive solely on the dry diets. Live food is essential for marine fish larvae during first feeding, because of their nutritional balance, exogenous enzyme production, digestibility and attractiveness (Kolkovski et al., 1997a). Most marine fish larvae rely on live foods for their first feeding due to the lack of sufficiently active digestive enzymes to thrive on compound diets (Cahu and Zambonino Infante, 2001). The important role of live foods during early development was confirmed in the weaning experiments, but the real mechanism of the larval digestion process needs more investigation. Even though the importance of algae as green water in cobia larvae rearing tanks has been first described by Liao et al. (2004) and confirmed by Faulk and Holt (2005; Gaumet et al. (2007) and Holt et al. (2007a), the mechanisms of those algae for the larvae are still not clearly understood.

Early co-feeding can lead to a decrease in water quality due to the deterioration of uneaten feed. Increase of water exchange may improve water quality but also increase the live foods loss from rearing system. Therefore, the appropriate moment to start co-feeding needs to be determined considering the balance of both factors in order to increase the economical benefit in practices.

*Improvement of appropriate formulated weaning diets and changing of nutritional requirements during the larval development*

The improvement by using a commercial dry feed (NRD®) instead of using minced trash fish followed by a home-made moist diet, has been proved in experiment 3 (Chapter 3). Evaluation of the effect of those diets on performance of cobia juveniles
during weaning showed that the local-made diets may not satisfy their high nutritional requirements. NRD® showed its advantages in a lower FCR, easier feeding management and storage compared to the local-made diets. The better growth, survival and gained biomass of the cobia juveniles fed NRD® in this experiment also indicated that cobia can accept dry diets rather than the home-made moist diet or minced trash fish during weaning stage.

Results from previous experiments on early co-feeding and effect of different formulated diets for cobia larvae and juveniles justified the evaluation of the effects of dietary DHA contents and DHA/EPA ratios on their performance. Eggs and newly hatched larvae of cobia contain high levels of DHA, EPA and ARA, up to 80% of the total fatty acids (Faulk and Holt, 2003). In addition, although tissue contents of DHA and other fatty acids decreased with larval growth (from 0 to 12 dph), the DHA/EPA ratio increased (from 4.6 to 6.6, respectively) meaning cobia larvae tend to preserve DHA. In this aspect, cobia is similar to mahimahi (Coryphaena hippurus) that DHA content of the larval tissues is retained despite a decrease of fatty acid content and type over time (Ostrowski and Divakaran, 1990). The positive effects of high levels of dietary DHA and DHA/EPA ratio on growth, survival and quality of cobia juveniles during weaning (Chapter 6) confirmed the importance of this fatty acid for their early development. Our results support the finding of Niu et al. (2008) that high dietary phospholipid levels improve growth and survival of early cobia juveniles. It is interesting that high requirement of dietary phospholipids and dietary DHA was also detected in juveniles of the fast growing Pacific bluefin tuna Thunnus orientalis (Seoka et al., 2008) and Mahimahi Coryphaena hippurus (Ostrowski and Divakaran, 1989).
Chapter 7

The high requirement of dietary DHA in marine fish larvae in general, was well explained by incapability of converting 18:3(n-3) or 18:2(n-6) into EPA or 20:4(n-6), because they contain very low or negligible Δ-5 fatty acid desaturate activity and EPA cannot be converted into DHA to meet the high demand of DHA during larval growth (Sargent et al., 1997). It has been demonstrated that bioconversion of n-3 fatty acid precursors to the higher chain DHA derivative does not occur in mahimahi Coryphaena hippurus larvae, at least during their first week of development (Ostrowski and Divakaran, 1990). Higher survival (in the transportation test) and lower cannibalism in the treatments fed the diets with a DHA content and DHA/EPA ratio higher than 37 mg g⁻¹ DW and 3.8, respectively, suggested that such high dietary fatty acid contents, close to that of their eggs of 41.3-42.8 mg g⁻¹ DW (Faulk and Holt, 2008), may fulfil the nutritional requirements of cobia juveniles. These results indicated that growth, survival and quality of cobia can be improved by dietary manipulation during the critical weaning period. Therefore, these data will be useful for the feed industry in formulation of appropriate weaning diets for this species.

