Adding value to New Zealand eels by aquaculture

by

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Attestation of Authorship

I hereby declare that this submission is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person (except where explicitly defined in the acknowledgements), nor material which to a substantial extent has been submitted for the award of any other degree or diploma of a university or other institution of higher learning.

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Jorge Andrés Hirt-Chabbert

Date

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Ethics

Eels were raised and handled according to widely accepted humane standards. Eels were slaughtered by an overdose of anaesthetic, a widely accepted method of humane slaughter.

Abstract

Freshwater eels are one of the world's most valuable cultured fish species. Although almost all worldwide eel production is derived from eel farms, no cultured eels are produced in New Zealand. Currently, the entire eel business in this country consists of catching shortfin (*Anguilla australis*) and longfin (*A. dieffenbachii*) in the wild and selling them on the local or international markets. However, changes in market specifications, production technology and wild eel stock restrictions have motivated an evaluation of culturing eels as a commercial route to contribute to regional economic development in New Zealand.

This thesis describes a series of feeding trials that aim to show how to add value to the native New Zealand shortfin eel by developing a market-oriented eel product through aquaculture, and to provide a better understanding of some eel culturing strategies that may help improve fish production and reduce the eel-farm operational work. All trials were undertaken indoors in recirculation aquaculture systems (RAS) and fish were fed commercial pelleted feed. Most of these experiments were developed on yellow shortfin eel at the Aquaculture Laboratory of the AUT University, but one experiment on feeding stimulants was done at the Unitat de Cultius Experimentals (IRTA, Centre de Sant Carles de la Ràpita, Spain) on glass eel and elvers of the European eel, because of the resources needed.

The main outcomes of this thesis are:
(1) The development of a New Zealand eel product aligned with international market specifications on the fat content.
It was possible to grow a fatty shortfin-eel product with 20 to 22% total fat starting from wild yellow eels with an initial fat content below 7%. From a human dietary perspective, the fat quality of the eel product obtained by culturing was appreciably superior (higher proportion of omega-3 fatty acids) than the fat of eels caught in the wild.

(2) The achievement of good values of mean growth rate (SGR= 1.1% day^{-1}) and feed conversion ratio (FCR ≤ 1.1) for rearing yellow shortfin eels.

These values compared favourably with other eel species of similar size range, which indicates a good potential for culturing.

(3) The rejection of a hypothesis that size grading can improve the proportion of fast-growers and reduce the high variation in individual growth rate of yellow shortfin eels.

It was not possible to improve the individual growth rate of small eels (slow-growers) by having no large eels (fast-growers) in the same tank. These findings suggest that the wide variability in the individual growth rate performance of the eels is not primordially a consequence of the social interaction (hierarchical position) among tank-mates.

(4) The confirmation of a hypothesis that feeding stimulants can improve the proportion of fast-growers and reduce the high variation in individual growth rate of juvenile eels.

The use of feeding stimulants showed a beneficial effect on the growth rate, size distribution homogeneity, feed intake and digestive function. Although this study was performed on the European eel, the results are encouraging enough to warrant research on the use of stimulants on shortfin eel as a culturing technique to obtain a rapid and uniform fish growth.

The outcomes of this research in conjunction with previous studies on the culture of shortfin glass eels and elvers indicate that the shortfin eel has good potential to be a cultured species. However, there are yet numerous aspects, mainly from a business and legislative perspective, which must be addressed before a new eel culture industry can be a reality in New Zealand.

Chapter 1

General introduction

1. Introduction

The term *eel* is widely used around the world to refer to hundreds of fish species with a snake-like shape. However, true eels are members of the Order Anguilliformes only, which include several groups of fish species like conger eels, moray eels and freshwater eels (Nelson, 1994). This study deals with the culturing of freshwater eels, a group of fish that belong to the genus *Anguilla*. Even though the members of the genus *Anguilla* are known worldwide as freshwater eels, strictly speaking they are not really freshwater, rather catadromus fish. Catadromus species are a particular group with an extraordinarily complex life cycle: they spend most of their life in freshwater but migrate to marine waters to breed. Throughout this study the term *freshwater eel* or just *eel* will be used to refer to the *Anguilla* spp.

Freshwater eels are very valuable fish species. Eel meat is much appreciated around the world, principally in Asian and European countries, where it is considered a delicacy and a very healthy food product (Sinha & Jones, 1975; Heinsbroek, 1991; Coello *et al*., 1999; Ottolenghi *et al*., 2004). Currently the global eel market trades around 270 000 tonne a year valued at over US$1.2 billion (FAO, 2010). Eel fisheries and aquaculture around the world are mainly limited to four freshwater eel species: the European eel (*Anguilla anguilla*, Linnaeus, 1758), the Japanese eel (*Anguilla japonica*, Temminck & Schlegel, 1847), the American eel (*Anguilla rostrata*, Le Sueur, 1821) and the shortfin eel (*Anguilla australis*, Richardson, 1841). Over 97% of worldwide eel production is based on farming of the European eel and the Japanese eel (FAO, 2010). Eel farming is a capture-based aquaculture activity. It is based on the practice of collecting juveniles' eels from the wild and subsequent on-growing in captivity to marketable size using aquaculture techniques. Due to their complex life cycle, research efforts have not yet managed to produce glass eel at a commercial scale (Ottolenghi *et al*., 2004).

Currently the eel business in New Zealand consists of catching eels in the wild and selling them on the local or international markets, but no cultured eel is produced in this country. However, changes in market specifications, production technology and wild eel stock restrictions have motivated an evaluation of

culturing eels as a commercial route to contribute to regional economic development in New Zealand (Watene, 2003; Jeff, 2003).

2. Freshwater eels around the world

Freshwater eels are fish with long, snake-like bodies which are cylindrical anteriorly and somewhat compressed posteriorly. They have a smooth and slippery body surface as a consequence of the minute scales embedded within their thick skin and the production of copious amount of slime. Dorsal and anal fins are confluent with the caudal fin. They have well developed pectoral fins but they lack pelvic fins (Sinha & Jones, 1975; Nelson, 1994; Tesch, 2003). All the freshwater eels belong to the genus *Anguilla*.

Class	Osteichthyes
Subclass	Actinopterygii (ray-finned fish)
Order	Anguilliformes
Family	Anguillidae
Genus	*Anguilla*

For all *Anguilla* species, the life cycle, although complex, is essentially the same and is marked by a series of defined stages (*Figure 1*). As catadromous fish, they migrate from rivers and inland bodies of water to the oceans to breed, and then the young return to freshwater.

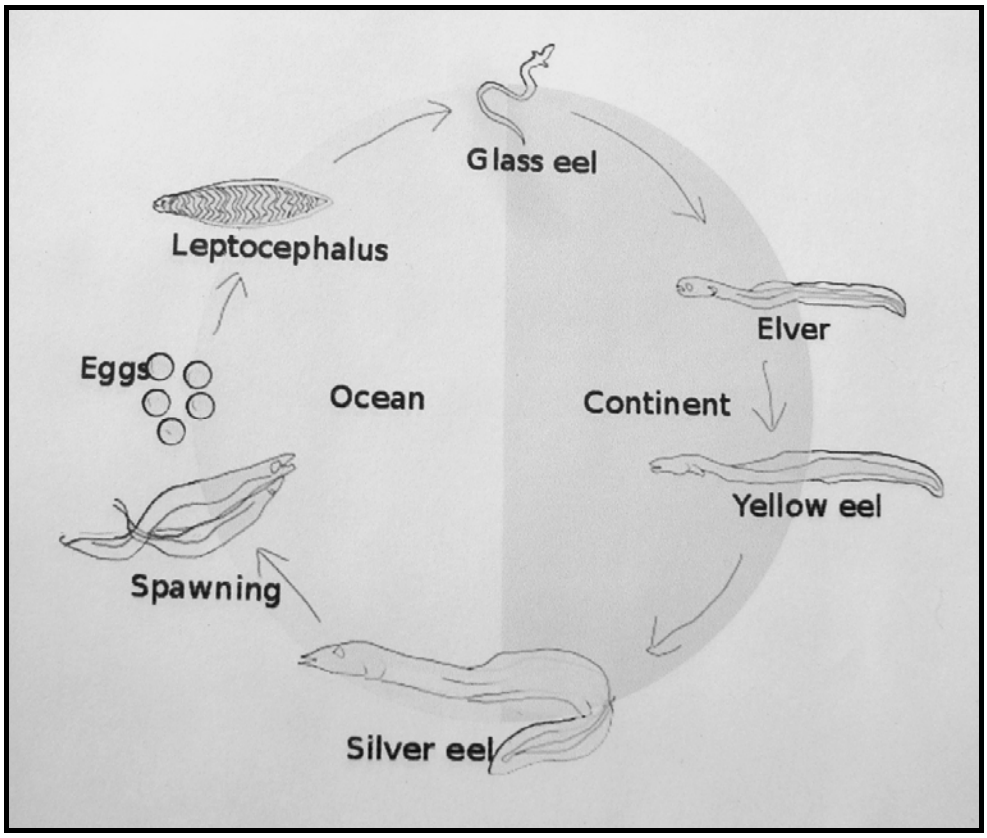


Figure 1- Life cycle of eel (Gissurardottir, 2006)

The different life stages of eels are defined after McKinnon (2006):

Leptocephalus – the oceanic pelagic larval eel, which migrate from the spawning area to the continental shelf. Narrow, deep-bodied, shaped like a willow leaf.

Glass eel – small, transparent juvenile eel formed by metamorphosis of leptocephalus. Metamorphisis occurs at sea, perhaps near the edge of the continental shelf. They are not fully pigmented juveniles. Marine and estuarine habitat.

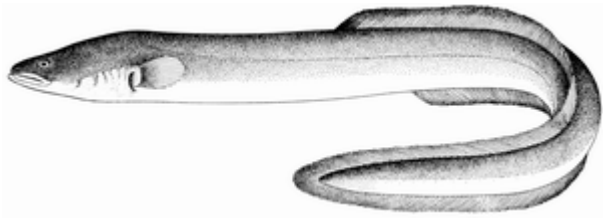
Elvers – fully pigmented juvenile eel, with a total length under 30 cm and less than 5 years old. Predominantly freshwater habitat.