The change in nutritional requirements of most marine fish larvae is related to their developmental stage (Cahu and Zambonino Infante, 2001). This has been clearly indicated in the case of cobia (Chapter 5 and Chapter 6). For the stages of 8-23 dph, Proton® seems to be more appropriate than the experimental diet containing a higher n-3 HUFA and crude protein content and having a higher DHA/EPA ratio (Chapter 5, experiment 1 and experiment 2). As the stomach of cobia is completely developed and differentiated at 20 dph (16.4 mm) (Faulk et al., 2007a), the experimental diet was better digested and hence more effective compared to Proton® or NRD® for cobia juveniles at the stage of 20-38 dph (Chapter 5, experiment 3). Cobia require high levels of dietary DHA and DHA/EPA during period 12-30 dph (Chapter 6), but in a
later stage of 56-296 dph, Ding et al. (2009) found no significant effect of dietary DHA/EPA ratios ranging from 0.9 to 2.1.

**Improvement of larval growth, survival and quality in cobia larviculture**

Natural copepods are regarded as excellent food for marine larvae due to their balanced macronutrients composition, high level of EFA including DHA and optimum DHA/EPA ratio (van der Meeren et al., 2008). Holt et al. (2007a) confirmed that cobia larvae grow faster when fed wild zooplankton (mostly copepods) compared to rotifers and *Artemia sp*. Cobia larvae rearing in a semi-intensive system in the United States fed natural zooplankton had SGR$_w$ of 19.2 % day$^{-1}$ for the first 28 dph (Weirich et al., 2004). Cobia juveniles in our experiment fed high levels of dietary DHA (21.12, 37.57 and 53.31 mg g$^{-1}$ DW) had body weights of 0.66, 0.77 and 0.80 g, respectively, at 30 dph. The quality of cobia juveniles, expressed as survival in the transportation test, was better in the treatments fed high levels of dietary DHA. The ones fed diets with a DHA content higher than 37 mg g$^{-1}$ DW had a survival of 100 % for 36 h. This result is very important as high mortality of juveniles and fingerlings during transport has been considered as one of the most serious problems in cobia aquaculture (Liao et al., 2004). Cobia has a high metabolic rate due to their active behavior and cannot resist to stressors. As a result, they require high levels of dissolved oxygen. The better vitality obtained by earlier co-feeding of Proton® (Chapter 5, experiment 1) and better survival in the transportation test by using high dietary DHA and DHA/EPA ratio (Chapter 6) are promising ways for further improvement of quality of cobia juveniles in practice.

Cannibalism is the main reason of low survival during early juvenile stage of larviculture of the fast growing species. High cannibalistic rate occurs in Pacific
bluefin tuna *Thunnus orientalis* juveniles during period of 14 to 30 dph (Sawada et al., 2005) or in mahimahi *Coryphaena hippurus* juveniles during period 20 to 30 dph (Lee, 1997). Cobia juveniles experienced similar high cannibalism during and post metamorphosis. Thus, besides avian predation, cannibalism is the main reason of poor survival in semi-intensive systems in outdoor ponds (Benetti et al., 2007; Weirich et al., 2004; 2007). Cannibalism is promoted by size differences (Baras et al., 2000b), that can be reduced effectively by frequent sorting in the case of mahimahi *Coryphaena hippurus* (Lee, 1997) or Pacific bluefin tuna *Thunnus orientalis* (Sawada et al., 2005). In our intensive, experimental conditions, cannibalism was affected by rearing density, feeding frequency (Chapter 3, experiment 1 and experiment 2) and dietary DHA levels and DHA/EPA ratios (Chapter 6). Applying these factors at hatchery scale i.e. reducing rearing density, increasing feeding frequency and using high dietary DHA and DHA/EPA ratio may contribute to reduce cannibalism especially during metamorphosis, when larvae are stressful and frequent grading needs to be minimized.
2. General conclusions

Rearing density affects growth and survival of cobia during weaning. Among three different rearing densities of 1, 2 and 4 weanlings L\(^{-1}\), the highest density resulted in lower growth and survival. Meanwhile, only continuous feeding resulted in lower cannibalism compared to the feeding frequency of every 4 or 2 h.