Yellow eel – eel residing in continental waters, with a size generally over 30 cm long and more than 5 years of age. This stage typically lasts several years. Predominantly freshwater habitat.

Silver eel – a sexually maturing eel, migrating to the oceanic spawning area. They usually reach the mature stage between 10-20 years. Marine habitat, but metamorphosis commences in freshwater.

The two most commercially important eel species produced around the world are: the European eel and the Japanese eel.

European eel *(Anguilla anguilla)*

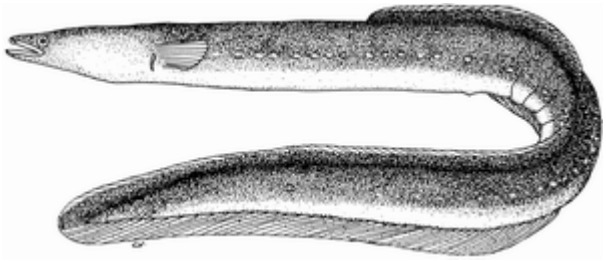


(Image from FAO (2007a) – Species Fact Sheets: *Anguilla anguilla)*

Anguilla anguilla inhabits rivers of North Atlantic, Baltic and Mediterranean seas (Ottolenghi *et al*., 2004). They spawn in the Sargasso Sea, and after reproduction the leptocephali larvae migrate (drifting by the Gulf Stream) to the coast of Europe where they metamorphose into glass eel (Tesch, 1977; van Ginneken *et al*., 2005). They live in freshwater for around 6 to12 years (30 to 40 cm, males) and 9 to 20 years (55 to 65 cm, females), turn into the silver stage, and then begin the migration to the ocean. European eels are thought to spawn at ocean depths of 400 to 700 m in mid-water in late winter and early spring (Bertelsen, 1967). These fish can live up to 85 years. They are important in commercial fisheries and aquaculture, gamefish, and in show aquariums (Ottolenghi *et al*., 2004). The species has undergone a sharp decline in recruitment, yield and stock. The European eel was listed in Appendix II of the

Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) in June 2007. This species has been categorised in the IUCN Red List of Threatened Species as Critically Endangered (CR) (Freyhof & Kottelat, 2008).

Japanese eel (*Anguilla japonica*)



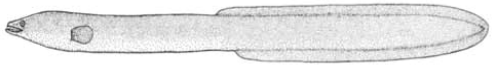
(Image from FAO (2007b) – Species Fact Sheets: *Anguilla japonica)*

Anguilla japonica inhabits East Asia, i.e. Japan, Taiwan, Korea, China and Northern Philippines (Ottolenghi *et al*., 2004). They spawn 3000 km south of their growing habitat in East Asia, near seamounts of the West Mariana Ridge (Kimura & Sugimoto, 1994; Kimura & Tsukamoto, 2006; Tsukamoto, 2006). The Japanese eel is a commercial aquaculture species, and one of the most appreciated and expensive food fishes in Japan (Frimodt, 1995).

3. New Zealand eels

Two main freshwater eel species occur throughout New Zealand, the shortfin (*Anguilla australis*, Richardson, 1841) and the endemic longfin eel (*Anguilla dieffenbachii*, Gray, 1842). Both species support important commercial and customary fisheries (McDowall, 2000; Jellyman, 2007) (*Table 1*). There is a minor third species, the Australian longfin eel (*Anguilla reinhardtii*, Steindachner, 1867) whose distribution is restricted to the northern areas of the North Island. This last species was reported for first time during the 1990s, and is not a significant component of New Zealand eel biomass (Chisnall, 2000; Jellyman, 2007) (*Table 1*).

Table 1 – Description of the three freshwater eel species that occur in New Zealand. Based on McDowall (2000) and Jellyman (2007).

	Shortfin eel	**Longfin eel**	**Australian longfin eel (Spotted eel)**
Scientific name	*Anguilla australis*	*Anguilla dieffenbachii*	*Anguilla reinhardtii*
Generic Māori name[1]	Tuna	Tuna	Tuna
Diagnostic features All three species • Body elongate • Dorsal and anal fin continuous with tail • No pelvic fins	• Dorsal-fin origin slightly in front of anal-fin origin  Image from MoF (2008a) • Vomerine teeth in roof of mouth form a short club-shaped mass between jaw teeth	• Dorsal-fin origin much further forwards than anal-fin origin  Image from MoF (2008a) • Vomerine teeth in roof of mouth in a long, pointed line between jaw teeth	• Dorsal-fin origin much further forwards than anal-fin origin • Vomerine teeth in roof of mouth form a long narrow band between jaw teeth
Distribution and habitat	Native Widespread throughout New Zealand. South-eastern Australia and some Pacific islands Principally a lowland species. Inhabit lowland lakes, wetlands, and low flowing rivers. They prefer slow-moving water (< 0.5 m s^{-1}) and fine substrata (mud).	Native - Endemic Widespread throughout New Zealand They are found in waterways further inland than *A. australis*. Inhabit high country lakes and rivers. Juveniles prefer fast-moving (> 0.5 m s^{-1}), and adults slow-moving water. They prefer coarse substrata (gravel and boulders)	Northern region of the North Island of New Zealand. Along the entire east coast of Australia and some Pacific island They prefer rivers rather than lakes and wetlands.

Colour	Golden-olive to olive-green on back and whitish-grey on belly. When maturing, the coloration intensifies to black on back and silvery on belly.	Dark-brown to grey-black on back, and yellowish-grey on belly. When maturing, more uniformly black on back and silvery belly.	Back and sides olive-green to brownish covered with brownish-black spotting, and silvery-brown ventrally. When maturing, the back loses spotting and the belly intensifies the silver.
Size	Maximum size (female) of about 110 cm and 3 kg. Males can reach about 60 cm.	Maximum size (female) of about 200 cm and 25+ kg. Males can reach about 70 cm	Maximum size (female) of about 165 cm and 14 kg Males can reach about 65 cm
Importance	An important commercial and customary fish. Important traditional food, highly valued by Māori.	An important commercial and customary fish. Important traditional food, highly valued by Māori.	A minor component of New Zealand fishery.
Status	Distribution and abundance compromised by habitat modification (e.g. wetland lost). Managed under the quota management system.	Distribution and abundance compromised by habitat modification (e.g. weirs and dams). Evidence of overexploitation. Managed under the quota management system.	Information is needed. They are included in the quota management system together with the shortfin eel.

[1] The Māori general name for eel is tuna; however there were at least 180 Māori names for the two main species of eels. Different names are used in different New Zealand regions, and according to size and colour (Jones, 2005).

Eels are an important cultural and commercial resource in New Zealand. They are prized as *taonga* (treasures) in Māori culture, and are important for *hui* (gathering), *tangi* (funerals) and other social activities (Statistics New Zealand, 2005). Commercially, New Zealand has been exporting eel products valued between $NZ 4.9 and 9.9 million per year (Free On Board values) during the last 15 years (SeaFIC, 2010). There is also a domestic market where the eels are sold mainly as a live product in fish shops.

To ensure that New Zealand eel species are sustainably utilised, they are managed under the New Zealand's Quota Management System (QMS) which monitors fish stock and annual catches. Each fishing year (from October to September of following year), the Minister of Fisheries states what quantity (quota) of freshwater eels may be caught. The quota values are stipulated by the Ministry of Fisheries on the basis of several indicators (e.g. fishing history, scientific studies, Māori observations), and aim to define the largest average annual catch that can be taken over time without reducing the stock's productive potential (maximum sustainable yield) (Statistics New Zealand, 2005).

The quantity of fish that can be taken for each fish stock, by both commercial and non-commercial fishers, is known as the Total Allowable Catch (TAC). The TAC for eels is made up by a combination of quantitative allowances: (1) non-commercial customary Māori uses (Customary), (2) recreational fishing, (3) commercial fishing (Total Allowable Commercial Catch - TACC), and (4) other unspecified sources of fishing-related mortality (e.g. poaching, illegal sale, misreporting, and injury of fish that are returned to the water) (Statistics New Zealand, 2005; Jellyman, 2007; MoF, 2009).

Customary eel fishing plays an important role in Māori culture. The use of the *tuna* (eel) takes many forms, varying according to tribal tradition, location, season and habitat. As noted above, this *taonga* is an important food source for use in customary Māori practice (e.g. *hui*, *tangi*, gift exchange) (Statistics New Zealand, 2005; MoF, 2009). Customary catches are not monitored (Jellyman, 2007).

Recreational eel fishing consists of any fish taken by non-commercial fishers under the amateur fishing regulations, and includes any eel harvest by Māori not taken under a customary permit (MoF, 2009). Recreational fishers can take six eels each a day and are limited to one fyke net per person, and unlike commercial fishers there is no maximum or minimum fish size restriction for non-commercial fishers. There is no quantitative information on the recreational harvest of freshwater eels, however the harvest by Māori might be significant (MoF, 2009).

Eels are the only New Zealand fish species captured in freshwater that are currently included in the Quota Management System (QMS). They were introduced to the QMS between 2000 and 2004. The initial catch limit was set below historic levels of commercial catch, and then they were further reduced in 2007 to increase the average size of eels and rebuild numbers (MoF, 2008b). Research has shown that the average size of both eel species had reduced compared to earlier times (MoF, 2010).

Besides the Total Allowable Catch (TAC) established in the QMS, eel fisheries are also managed using size limits, fishing equipment controls, area closures and voluntary measures to further ensure that the eel fishery is sustainably used (MoF, 2009). Currently commercial fishers are prohibited from taking or possessing eels below 220g and above 4 kg on a nationwide basis. In 1981, the minimum legal size of eel that could be caught was set at 150 g, and since September of 1993 this minimum was reset to 220 g (Regulation 31F(4) of the Fisheries (Commercial Fishing) Regulation 2001). Likewise, a 4 kg upper size limit was introduced in the South Island in November 1995, and this was extended to the whole country on April 2007 (Regulation 50 of the Fisheries (Commercial Fishing) Regulation 2001) in order to improve the number and size of female eels migrating to spawn at the end of their life. Apart from small quantities of glass eels that can be caught for research purposes, it is not legal in New Zealand to catch or export glass eels (Jellyman, 2007).

The South Island eel fishery was introduced to the QMS on October 2000, but unfortunately the eel quota was set for both shortfin and longfin species combined. Thus, both eel species of the South Island are managed as a unique stock (Jellyman, 2007; MoF, 2009). On the other hand, the North Island eel species were added to the QMS on October 2004, and their quotas were set separately for each species. Thus, the shortfin and longfin eel stock are managed independently in the North Island.

All the fish species included in the QMS have a code (established for the Ministry of Fisheries) as an easy way to manage the different fish stocks. This code can represent a particular fish species or a group of fish species. In the case of the New Zealand eels, the following codes were established: (1) ANG = both longfin and shortfin eels stocks of the South Island; (2) SFE = shortfin eel stock of the North Island; (3) LFE = longfin eel stock of the North Island (MoF, 2009).

Commercial catch of freshwater eels in New Zealand started in the 1960s and reached its historical peak in the following decade, with a capture above 2000 tonne per year in 1972. During the 1980s and 1990s, commercial annual catches fluctuated generally around 1200 to 1500 tonne (MoF, 2010), and the last ten years have seen a marked decline in the commercial fishery catches of eel species in New Zealand, with annual captures of less than 520 tonne in 2008/2009 (*Figure 2*). This reduction in catch has been attributed mainly to the more restrictive fisheries management (the progressive introduction of all eel stock into the QMS), loss of experienced fishers and processors choosing to leave the fisheries, varying overseas markets, some reductions in areas available to commercial fishers, droughts, and availability of eels (Statistics New Zealand, 2005; Jellyman, 2007; MoF, 2009).

During the 2008/09 fishing year, the total allowable catch (TAC) set for the South Island eel fisheries (ANG) was 539 tonne, of which the commercial catch (TACC) represented 421, customary fishing 107 and recreational fishing 11 tonne (MoF, 2009) (*Figure 3*). For the same period in the North Island, the TAC was 501 tonne for shortfin eel (SFE) and 170 tonne for longfin eel (LFE). The

TAC of the SFE quota was distributed as follow: TACC 347 tonne, customary 77, recreational 64 and other sources of fishing-related mortality 13 tonne. The TAC of the LFE quota was distributed as TACC 82 tonne, customary 47, recreational 33 and other 8 tonne (MoF, 2009) (*Figure 3*). Statistics for the period 2008/09 indicate that the commercial harvest of the eels did not reach 60% of the total allowable commercial catch (*Figure 3*). There is no relevant information regarding the customary and recreational catches (MoF, 2009).

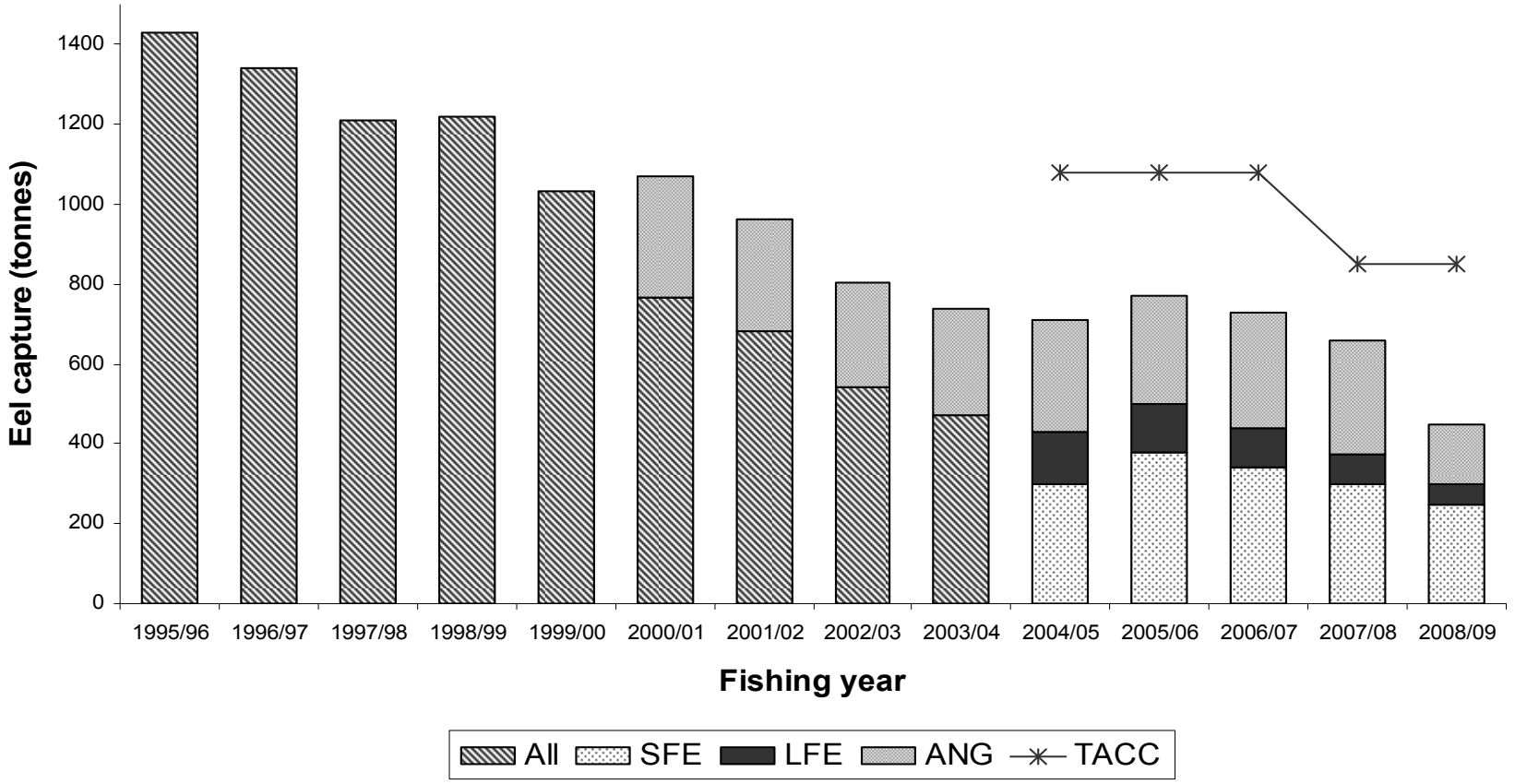
The shortfin eel has always been the dominant species caught. Even though the longfin eel has sometimes contributed as much as 45% of the total annual catch, the proportion over recent years has consistently been about 66% SFE and 33% LFE (Jellyman, 2007). In the fishing year 2008/09 the quantity of longfin eel caught in the North Island (LFE) was less than 20% of the total (*Figure 3*). Likewise, North Island catches have always exceeded South Island catches, the relative contributions over the past 14 years being 64% and 36%, respectively (Jellyman, 2007).

At its peak in the 1970s, there were up to 35 factories processing eels; today the number has dropped to 4 main licensed fish receivers (LFRs) which also hold much of the quota (MoF, 2009). Two of these fish receivers are located in the North Island (Te Kauwhata and Levin factories) and two in the South Island. Up to May 2008, an eel processing plant was also operating in Whenuapai (Auckland) by Aotearoa Fisheries Ltd., but for reasons of economic viability it was closed in connection with further reduction of the commercial catch limit in the North Island from October 2007.

New Zealand eels were exported for the first time in 1965, and from then on practically the entire eel fishery has been export-driven, with predominant markets in Europe and Asia (MoF, 2009). Eel export statistics from the last decade indicate a fluctuation of the eel product quantity between 572 and 850 tonne (*Figure 4*), and the eel product FOB value (NZ$) between 7.4 and 4.6 million. Year 2009 was one of the lowest eel export years with 573 tonne exported for a value of NZ$ 4.9 million (SeaFic, 2010).