Cobia larvae can ingest and digest UAF from first feeding and replacing enriched rotifers by UAF as starter food has very little effect on growth till 8 dph and appears to have no significant negative effect on larval quality, growth or survival by 18 dph.

Early co-feeding of Proton\(^\circ\) from 8 dph was possible and resulted in better growth, but not in higher survival of the juveniles compared to the ones co-fed from 13 or 18 dph. However, the experimental diet with higher n-3 HUFA content and DHA/EPA ratio compared to Proton\(^\circ\) did not have any effect in the co-feeding trial for 8-23 dph. On the other hand, during weaning stage (20-38 dph), it effectively supported growth, survival and quality of the juveniles.

The use of the NRD\(^\circ\) resulted in better growth performance and survival compared to the minced trash fish and moist home-made diet for the weaning stage.

Cobia larvae (0-12 dph) tend to retain DHA rather than other fatty acids. High levels of dietary DHA and DHA/EPA ratio effectively improved growth, survival and quality of the juveniles (12-30 dph). Cannibalism was negatively correlated with dietary DHA level.
3. Future researches

The successful use of UAF from first feeding of cobia larvae opens a possibility to simplify the rearing protocol and to reduce the production cost. However, more research on the use of UAF is still needed such as improving UAF production, elongating time of umbrella-stage, increasing their availability in the water column of the larval rearing tanks and/or co-feeding with appropriate formulated diets.

Results of co-feeding and weaning studies indicated that it is necessary to conduct more research on nutritional requirements of different development stages of cobia larvae and juveniles. Development of the appropriate formulated diets with efficient digestibility, especially for early co-feeding and weaning needs to be addressed.

Due to the effectiveness of high dietary DHA levels and DHA/EPA ratio for cobia larvae and juveniles during weaning, the optimal DHA level and the appropriate ratio of DHA to other EFA needs to be clarified.
References
References


References


References


References


168


References


symposium on cage aquaculture in Asia. Asian Fishery Society, Manila, Philippines, Zhejiang University, Hangzhou, China, pp. 42-47.


References


Ostrowski, A.C., Divakaran, S., 1989. The amino acid and fatty acid compositions of selected tissues of the dolphin fish (Coryphaena hippurus) and their nutritional implications. Aquaculture 80, 285-299.


References


Van der Meeren, T., 1991a. Selective feeding and prediction of food consumption in turbot larvae (Scophthalmus maximus L.) reared on the rotifer Brachionus plicatilis and natural zooplankton Aquaculture 93, 35-55.


References


Summary

Samenvatting
Summary

Cobia is a potential fish species for marine aquaculture due to its rapid growth and succulent flesh. However, shortage of high quality fingerlings remains a bottle neck for further propagation of the species. This PhD thesis aimed to study the effects of feeds, feeding frequency and rearing density on the performance of cobia larvae and early juveniles, with focus on the co-feeding and weaning stage.

Background information of the research and development of cobia aquaculture is presented in the literature study (Chapter 2). The data demonstrate the advantages of cobia in aquaculture and its recent rapid development. Besides a global overview, the present situation of research, development and prospects of cobia aquaculture in Vietnam are also presented to highlight the necessity of this study.

In order to improve husbandry techniques during weaning, the effects of rearing density (1, 2 or 4 weanling L\(^{-1}\)) and feeding frequency (continuous, every 2 and 4 h feeding) on growth and survival of cobia juveniles from 20 day post hatch (dph) till 35 dph were studied (Chapter 3). At a feeding frequency of 5 times a day, growth and survival of cobia juveniles were reduced at the density of 4 weanling L\(^{-1}\) \((P<0.05)\), while feeding frequency had no significant effect \((P>0.05)\) at a density of 1 weanling L\(^{-1}\). The effects of rearing density and feeding frequency on cannibalistic mortality and size variation were also evaluated and discussed as a potential method to improve survival. In addition, a comparison of home-made moist diet or minced trash fish and a commercial dry feed (NRD\(^\circledR\), INVE Aquaculture SA) indicated that growth and survival of cobia juveniles fed NRD\(^\circledR\) was significantly higher \((P<0.01)\). These results
revealed the advantages of NRD® and acceptance of the dry diet of cobia juveniles during weaning stage.

As cobia larvae grow very fast, they may need high amounts of nutrition from the onset of exogenous feeding onwards. The use of freshly-hatched *Artemia franciscana* shortened the rotifer feeding period, but could not completely replace rotifers. Umbrella-stage of *Artemia franciscana* (UAF) was tested as feed for further shortening and eventually completely substituting enriched rotifers (Chapter 4). In this trial, UAF was used as sole feed from the onset of exogenous feeding compared to the use of enriched rotifers for the first 2 days followed by UAF and the sole use of enriched rotifers as control. The results revealed the capability of cobia larvae to ingest and digest UAF although the feeding incidence in the UAF treatment was significantly lower \((P<0.05)\) on the 1st feeding day. Replacing enriched rotifers by UAF as starter food for cobia larvae had very little effect on larval growth by 8 dph and appeared to have no significant negative effect on larval quality, growth or survival by 18 dph.

Successful use of UAF as an option for earlier substitution of enriched rotifers allows simplifying the rearing protocol for cobia larvae, but at the same time, might result in too limited provision of DHA needs. In order to balance and improve the nutritional condition of the larvae, early co-feeding was implemented (Chapter 5). The use of Proton® and the experimental diet (INVE Aquaculture NV) along with live food from 8 and 13 dph were compared to 18 dph which served as a control. Early co-feeding of Proton® from 8 dph resulted in better growth \((P<0.05)\), but not in higher survival of cobia juveniles. No significant difference in terms of growth, vitality and survival was detected between treatments fed the experimental diet. The study suggested that early
co-feeding of Proton® from 8 dph is possible and the digestibility of the diet seems to be more important than the composition for cobia at the stage of 8-23 dph. However, the use of an experimental diet with a higher n-3 HUFA content and DHA/EPA ratio compared to Proton® and NRD®, resulted in bigger size and weight ($P<0.05$) of the juveniles at the stage of 20-38 dph. Survival of the juveniles fed Proton® was the lowest ($P<0.05$) and there were no significant differences ($P>0.05$) in the coefficient of size variation as well as the cumulative stress index in a salinity challenge test between the treatments. The daily mortality of all 3 treatments had two peaks: one at the beginning of the experiment and one when live food feeding was discontinued. This result indicates that the nutritional requirements of cobia are age dependent and prolongation of live food co-feeding during weaning may be necessary for growth and survival improvement.

The high DHA content in yolk-sac larvae suggests that cobia larvae may require high dietary levels of this fatty acid. Cobia larvae retain DHA rather than other fatty acids in their body: the content of DHA and other fatty acids in their tissues decreased according to larval growth (0-12 dph), but the DHA/EPA ratio increased from 4.6 to 6.6. Effects of dietary DHA levels of 21.12, 37.57 and 53.31 mg g$^{-1}$ DW with DHA/EPA ratios of 3.6, 3.8 and 6.0, respectively on growth, survival and quality of cobia juveniles (12-30 dph) were evaluated (Chapter 6). The results indicated that higher levels of dietary DHA and DHA/EPA ratios resulted in a better specific growth rate (varying from 22.60-23.77 % day$^{-1}$). Cannibalism was the highest in the treatment with low dietary DHA. Levels of DHA and DHA/EPA ratio in the juvenile tissues could not be correlated with dietary contents. Although no differences were noticed among treatments in the salinity tests, best survival in the transportation test was recorded in the highest DHA treatment.
In conclusion, our study revealed that cobia larvae are able to ingest and digest UAF from first feeding onwards. Substitution of enriched rotifers by UAF has little effect on larval growth by 8 dph and appears to have no significant negative effect on larval quality, growth or survival by 18 dph. Early co-feeding of Proton® from 8 dph is possible and resulted in better larval growth, but not in higher survival. During the weaning period, growth and survival of cobia juveniles are reduced at a density of 4 weanling L⁻¹. Use of NRD® resulted in better growth and survival compared to the home-made moist diet and minced trash fish. High levels of dietary DHA and DHA/EPA ratio effectively improve growth, survival and quality of the juveniles for the stage of 12-30 dph. Researches on further improvement of UAF and appropriate formulated diets with an optimal DHA content and DHA/EPA ratio for co-feeding and weaning need to be addressed.
Samenvatting