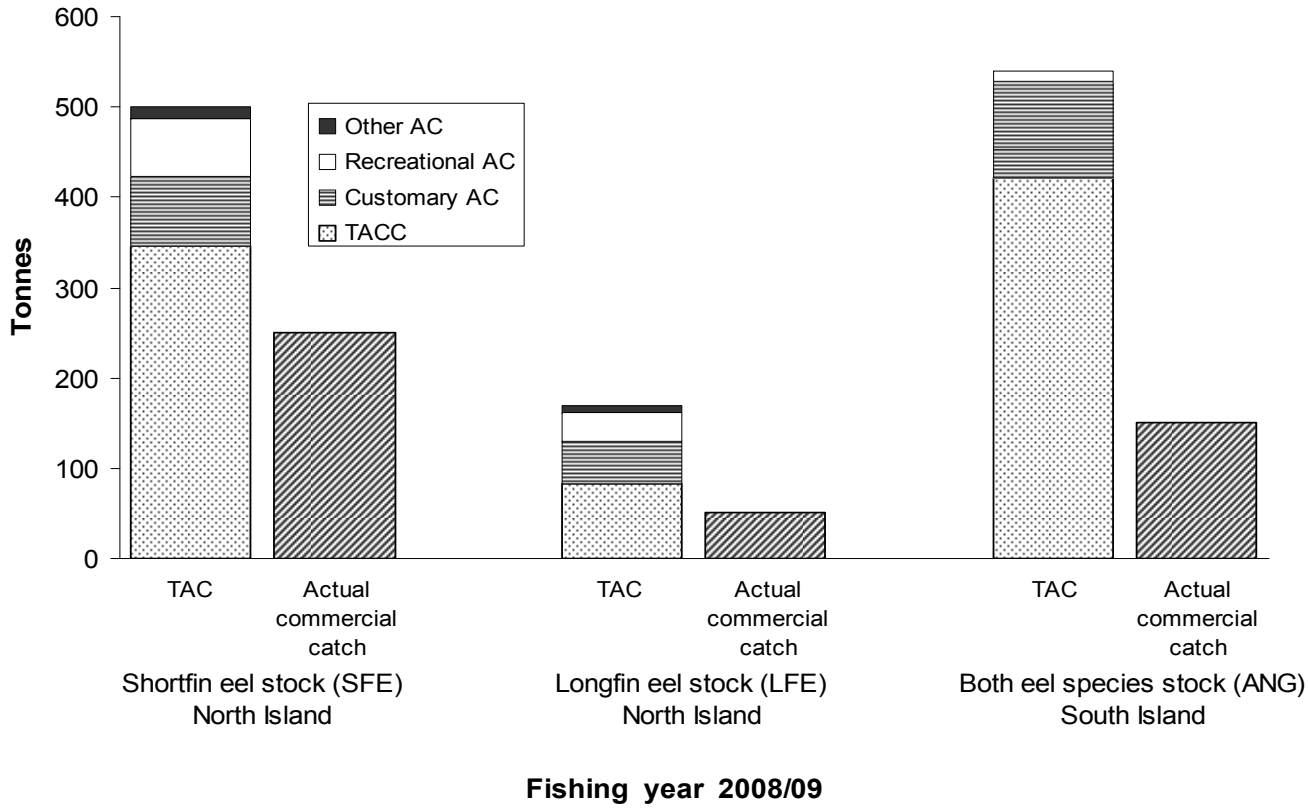
Eel products are exported mainly as live fish or frozen, making up 39% and 59% respectively of all eel products exported during the period 1999-2009 (*Figure 4*). There is also a small quantity that is exported chilled or smoked (*Figure 4*). Around 75% of all frozen eel products are exported to Europe, mainly Belgium, Germany and United Kingdom (*Figure 5a*). The principal importers of the New Zealand live eels are the Republic of Korea, Taiwan, Hong Kong and Belgium (*Figure 5b*). Chilled and smoked eels are sold mainly in Australia and the USA (*Figure 5c* and 5*d* respectively).

Figure 2 – New Zealand Commercial eel catches from fishing years 1995/96 to 2008/2009. Data obtained from New Zealand Ministry of Fisheries (MoF, 2009).



All: all eel species from both islands; SFE: shortfin eel from North Island; LFE: longfin eel from North Island; ANG: both eel species from South Island; TACC: Total Allowable Commercial Catch.

Figure 3 – Allowable eel catches by category and species in the North and South Island for fishing year 2008/09, in relation to actual commercial catches in the same period. Data obtained from New Zealand Ministry of Fisheries (MoF, 2009).



TAC: Total Allowable Catch; TACC: Total Allowable Commercial Catch; Customary AC: Customary allowable catch; Recreational AC: Recreational allowable catch

Figure 4 – Quantities (tonne) of New Zealand eels exported by product during the period 1996 to 2009. Official export figures collected by NZ Customs and supplied by Statistics New Zealand (SeaFIC, 2010).

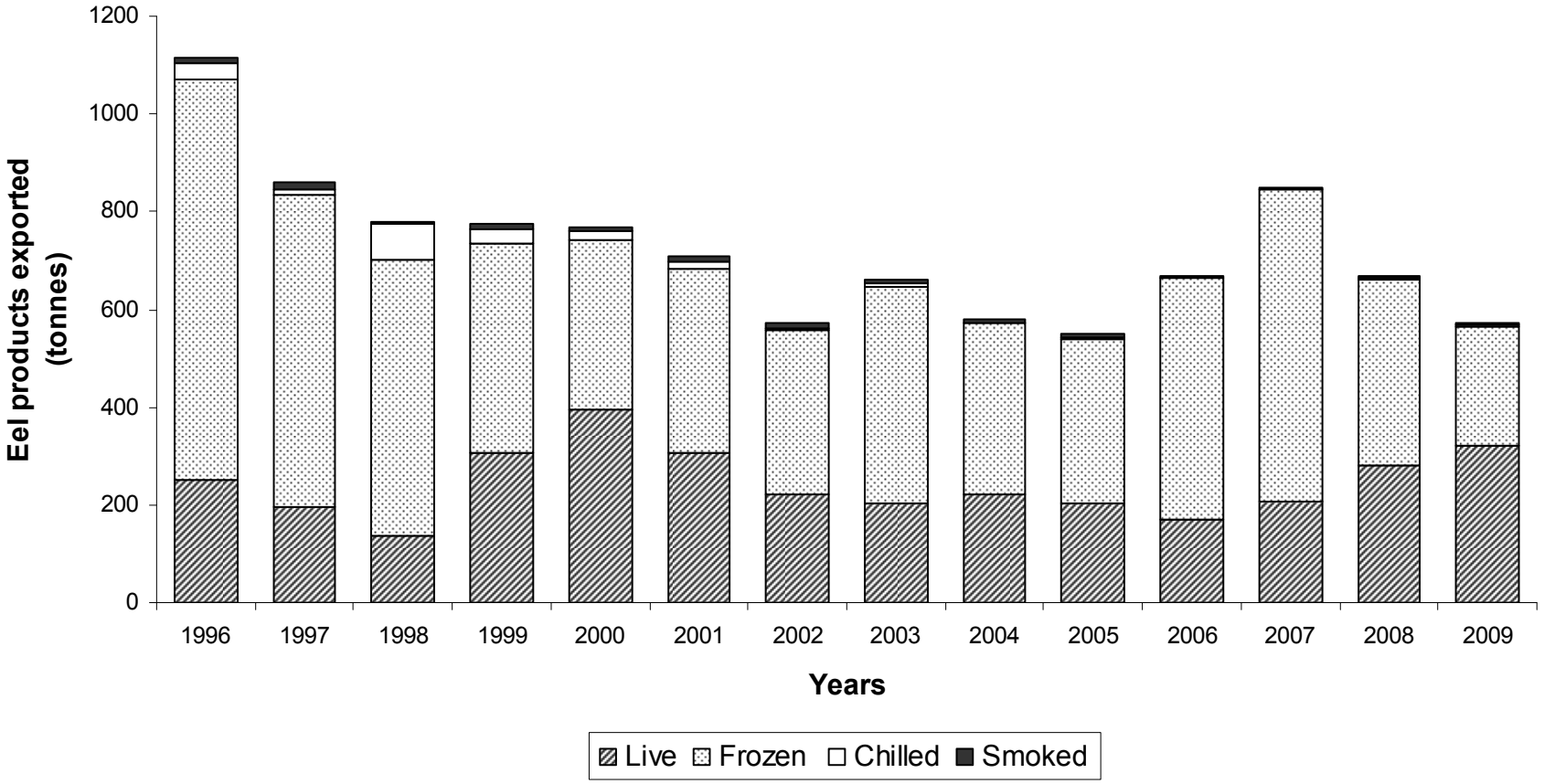
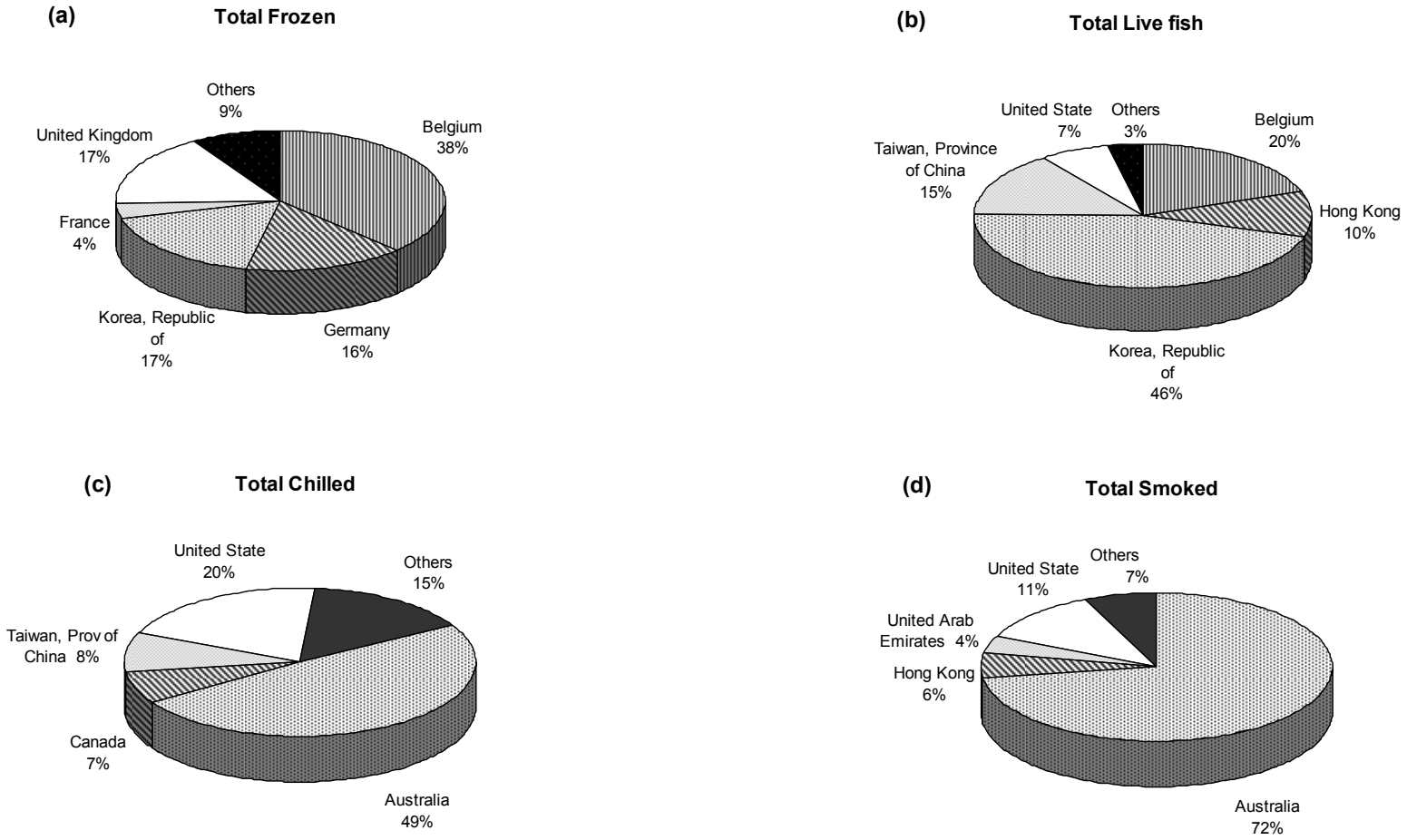


Figure 5 **–** Main importer countries of New Zealand eel products in 2009. (a) Frozen eel product, (b) Live eel product, (c) Chilled eel product and (d) Smoked eel product. Official export figures collected by NZ Customs and supplied by Statistics New Zealand (SeaFIC, 2010).