Cobia is een vissoort met potentieel voor mariene aquacultuur omwille van zijn snelle groei en sappig vlees. Het tekort aan juvenielen van goede kwaliteit blijft echter een Bottle Neck voor de verdere uitbreiding van deze soort. Deze doctoraatsthesis had als doel het bestuderen van de effecten van voeder, voederfrequentie en visdensiteit op de prestaties van cobia larven en jonge juvenielen met een focus op de co-feeding en speenstadium.

In de literatuurstudie wordt achtergrond informatie over het onderzoek en ontwikkeling van de cobia aquacultuur gegeven (Hoofdstuk 2). De gegevens tonen de vooruitgang in cobia aquacultuur en de recente snelle ontwikkeling aan. Naast een overzicht op wereldschaal, wordt ook de huidige status van het onderzoek, ontwikkeling en vooruitzichten van cobia aquacultuur in Vietnam belicht om de noodzaak van deze studie te onderschrijven.

Om de broedhuistechnieken tijdens het verspenen te verbeteren, werden de effecten van visdensiteit (1, 2 of 4 juvenielen L⁻¹) en voederfrequentie (continu, om de 2 of 4 uur) op groei en overleving van cobia juvenielen tussen 20 dagen na ontluing (dno) en 35 dno bestudeerd (Hoofdstuk 3). Bij een voederfrequentie van 5 keer per dag, werd de groei en de overleving van de cobia juvenielen gereduceerd bij een visdensiteit van 4 juvenielen L⁻¹ \( (P < 0.05) \), terwijl de voederfrequentie geen effect had bij een visdensiteit van 1 juveniel L⁻¹. De effecten van visdensiteit en voederfrequentie op kannibalisme en groottevariabiliteit werden ook gemeten en besproken als een mogelijke methode om de overleving te verhogen. Daarnaast werd een huisbereid vochtig dieet, vermalen visafval samen met niet-commerciële vis en een commercieel droog voeder (NRD®, INVE Aquaculture NV) vergeleken. Daarbij
was the growth and survival of cobia juveniles fed with NRD® significantly higher \((P<0.01)\). These results showed the benefits of NRD® and the inclusion of the dry diet by cobia juveniles during the yolk-sac stage.

As cobia larvae grow very quickly, they likely have high needs for food from the beginning of exogenous feeding. The use of newly hatched \textit{Artemia franciscana} shortened the period of feeding on rotifers, but was insufficient to replace the rotifers completely. The \textit{paraplu}-stage of \textit{Artemia franciscana} (PAF) was tested to further shorten the period of feeding on rotifers or even replace a part of them completely (Chapter 4). In this test, PAF was used as the only food from the beginning of exogenous feeding in comparison with the use of enriched rotifers for the first 2 days followed by PAF and with the exclusive use of enriched rotifers as control. The results showed that cobia larvae are able to take up and digest PAF although the intake frequency was significantly lower \((P<0.05)\) during the first day. Replacing enriched rotifers by PAF as a starter food for cobia larvae had a very small effect on larval growth until 8 dno and had no significant negative effect on larval quality, growth or survival until 18 dno.