4. Eel aquaculture

Aquaculture of eels is not a modern concept. Over 2000 years ago elvers were reported to have been transported and reared in outdoor ponds in Macedonia (Beumer, 1983). But the development of an eel farming industry started in Japan in 1879 (Matsui, 1952) with the culture of the Japanese eel, and a few years later in Italy and France with the European eel (Gousset, 1990). However, production of cultured eels around the world has been minimal in relation to wild eel production until the 1970s, when there was a boom in cultured eel production. This was the result of advances in culture techniques (Gousset, 1990; Heinsbroek, 1991). Currently, eel aquaculture is a highly specialized industry with well developed fish farming technologies, but these relate almost exclusively to the two main commercial eel species, Japanese and European (Gousset, 1992; Liao *et al*., 2002; Din *et al*., 2004).

The extraordinarily complex life cycle of eels makes it as yet impossible to produce their glass eel stage at a commercial scale, and consequently eel culture is a capture-based aquaculture activity. Farmers have several options as to the stage of life cycle that may start the culture: glass eel, elvers or yellow eels. Usually, they begin with the glass eel. The fact that eel farming is totally dependent on the availability of the wild juvenile stage (the seedstock as it were) has transformed the glass eel into a high-value product. The demand for glass eels is increasing worldwide and consequently, eels are over-exploited and their wild populations have declined over the last decade (Ottolenghi *et al*., 2004; ICES, 2005, Freyhof & Kottelat, 2008).

Glass eels are very delicate. During capture these young fish are exposed to high levels of stress, thus the method of fishing and manipulation used has a significant impact on the survival and quality of this seedstock (Ringuet *et al*., 2002). The method of capture should be as passive as possible to limit damage and minimize losses. Likewise, good management is required for the transportation and acclimation of the glass eel or elvers to the aquaculture facilities. At present, there are a series of protocols more or less standardised worldwide that protect the health of the fish (Ottolenghi *et al*., 2004), although these protocols are unfortunately not always followed. Rough handling can

expose the fish to hypoxia, long period of fasting and unsuitable water quality, which can lead to high mortality, the development of pathological conditions or difficulties in feeding the fish (Rodríguez *et al*., 2005a).

Eel culture around the world employs a variety of well-established production systems, ranging from low-density pond system, to semi-intensive pond/tank accelerated temperature culture, to a high-density recirculation tank system (Usui, 1991; Gooley, 1998; Ingram, 2002; Watene, 2003; Gooley & McKinnon, 2004). These culture systems generally use freshwater, but some of them use brackish water or seawater (Jellyman, 1995; Ottolenghi *et al*., 2004).

The pond culture is the traditional eel production system. It was originated in Japan, and it is currently used principally in Asia and Mediterranean European countries. Glass eels are grown to market size using different sized ponds to complete all the production system (Usui, 1991). When the glass eels arrive at the farm, they are placed into training ponds. During this stage a 20 to 50% of glass eel mortality may occur; weak, stunted or diseased eels must be removed as soon as possible. After approximately one month in the training ponds, the glass eel are graded and transferred to bigger ponds where they become elvers. The elvers are then placed in outdoor fingerling ponds until they are large enough to go into the adult ponds (Usui, 1991).

The pond/tank accelerated temperature culture uses warmer water than ambient to increase the metabolic growth rate of the eels and consequently reduce the time to reach market size. It is well known that eels grow faster in warmer waters, and a temperature range of 24 to 26 °C is believed to be optimum (Seymour, 1989; Heisnbroek, 1991; Ingram *et al*., 2001; among others). The increased water temperature can be achieved by the use of different heating systems: (1) thermal water sources (geothermal, or heated effluent from industry), (2) enclosing ponds to utilise solar energy (greenhouse system), or (3) heat pumps or heat exchangers (Gousset, 1992; Jellyman, 1995; Kobayashi *et al*., 1999; Gooley & McKinnon, 2004).

The indoor recirculation aquaculture system (RAS) is a land-based intensive system which reuses water (closed loop) with mechanical and biological treatment between each use (Gooley & Gavine, 2003). The RAS started experimentally in 1978 in Denmark, and it was developed on a commercial scale in the early 1980s in Netherlands, Denmark, Germany and Spain (Gousset, 1990; Ottolenghi *et al*., 2004). Currently, most eel farming in Europe, apart from the traditional forms of eel culture in Italy, is done in recirculation systems (Heinsbroek, 1991; Ottolenghi *et al*., 2004; R. Barrera, VALAQUA S.A., Puçol, Spain, *personal communication*, 2009). *Annexe 1: Eel farm: Valenciana de Acuicultura S. A.* shows a commercial eel farm based on a recirculation system.

The basic components of a recirculation aquaculture system are: (1) rearing tanks that hold the cultured eel; (2) mechanical filters (e.g. sand filters, drum/microscreen filters, plated settlement tanks, etc.) to collect solid organic material (faeces, uneaten feed) that is then washed out; (3) biological filters (e.g. rotating drum/disc filters, trickling filters, upwelling filters, fluidised bed filters, etc.) to remove dissolved metabolic wastes (e.g. ammonia and nitrites) through microbial digestion; (4) sterilisation units (e.g. ultraviolet light filters, ozone generators, etc.) to destroy pathogens; (5) an overflow/storage unit, and (6) miscellaneous pumps, pipes and valves to recirculate the water through the system. Added components are the mechanical aeration or oxygenation to increase dissolved oxygen levels; a heating unit (mostly in the overflow/storage unit); and a control unit which monitors the level of water, dissolved oxygen concentration, pH, and water temperature. The control unit can detect critical conditions and be switched on with an alarm or emergency device (Gousset, 1990; Gooley, 1998; Liao *et al*., 2002). All these components have to be balanced to obtain optimum performance of the RAS. For example, increasing levels of water recirculation, stocking density, feed rates and water temperature should be accompanied with increasing filtration and supplementary aeration (Gooley, 1998).

Intensive recirculating culture systems provide controlled environment conditions for year-round production at a high density, require less water than

conventional aquaculture, can be installed in a relatively small area, reduce discharge of nutrient-rich effluent, and also reduce to a minimum the possibility of the escape of exotic stocks and disease pathogens to natural waterways (Heinsbroek & Kamstra, 1990; Gooley, 1998; Liao *et al.*, 2002; Gooley & Gavine, 2003). One of the disadvantages of a recirculation aquaculture system is the high initial capital investment required, and the necessity for skilled staff to carefully monitor and maintain the different components of the system (Liao *et al*., 2002).

Almost all intensive eel culture systems around the world are based on artificial feeds; which are mostly high-energy, protein rich and mainly composed of high-quality marine raw material (Brusle, 1990; Gooley, 1998; Luzzana *et al*., 2003). The artificial feed can be presented in the form of moist paste, or dry pellets of different sizes according to the developmental stage of the fish (Gooley, 1998). In modern intensive eel culture, the use of moist paste has become almost totally obsolete, and eel farms rely on the use of pelleted feeds (De Silva *et al*., 2001). Pelleted feed is easier to store and handle, and results in better conversion rates and less water pollution that paste feeds (Brusle, 1990; Gousset, 1992; Roncarati *et al*., 1998).

Starter feeding of eels onto artificial feed (weaning) is one of the key aspects of rearing eels (Degani & Levanon, 1986, Kamstra & Heinsbroek, 1991; Ajuzie & Appelbaum, 1993; Degani & Gallagher, 1995; R. Barrera, VALAQUA S.A., Puçol, Spain, *personal communication*, 2009). Non-acceptance of the feed can lead to mortality and retarded growth, and therefore affects the overall production. The protocol used for weaning glass eel onto pelleted feeds has undergone many changes over the years (Heinsbroek, 1991). Currently the most common process consists of first feeding eels with fish roe (e.g. cod (*Gadus morhua*) roe in Europe; carp (*Cyprinus carpio*) roe in Australia) and gradually weaning from roe to a suitable artificial dry diet (De Silva *et al*., 2001b).

The use of feeding stimulants may facilitate the acceptance of these artificial diets currently used by eel farmers. Feeding stimulants are specific compounds or ingredients added to the feed to enhance the diet palatability and,

consequently, its acceptability by the fish. As a result of the improvement in the diet acceptability, the fish can adapt earlier to a dry diet during the weaning period, and attain a higher overall feed consumption and growth rate (Mackie & Mitchell, 1983; Degani & Levanon, 1986; Kamstra & Heinsbroek, 1991; Heinsbroek & Kreuger, 1992).

The composition of the dry feeds is calculated not only in quantitative and energy terms but also in qualitative terms (e.g. essential amino acids, essential fatty acids, vitamins) (Halver & Hardy, 2002). Likewise, eel aquaculture must consider not only the performance of the diets in terms of food conversion efficiencies and growth rate, but also product quality. The concentration of body fat and its composition (fatty acid profile) are among the main factors that define the flesh texture, flavour and aroma of the eel product (Haard, 1992; Shearer, 1994; Fjellanger *et al*., 2001). The development of commercial pelleted feeds focusing on fish flesh quality assists in the successful marketing of the product (Ottolenghi *et al*., 2004).