The successful use of PAF as an option for early replacement of enriched rotifers allows for a simplification of the rearing protocol for cobia larvae, but can simultaneously lead to a low supply of DHA. To improve the nutritional condition of the larvae, early co-feeding was applied (Chapter 5). The use of Proton® and the experimental diet (INVE Aquaculture NV) after the administration of live food from 8 and 13 dno was compared with the co-

\textit{Summary/ Samenvatting}

Daar cobia larven heel snel groeien, hebben ze heel waarschijnlijk hoge behoeften aan voeder vanaf het begin van het exogene voeden. Het gebruik van pas ontloken \textit{Artemia franciscana} verkortte de periode waarbij rotiferen worden gevoederd, maar was onvoldoende om de rotiferen volledig te vervangen. Het paraplu-stadium van \textit{Artemia franciscana} (PAF) werd getest om de periode waarbij rotiferen worden gevoederd verder te verkorten of om zelfs aangerijkte rotiferen volledig te vervangen (Hoofdstuk 4). In deze test werden PAF gebruikt als enige voeder vanaf het begin van exogene voeden in vergelijking met het gebruik van aangerijkte rotiferen gedurende de eerste 2 dagen gevolgd door PAF en met het uitsluitend gebruik van aangerijkte rotiferen als controle. Uit de resultaten bleek dat de cobia larven in staat zijn om PAF op te nemen en te verteren alhoewel de opnamefrequentie significant lager \((P<0.05)\) was tijdens de eerst dag. Het vervangen van aangerijkte rotiferen door PAF als startvoeder voor cobia larven had een heel klein effect op de larvale groei tegen 8 dno en had geen significant negatief effect op de larvale kwaliteit, groei of overleving tegen 18 dno.

Het succesvol gebruik van PAF als een optie voor eerdere vervanging van aangerijkte rotiferen laat een vereenvoudiging van het opfokprotocol voor cobia larven toe, maar kan terzelfdertijd leiden tot een te lage toediening van DHA. Om de nutritionele conditie van de larven te verbeteren, werd vroege co-voedering toegepast (Hoofdstuk 5). Het gebruik van Proton® en het experimenteel dieet (INVE Aquaculture NV) naast het toedienen van levend voeder vanaf 8 en 13 dno werd vergeleken met het co-
voederen vanaf 18 dno, wat als controle diende. Vroege co-voeding met Proton® vanaf 8 dno leidde tot een betere groei \((P<0.05)\), maar niet tot een hogere overleving van de cobia juvenielen. Er werd geen significant verschil gevonden in groei, vitaliteit en overleving tussen de behandelingen die het experimenteel dieet werden gevoederd. Deze studie gaf aan dat vroeg co-voeden met Proton® vanaf 8 dno mogelijk is en dat de verteerbaarheid van het dieet belangrijker blijkt dan de samenstelling voor cobia in het stadium tussen 8 en 23 dno. Het gebruik van een experimenteel dieet met een hoger n-3 HUFA gehalte en DHA/EPA verhouding vergeleken met Proton® en NRD®, leidde tot grotere en zwaardere \((P<0.05)\) juvenielen in het stadium tussen 20 en 38 dno. De overleving van de juvenielen gevoederd met Proton® was het laagst \((P<0.05)\) en er waren geen significante verschillen \((P>0.05)\) in de grootte variatie coefficiënt en in de cumulatieve stress index in de saliniteitsstresstest tussen de behandelingen. De dagelijkse mortaliteit in de 3 behandelingen vertoonde 2 pieken: één in het begin van het experiment en één toen het voederen met levend voer werd beëindigd. Dit resultaat laat vermoeden dat de nutritionele behoeften voor cobia leeftijdsafhankelijk zijn en dat de verlenging van de periode waarin levend voer wordt ge-covoederd tijdens het spenen waarschijnlijk noodzakelijk is voor het verbeteren van de groei en overleving.