Eels appear to be more susceptible to diseases and resultant mortality than many other aquaculture species (Ottolenghi *et al*., 2004). They are susceptible to numerous disease agents: (1) parasites, such as *Ichthyophthirius multifiliis* (protozoan), *Trichodina* spp. (ciliates), *Pseudodactylogyrus* spp. (monogeneans, Grano-Maldonado *et al*.(2011)), *Myxidium* sp. (microspora) and *Anguillicola* spp. (nematode); (2) fungi, such as *Saprolegnia* spp. (cotton cap disease); (3) viruses, such as Herpes and Rhabdoviruses; and (4) bacteria, such as *Aeromonas hydrophila* (red fin disease), *Vibrio anguillarium* (red eel pest). However, most of their diseases can be minimised by careful control of the status of imported glass eels and elvers, and by good management and husbandry systems that manage water quality, temperature and oxygen concentration. Any stress can contribute to the development of a disease. The diseases outbreaks of most common pathogens are mainly treated with salt baths, temperature changes, a formaldehyde bath and/or antibiotics. Currently, the trend is to use exclusively ecological treatments (salt bath and temperature changes) (R. Barrera, VALAQUA S.A., Puçol, Spain, *personal communication*, 2009).

5. Eel aquaculture in New Zealand

5.1 Earlier attempts at farming eels

From 1971 to 1973, six private eel farms were established in New Zealand. These farms were small scale operations with production generally under 10 tonne a year (Watene, 2003). Four of them were outdoor pond-based on the traditional Japanese culture system (located at Kerikeri, Brookby, Te Kaha and Flag Swamp), and two were indoor farms that used heated water (located at Meremere and Pakuranga). The Meremere farm used waste-heat from the Meremere coal-fired electricity generating station, and the Pakuranga farm used a recirculation aquaculture system with heaters to keep the water temperature between 23 and 24 °C (Jellyman & Coates, 1977).

By late 1975, all these ventures were closed. They did not prosper for a variety of reasons including high feed costs and unsuitable food types for the different eel stages, disease problems resulting in high mortality, irregular supply of glass eels, generally slow and highly variable growth rate, depressed export prices, poor water quality, and unfamiliarity with the culture requirement of the New Zealand species (Waugh, 1980; Jones *et al*., 1982; Jellyman, 1999).

In conjunction with the development of these New Zealand eel farms, a significant fishery for glass eel became established in the Waikato River to supply the seedstock to the farms, and also a small surplus catch of glass eels was allowed for export to Japan. However, it was sometimes difficult to obtain enough glass eels to provide to the local farms, and thus the export of glass eels was banned (Jellyman, 1979). In 1981 the New Zealand government prohibited the capture of freshwater eel smaller than 150 g, effectively banning the harvest of glass eels (MoF, 2009).

The Te Kaha farm (Bay of Plenty) reopened in 1977 under the management of the Ministry of Agriculture and Fisheries (MAF) as an aquaculture facility and pilot production station (Waugh, 1980; Sorrenson, 1981). The research effort was concentrated on developing techniques for farming shortfin eels in outdoor ponds, and also on a two-year pilot production programme which harvested 1.4 tonne of eels (Jones *et al*., 1982). The Te Kaha aquaculture station remained

operational until 1982, when it closed because of poor economics (Jellyman, 1995; Watene, 2003).

5.2 Current research and development for a new eel culture industry

Currently, there is a renewed interest in culturing freshwater eels in New Zealand. Research Institutes and the Seafood Industry of New Zealand have started research programmes with the objective of developing a cultured eel product. This fresh motivation is a consequence of a combination of factors:

(1) There has been a decline in the wild eel stock around the world and a significant reduction in supplies of European and Japanese glass eels (Rodríguez *et al*., 2005a). This decline has placed a commercial premium on the development of intensive eel culture and the associated utilisation of other anguillid glass eel seedstock (Ingram *et al.*, 2001; Gooley & McKinnon, 2004).

(2) The entire New Zealand wild eel stocks are now incorporated in the quota management system (QMS) with a concomitant reduction of the Total Allowable Catch (TAC) (Statistics New Zealand, 2005; Jellyman, 2007). These changes have prompted the eel processing industry to consider the culture and fattening of wild eels as a way of adding value and consequently improve the returns of the limited eel stock now available (Chisnall & Martin, 2002; Jeff, 2003; Jeff & Watene, 2003; J. Jameson, AFL, Auckland, New Zealand, *personal communication*, 2005).

(3) The new *New Zealand Aquaculture Industry Strategy* proposes to develop new aquaculture species and high quality, value-added products in order to grow the national revenue to a billion dollar industry by 2025 (Burrell *et al*., 2006). Currently, New Zealand's aquaculture production is dominated by three species: the green-lipped mussel (*Perna canaliculus*), the Chinook king salmon (*Oncorhynchus tshawytscha*) and the Pacific oyster (*Crassostrea gigas*). To develop a wider national aquaculture industry, it is necessary to increase the spectrum of New Zealand high-value cultured species (Bruce, 2006). Eels is one of these species.

In the last 30 years, there have been considerable advances in eel culture technology and techniques around the world. Overseas research has revealed optimal rearing temperature for a number of eel species, improved artificial feed and other technologies that have reduced labour costs and improved water quality (Jeff, 2003). The development of recirculation systems for culturing eels at high density in heated water now provides much greater production efficiency than in 1970s (Jellyman & Lockman, 2003). Nevertheless, all these improvements are related almost exclusively to the two main commercial eel species, Japanese and European (Gousset, 1990; Heinsbroek, 1991; Gousset, 1992; Liao *et al*., 2002; Din *et al*., 2004).

Although the new eel farm technologies developed overseas greatly improve the prospect of establishing commercially viable eel aquaculture operations in New Zealand, further research is needed to assess the applicability of the overseas studies to the shortfin and longfin eel species (Ingram *et al*., 2001; Ingram, 2002; Jeff, 2003; Gooley & McKinnon, 2004).

In the last decade, New Zealand and Australian researchers have conducted a number of studies related to the nursery of glass eel and elvers of shortfin (*Anguilla australis*) and longfin (*Anguilla dieffenbachii*) eels. These scientists addressed aspects such as: the basic husbandry requirements for rearing glass eels and elvers in tanks and earthen ponds (Gooley *et al*., 1999; Ingram *et al,* 2001; Jellyman & Taylor, 2001; Gooley & Ingram, 2002; Jellyman & Lokman, 2003); the weaning of the glass eel with different types of fish roe (De Silva *et al*., 2001b); the effects of different experimental diets on growth rate, feed efficiency, survival and proximate body composition of eel elvers (De Silva et al., 2001a; Engin & Carter, 2005, 2006); and the quality of the glass eels harvested in New Zealand and the effect of salinity and temperature on their growth and survival (Kearney *et al*., 2008; Kearney, 2009).

There are a few studies on husbandry and nutrition of yellow eels. De Silva *et al.* (2000) and Gunasekera *et al.* (2002) studied the protein and lipid digestibility of some experimental diets in yellow shortfin eels, respectively. The influence of different rations of natural food (squids – *Nototadarous* spp.) and water

temperature on the growth rate of shortfin and longfin eels were evaluated by Graynoth & Taylor (2000). Research into the fattening of wild New Zealand yellow eels with artificial feeds is limited. Tomiyama *et al.* (1979) studied the fat content and growth rate of shortfin wild eels (average weight 135 g) reared for 34 days on an artificial paste diet. In 2000 and 2001, Chisnall & Martin (2002) carried out pilot trials to fatten shortfin eel of around 220 g in dairy farm and factory wastewater ponds. Subsequently, the same researchers (Martin & Chisnall, 2004) studied the weaning of yellow shortfin eels on to formulated wetmix feeds, and also performed some preliminary studies on the use of pelleted feeds. These studies indicated that yellow shortfin eels can be successfully weaned to artificial feeds, but further experiments are needed on production aspects such as growth rate, size variation, feed conversion ratio and the final eating quality of the cultured eel product.

In addition to the nursery and grow-out studies for developing eel farms, New Zealand research institutes have been working on spawning and reproduction of shortfin eels in order to obtain a future self-sustaining eel aquaculture industry. Researchers of the Mahurangi Technical Institute (MTI) successfully bred *A. australis* for the first time in captivity, and the challenge now is keeping the larval (leptochephalus) eel alive for 100 days by which time they will have matured into glass eels (MTI, 2010).

6. Aim and structure of the thesis

6.1 Aim of the thesis

This thesis aims to show how to add value to the native New Zealand shortfin eel by developing a market-oriented eel product through aquaculture, and to provide a better understanding of some culturing strategies for eels that may help improve fish production and reduce eel-farm operational work.

The specific objectives are:

(1) to examine the final eel product quality, in terms of body proximate composition with a focus on the fat content and fatty acid profile, of wild yellow shortfin eel cultured in a RAS.

(2) to examine the growth rate, feed efficiency and fish size variability of wild yellow shortfin eel cultured in a RAS.

(3) to examine the effect of size grading on the individual growth rate of wild yellow shortfin eel cultured in a RAS.

(4) to examine the effect of feeding stimulants on the individual growth rate of juvenile European eels cultured in a RAS.

During preliminary research about the eel business in New Zealand – to define which aspect of eel culture should be addressed in this thesis – it was noted that New Zealand eel companies were interested in exploring the possibility of increasing the fat content of their wild eels by aquaculture. The reason for that was that wild New Zealand eels were sold at a low price in the international market because their low fat content did not match the specifications of the high quality eel products.