Het hoge DHA gehalte in de dooierzaklarven laat vermoeden dat cobia larven een hoge behoefte hebben aan dit vetzuur in het voedsel. Cobia larven behouden eerder DHA dan andere vetzuren in hun lichaam: de inhoud van DHA en andere vetzuren in hun weefsels verlaagt tijdens de larvale groei (0-12 dno), maar de DHA/EPA verhouding verhoogd van 4,6 tot 6,6. De effecten van verschillende DHA gehaltes in het voeder (21,12; 37,57 en 53,31 mg g\(^{-1}\) DW) met DHA/EPA verhoudingen (respectievelijk: 3,6; 3,8 en 6,0) op de groei, overleving en kwaliteit van cobia
juwenielen (12-30 dno) werd geëvalueerd (Hoofdstuk 6). De resultaten toonden aan dat hogere DHA gehalte in het voeder en DHA/EPA verhoudingen leidden tot een betere specifieke groeisnelheid (variërend van 22,60 tot 23,77 % dag⁻¹). Het kannibalisme was het hoogst in de behandeling met een laag DHA gehalte. Er was geen correlatie tussen de DHA gehalte en DHA/EPA verhoudingen in de weefsels van de juwenielen en de gehaltes in het voeder. Niettegenstaande er geen verschillen werden gevonden tussen de behandelingen in de saliniteitsstresstesten, werd de beste overleving geobserveerd tijdens de transporttest in de behandeling met het hoogste DHA gehalte.

Tot besluit; onze studie toonde aan dat cobia larven in staat zijn om PAF op te nemen en te verteren vanaf de eerste voedering. Vervanging van aangerijkte rotiferen door PAF heeft weinig effect op de larvale groei tegen 8 dno en heeft blijkbaar geen significant negatief effect op de larvale qualiteit, groei of overleving tegen 18 dno. Het vroeg co-voederen van Proton® vanaf 8 dno is mogelijk en leidde tot een betere larvale groei en qualiteit, maar niet tot een hogere overleving. Tijdens de speenperiode werd de groei en overleving van cobia juvenielen gereduceerd bij een visdensiteit van 4 juvenielen L⁻¹. Het gebruik van NRD® resulteerde in een betere groei en overleving in vergelijking met een huisbereid vochtig dieet en vermalen visafval samen met niet-commerciële vis. Hoge gehaltes aan DHA in het voeder en DHA/EPA verhoudingen verbeteren aanzienlijk de groei, overleving en qualiteit van de juvenielen in het stadium tussen 12 en 30 dno. Verder onderzoek naar het verbeteren van PAF en naar een geformuleerd dieet met een optimaal DHA gehalte en DHA/EPA verhouding om te co-voederen en spenen moet ondernomen worden.
Curriculum vitae
Curriculum vitae

1. Personal information

   Name: Nhu Van Can
   Gender: Male
   Nationality: Vietnamese
   Date of birth: 18-05-1969
   Place of birth: Thanh-hoa, Vietnam
   Marriage status: Married

2. Academic qualifications

   1992 to 1997

   - Bachelor of Science in Aquaculture - University of Fisheries
     
     - Thesis subject: A survey on distribution and production of Japanese shrimp
       Penaeus japonicus in coastal areas of Nghe-an Province

   1997 to 1999

   - Master of Science in Aquaculture (Postgraduate Research and Training
     Program in Marine Aquaculture, a joint cooperation between Fisheries
     University and Norwegian University of Science and Technology)
     
     - Thesis subject: Research on acclimatization and culture quality of the rotifer
       Brachionus plicatilis (L-strain) from Norway fed on different diets under the
       conditions of Nha-trang, Vietnam

   2005 to present:

   - Registered for PhD at Laboratory of Aquaculture and Artemia Reference
     Center, Ghent University
     
     - Thesis subject: Optimization of the larviculture of the tropical fish cobia
       Rachycentron canadum in Vietnam
3. Working Experiences

**Working Positions:**

1999 to 2002:  Research assistant in the Marine Component of the Project SRV 0330 (phase 1) - Research Institute for Aquaculture No.1

2002 to 2005:  Head of the Marine Hatchery - Research Institute for Aquaculture No.1 (RIA-1)

2005 to 2007:  Director of Aquaculture Research Sub-institute for North Central - Research Institute for Aquaculture No.1 (ARSINC-RIA1).

2007-2008:  Secretary of Scientific Committee of RIA-1

2008 to present:  Head of Department of Science and Information, RIA-1

**Involvements in Research and Development Activities**


e)


4. Publications

4.1 Research Papers in Peer-reviewed Journals


4.2 Papers Presented in International Conferences

Oral presentations:


Poster presentations

### 4.3 Other publications:


Acknowledgements

First of all, I would like to express my deep gratitude to Prof. Patrick Sorgeloos - my promoter, for his guidance, scientific orientation and kind support through my doctoral studies at the Laboratory of Aquaculture and Artemia Reference Center, Ghent University. I am grateful to Prof. Patrick Sorgeloos for providing me the technical training in MRS (Italy) and for giving me the most convenient conditions for my study. During 4 years studying, I have been frequently receiving his encouragements, detailed instructions, relevant comments, updated knowledge and information despite of his tight schedule. I gained a lot of knowledge, experiences and improved my scientific abilities. I hope I could receive his support and instructions in the future.

I am grateful to Dr. Tran Mai Thien and Dr. Le Thanh Luu - my local promoters for giving me opportunity and support for my studies in Belgium. I highly appreciate my local promoters for promoting and providing me advantageous conditions for my research. I always felt supported even when I was studying abroad.

I thank Mr. Kristof Dierckens for detailed instructions, useful suggestions and relevant comments during my 4-years study. I appreciate his enormous effort correcting all my research papers and my thesis. I learnt a lot from him on writings, as well as strict discipline under his supervision.

I would also like to thank Prof. Peter Boosier for his support, encouragement and for giving me the convenient conditions for my studies at the Laboratory of Aquaculture and Artemia Reference Center. I appreciate great help and supports from staff of the Laboratory of Aquaculture and Artemia Reference Center: Magda Vanhooren, Marc Verschraeghen, Tom Baelemans, Alex Pieters, Dorinda Tack, Caroline Van Geeteruyen, Geert Vandewiele, Jean Dhont and Mathieu Wille for administrative and technical issues for my study. After four years the Laboratory of Aquaculture and Artemia Reference Center feels like family to me and I am looking forward to the future co-operations.

I am grateful to the members of the examination and reading committee for critical reviews and valuable comments to improve this thesis.
During 4 years study, I received great help of friends, colleagues and professors from different institutions/organizations. I am thankful to Veerle Courtens, Umbecto Capiferri, Lenzi Francesco, Tania De Wolf and all staff of MRS for friendly help, sharing experiences and support during my training in Mariculture de Rosignano Solvay (Italy). Special thanks to Christel Nys (INVE technologies NV) for her cooperation in the experimental diet formulation and support. I am grateful to Prof. Helge Reinertsen, Prof. Elin Kjørsvik, Ms. Kjersty Rennan Dahl from SeaLab, Norwegian University of Science and Technology (NTNU), Norway for their kind support and help for my analytical works.

Most of my experimental work was implemented at ARSINC and Qui-kim Station (RIA-1) where I received great help from the staff of these research centers. I thank also my students: Hong, Minh, Huyen, Huong, Thuy, Duc for their assistances on my experimental work. I never forget the useful support and sharing experiences from my friends, colleagues in RIA-1 and in the Laboratory of Aquaculture and Artemia Reference Center: your friendship, assistance and fun facilitated my study and make me enjoy life during my moments of hard working and being far away from my family.

I acknowledge the Belgian Technical Cooperation (BTC/CTB) for my scholarship under the mixed PhD program between the Laboratory of Aquaculture & Artemia Reference Center, Ghent University, Belgium and the Research Institute for Aquaculture No1 (RIA-1), Hanoi, Vietnam. The infrastructure and equipments for the research experiments were partly co-funded by NORAD under the project SVR 0330.

Finally, I dedicate this thesis and all my love to my dear wife, Lam Hong and two lovely little daughters, Thu Trang and Ha Thu for their patience and understanding, never ending love and support!

Ghent, November 2009

Nhu Van Can