On the basis of this interest in the fat content, several meetings between New Zealand eel companies and AUT University were held in order to establish a plan of mutual collaboration for the development of a fatty eel product. This resulted in a memorandum of understanding between the eel company, Aotearoa Fisheries Ltd. (AFL), and AUT University to develop feeding trials with

wild yellow eels. Likewise, it was decided to perform the feeding trials only with shortfin eels because preliminary studies indicated a better adaptation of this species to the culturing environment than the longfin eel. These trials were going to be carried out in a commercial scale recirculation aquaculture system at the AFL eel processing plant, located in Whenuapai (Auckland). Unfortunately external problems did not allow the partnership to proceed. The eel company was forced to withdraw its participation in this thesis study at the last minute, because the entire basis of its existing business model was adversely affected by further reduction of the Total Allowable Commercial Catch – TACC of the eel species implemented by the Ministry of Fisheries in October of 2007. As a result of that, AFL decided to close its eel plant.

The withdrawal of the eel company as a supporter of this study forced a search for alternatives as to where to conduct the eel feeding trials. It was ultimately decided to build a RAS in the Aquaculture Laboratory of the AUT University (Auckland, New Zealand). The construction of this RAS demanded a great deal of energy and time and was carried out entirely by the author.

Given the limited space available in the AUT Laboratory, it was only possible to build three recirculation modules, each conformed by three 160-L circular tanks and one media box biofilter (*Annexe 2*). Although the 3-modules RAS was suitable for the development of the feeding trials, its relatively small size limited the number of eel specimens that could be reared simultaneously in a given experiment. Therefore, to increase the robustness of the data, it was decided to tag each fish specimen (*Annexe 3*). By tagging each fish, it was possible to obtain individual values of growth rate instead of a tank mean-value, and also to track the rearing history of any individual eel in relation to its interaction with different fish-size tank mates.

6.2 Structure of the thesis

The thesis describes the set of experiments conducted in the following three chapters (*Chapters 2, 3 and 4*), and a general discussion in the last chapter (*Chapter 5*). Moreover, it includes three Annexes with additional information sourced from this doctoral study.

Chapters 2, 3 and 4 can be read as stand-alone units, and each has a format similar to a journal research article. The experiments described in *Chapters 2* and *3* were undertaken at the Aquaculture Laboratory of the AUT University. However, the experiments in *Chapter 4* required resources and logistics well beyond the capacity of the AUT laboratories. Fortunately, I was invited to conduct these experiments in the Unitat de Cultius Experimentals, Institut de Recerca i Tecnologia Agroalimentaries (IRTA, Sant Carles de la Ràpita, Spain).

The contents of these chapters are now summarised.

Chapter 2 - Growth and body composition of yellow cultured New Zealand shortfin eels (*Anguilla australis*).

The objective of this chapter is to investigate the effects of two commercial pelleted diets on growth performance, feed efficiency and product quality of yellow shortfin eels (*Anguilla australis*). The fish quality, defined by body proximate composition and fatty acid profile, of the cultured eels is studied for both diets and also compared with wild shortfin eels of a similar size.

Chapter 3 - Effect of size grading on the growth performance of shortfin eels (*Anguilla australis*) during its yellow stage.

The objective of this chapter is to evaluate the effect of size grading on the individual growth performance during the yellow stage of shortfin eel (*Anguilla australis*). The hypothesis tested is that small eels can improve their growth rate in the absence of large eels.

Chapter 4 - Effects of feeding stimulants on the feed consumption, growth and survival of different developmental stages in the European eel (*Anguilla anguilla*)

The objective of this chapter is to evaluate the effects of using feeding stimulants at different concentrations on feed consumption, growth rate and survival of cultured European eels during the glass eel and elver stages. The search for culture methods to increase the proportion of fast growing eels and to reduce the variation in eel growth rate has led to the use of feeding stimulants as a potential solution to this global problem in eel culture. Even though this chapter provides specific information on the performance of the European eel, its results and the knowledge gained in the process may be applied in the future to similar research on the shortfin species.

Chapter 5 – General Discussion

Presents a general discussion about the outcomes of these experiments and the viability of developing an eel culture industry in New Zealand.

Chapter 2

Growth and body composition of cultured yellow New Zealand shortfin eels (*Anguilla australis*)

1. Introduction

Freshwater eels are high value food species with a well-developed European and Asian markets. Even though the global eel industry is strongly dominated by cultured eels (FAO, 2010), there are no eel farms in New Zealand. The eel business in New Zealand consists of catching shortfin (*Anguilla australis*) and longfin (*Anguilla dieffenbachii*) eels in the wild and selling them live, frozen, fresh or smoked on the local and international markets.

Although there is a profitable international eel market, New Zealand wild eels are either not accepted in some markets or accepted as a lower quality and less valuable product. This is because their body fat concentration (or content as it is commonly known) is generally below 10%, not high enough to match the demands of high-value markets of cultured fatty eels (Sumner & Hopkirk, 1976; Jellyman & Coates, 1977; Tomiyama *et al*., 1979; Sumner *et al.,* 1984; Jeff, 2003; J. Jameson, AFL, Auckland, New Zealand*, personal communication*, *2005*). Market-size European eels contain over 20% body fat by weight (Luzzana *et al*., 2003; Özogul *et al.,* 2005; Heinsbroek *et al.,* 2007; R. Barrera, VALAQUA S.A., Puçol, Spain, *personal communication*, 2009) and the Japanese eels from 13 to 20% (Ozaki *et al*., 2008; Oku *et al*., 2009).

The body fat content and its fatty acid profile are among the main factors that define flesh texture, flavour and aroma of any fish product (Haard, 1992; Shearer, 1994; Fjellanger *et al*., 2001). These organoleptic properties markedly affect consumer acceptance of eels (Usui, 1974; Otwell & Rickards, 1981/1982; Coello *et al*., 1999). Likewise, consumers are purportedly interested in the healthy (nutritional) quality of the food products (Garcia-Gallego & Akharbach, 1998; Coello *et al.,* 1999). A diet that includes fish fats is widely recommended, as they aid in minimising the development of degenerative diseases, notably cardiovascular disease. This is due to their relatively high content of polyunsaturated fatty acids (PUFA) belonging to the omega-3 family (Bang & Dyerberg, 1985; Kinsella, 1987; Leaf, 1989; Vazques & Sanchez-Muñiz, 1994; Lie, 2001; Fjellanger *et al.,* 2001), notably eicosapentaenoic (EPA) and docosahexaenoic acids (DHA). These are esterified along with other fatty acids in the form of triacylglycerols, the formal name for fat.

Through aquaculture, it is possible to manipulate fish composition to make a market-oriented product (Haard, 1992; Morris, 2001). By culturing shortfin eels, the New Zealand aquaculture industry could increase the fat content and maintain or increase the proportion of the beneficial polyunsaturated fatty acids of the fish. At the same time it should be possible to reduce variation in chemical and physical properties in the same way that any farmed product is less variable than wild-type equivalents.

At present, the prospect of farming New Zealand shortfin eels is generating interest from research institutes and the wider seafood industry. However, to successfully develop an eel aquaculture industry in New Zealand, a marketable eel must be produced in a profitable way. This will involve research in essential aspects, such as feed efficiency, growth rate and product quality.

A number of studies have been conducted on the effects of different diets and water conditions in relation to the growth rate, survival and body composition of the glass eel and elver stages of the shortfin eel life cycle (Gooley *et al*., 1999; De Silva *et al*., 2001a; De Silva *et al*., 2001b; Gooley & Ingram, 2002; Engin & Carter, 2006; Kearney, 2009; among others). By contrast, studies on the growth rate, feed efficiency and body composition of market-size cultured shortfin eels are limited. Tomiyama *et al.* (1979) studied the fat content and growth rate of wild shortfin eels (average weight 135 g) reared for 34 days on an artificial paste diet. In 2000 and 2001, Chisnall & Martin (2002) did pilot trials to fatten shortfin eel of around 220 g in dairy farm and factory wastewater ponds. Subsequently, the same researchers (Martin & Chisnall, 2004) studied the weaning of yellow shortfin eels onto formulated wetmix diets, and also did some preliminary study on the use of pelleted diets. These studies indicated that yellow shortfin eels can be successfully weaned to artificial diets, but further experiments are needed on production aspects such as growth rate, size variation, feed conversion ratio and the final eating quality of the cultured eel.

The objective of the present study was to investigate the effects of two commercial pelleted diets on the growth performance and product quality of

yellow shortfin eels. The fish quality (defined by body composition and fatty acid profile) of the cultured eels was studied for both diets and also compared to wild shortfin eels of a similar size.

2. Materials and methods

2.1 Cultured shortfin eels

2.1.1 Origin of fish and their maintenance

The yellow shortfin eels cultured in the present study were collected by the use of a fyke net from Lake Waikare, North Island, New Zealand (37°26′29″ S and 175°11′56″ E) in August 2008, and transported by road (1.5 hours) to AUT University's Aquaculture Laboratory. On arrival, the eels were transferred to 160-L tanks that were connected to freshwater recirculation modules *(Annexe 2).*

Fish were maintained under 12 hours light: 12 hours dark regime, and each tank was covered partially with a plastic lid to reduce the intensity of the light. All tanks were provided with individual flow of water at 3 to 5 L min^{-1}, and constant aeration. For the first 10 days the water temperature was increased gradually from 15 °C to 25.5 °C, and then maintained at 25.5 ± 1.6 °C. The pH and dissolved oxygen were monitored by a Portable Multimeter 340i (WTW Wissenschaftlich - Technische Werkstätten GmbH, Wheilheim, Germany), and maintained at 7.2 ± 0.3 and 6 to 8 mg L^{-1} (70 to 100% saturation) respectively. The concentrations of total ammonia (NH_3/NH_4^+), nitrite (NO_2^-) and nitrate (NO_3^-) were tested by an Aquarium Pharmaceuticals API Water Test Kit (Chalfont, Pennsylvania, USA). Temperature, oxygen and ammonia concentrations were recorded daily, and nitrate, nitrite and pH were monitored weekly. Every day, the biofilter was cleaned, and 15% of the water was renovated in each recirculation module.

The eels were acclimated to the RAS environment for a period of 15 days before the start of the rearing experiment.

2.1.2 PIT (Passive Integrated Transponder) tagging

At the start of the acclimation period all eel specimens were individually marked with PIT tags. As mentioned in *Chapter 1*, it was decided to PIT tagging each fish in order to increase the robustness of the data.

A PIT tag is an electronic microchip encased in biocompatible glass with a unique alphanumeric code that can be read by a scanner (Gibbons & Andrews, 2004). It is inserted by surgical incision under the animal's skin, usually into muscle or the body cavity. They are among the best candidates for tagging fish, because of their small size and mass, their long functional life (> 10 years), the availability of millions of individual codes, and because the animal does not have to be sacrificed for the tag number to be read (Acolas *et al*., 2007; Jellyman *et al*., 2007).

In order to use PIT-tagged fish in this study, it was necessary to confirm that the tagging methodology would not affect the growth and survivorship of the eels, and that the specimens would have a high PIT-tag retention rate. Because there were no studies on the effect of the PIT tag in the growth rate and survival of shortfin eel, a prior experiment was done to test the suitability of using the PIT-tag methodology in this rearing trial (*Annexe 3*).

The results of the *Annexe 3* experiment showed no significant differences in growth rate (length and weight) and survival between the tagged and untagged shortfin eels. The tagged fish showed a lower growth rate on the first sampling event, but these initial lower rates did not lead to significant differences at the end of a 42-day experiment.

Thus, it was concluded that PIT tagging was a suitable method for marking yellow shortfin eels. Likewise, it was decided to always tag the eels at the start of the acclimation period (15 days before the rearing experiment began) to avoid the initial lower growth rate of the tagged fish.

2.1.3 Diets

During the acclimation period the eels were fed hoki (*Macruronus novaezelandiae*) roe. When the experiment began, the fish were adapted to commercial pelleted feeds through a 2-week weaning period. These artificial diets progressively replaced hoki roe such that after 2 weeks only pelleted feed was provided.

Artificial pelleted diets with a protein content of 47% were chosen on the basis of prior studies on protein requirements and best growth performance of other eel species (*A. anguilla*, Degani & Gallagher, 1995; *A. japonica*, Okorie, 2007; *A. rostrata*, Tibbetts *et al*., 2000, 2001), and also on an earlier investigation on *A. australis* (Engin & Carter, 2006). The diets chosen had a high fat content because the intention was to grow a market-size fatty eel.

Two commercial pelleted diets were tested; a grow-out feed formulated for European eel, Eel 4726 Alitec (sourced from Alitec S.A., Puerto Montt, Chile), and a grow-out feed formulated for Pacific salmon, Orient 50 Skretting (sourced from CRT Co-operative, Christchurch, New Zealand). The proximate composition and main ingredients of these diets were provided by the manufacturing companies (*Table 1*). Both diets were mainly based on fish meal and fish oil with a high level of omega-3 fatty acids. The choice of the commercial eel diet was based on the assumption that an eel diet used for the European eel might also yield a good performance in the New Zealand shortfin eel. On the other hand, the commercial salmon diet was chosen because it was readily available in New Zealand.

2.1.4 Experimental procedures

A total of 112 PIT-tagged shortfin eels were selected for the experiment, and distributed uniformly among eight 160-L circular rearing tanks, 14 fish per tank at a density of 9 to 10 kg m^{-3}. Two different dietary treatments were evaluated; four tanks (randomly selected) were assigned the commercial eel diet and the other four, the commercial salmon diet. At the start of the experiment, there were no significant differences between the size of fish fed the eel diet (103.1 ± 10.7 g, mean ± SD) and the salmon diet (104.8 ± 11.5 g).

Eel were fed to apparent satiation twice daily from Monday to Friday, and once a day on weekends. Each ration of extruder pellets provided to the eels was weighed, deposited in feeding stations (*Annexe 2*), and after approximately 40 minutes, uneaten food was removed and kept for later calculation of feed intake. During the course of the 86-day experiment, the biofilter pads were

cleaned everyday, and the tanks checked daily for dead or dying fish. If any were found, they were removed and recorded as a death.

2.1.5 Sampling, data collection and growth parameters

The individual weight and general condition of the eels were recorded on Days 0, 40 and 86 of the experiment. On Day 39, all the non-growing eels (null or negative growth rate) and also the particularly slow-growing eels (specific growth rate < 0.3% day^{-1}) were discarded from the experiment, because they were considered commercially unviable fish. Therefore, from that point to Day 86 the experiment was carried out only with fish that were growing well in the tanks.

Samples of eels were collected for biochemical analysis when the wild yellow eels arrived to the AUT University's Aquaculture Laboratory, and at the end of the experiment. On arrival at the AUT laboratory, ten wild fish ranging from 90 g to 120 g, coded as the WI group, were randomly selected, slaughtered by an overdose of benzocaine, wrapped whole in aluminium foil, and held frozen at -20 °C until further proximate analysis of composition and fatty acid profile. At Day 86, two fish-size groups were established for biochemical sampling: medium eels (220 g to 300 g), and large eels (400 g to 600 g). For each of the dietary treatments, five medium-size eels and five large-size eels were randomly taken from the tanks, slaughtered and kept frozen until analysed. These fish were coded as the EFM group (medium fish fed eel diet), the EFL group (large fish fed eel diet), the SFM group (medium fish fed salmon diet), and the SFL group (large fish fed salmon diet). A timeline of production and sampling events is presented in *Table 2*.

The following parameters were calculated:

SGR (Specific growth rate, % day^{-1}) = 100 (ln BW_f – ln BW_i) / days

CV (Body weight coefficient of variation, %) = 100 (SD/ mean body weight)

Mortality rate (%) = 100 Deaths of fish / Initial fish stock

BG (Biomass gain, percent of initial biomass) = 100 (B_f –B_i) B_i

FI (Feed intake, percent of initial biomass) = 100 (Tank feed weight consumed (g) / B_i)

FCR (Feed conversion ratio) = tank feed weight consumed (g) / (B_f –B_i)

Where BW_i and BW_f are the initial and final body weight of each individual (g); B_i and B_f the initial and final tank stocked biomass (g); SD = standard deviation.

The BG, the FI and the FCR were calculated only in *Period 2* of the experiment, because during two weeks of the Period 1 the eels were fed with a variable mixture of pelleted feed and hoki roe (weaning stage). Therefore, the calculation of the feed efficiency for the *Period 1* (day 0 to day 40) was not indicative of each artificial diets performance by itself but of the mixed roe and pellet effect.

2.2 Wild shortfin eels collected for body biochemical analyses

In October 2009, five medium-size eels (220 to 300g, coded as the WM group), and five large-size eels (400 to 600g, coded as the WL group) were collected from Lake Waikare. Individual weights and lengths were recorded. After slaughter with benzocaine, the whole fish were held frozen (as indicated previously) for later body composition analyses.

2.3 Body proximate composition and fatty acid analyses

The collected samples of the seven fish groups previously defined (WI, EFM, EFL, SFM, SFL, WM and WL) were compared in their proximate body composition and fatty acid profile. The analyses were carried out individually for the whole body of each fish (gutted, head-off, skin-on), except for the WI group where a pool of two fish was tested as one replicate. There were nominally five replicates (n = 5) in each of the seven treatments.

The frozen whole-fish replicates were first held at laboratory temperature for approximately 30 minutes. Then the semi-frozen fish were gutted and decapitated. The remaining body carcasses were cut transversally in pieces 3 to 4 cm long, and then comminuted by two passes through a standard worm-drive mincer with a 4-mm cutting plate fitted to a domestic food mixer (Kenwood KM210, Havant, UK). For the WI treatment category, two eels were minced together as one replicate because their small size did not yield a sample large

enough for all the analyses with only one individual. The minced samples were immediately frozen in liquid nitrogen in lots suitable for each analytical method, and stored at -80 °C.

The moisture, protein, total fat and ash of the shortfin eel specimens were analysed. All determinations were done in duplicate and the means of duplicates were the units of replication for statistical analysis. Moisture was determined by heating weighed samples in an oven for 18 h at 100 °C. The dried samples were cooled in a desiccator then reweighed to calculate percent moisture by weight. Ash was similarly determined after heating samples held in ceramic crucibles in a furnace for 6 h at 550 °C. Total fat was determined by chloroform/methanol extraction according to Bligh & Dyer (1959), and protein by the Kjeldahl method using a UDK 126 A distillation apparatus (VELP, Usmate, Italy). A conversion factor of 6.25 was used to estimate protein from the determined nitrogen.

The fatty acid profile was determined on the fats extracted with chloroform/methanol. These extracts were held at -80 °C in air-tight glass vials under a nitrogen atmosphere until thawed for methyl ester preparation. Methyl esters were prepared as follows. For each treatment, duplicate aliquots of fat around 50 µL were added to 3 mL of 5% sulphuric acid solution in 1:1 methanol:toluene, in sealed glass tubes with Teflon-lined caps. The tubes were heated in an oven at 100 °C for 1 hour. After cooling, 5 mL of saturated salt solution was added, the mixture shaken, then centrifuged at low speed to separate the phases. The upper toluene layer was recovered, followed by an additional 1 mL of toluene to recover more fatty acid esters. The combined toluene extract was dried over anhydrous Na_2SO_4 before gas chromatography. This was carried out on a Shimadzu GC-2010 chromatograph (Shimadzu, Kyoto, Japan) equipped with a SGE SolGel-WAX capillary column, 30 m long, 0.25 mm internal diameter, 0.25 µm film thickness (SGE, Melbourne, Australia). The injector port was maintained at 235 °C. The oven temperature profile was from an initial 170 °C, rising to 225 °C at a rate of 1 °C per minute over 55 minutes. Hydrogen was the carrier gas at 1.21 mL per minute. The flame ionization detector was held at 325 °C. A 1 µL sample of each duplicate methyl

ester preparation was injected into the instrument before a 75:1 split ratio to prevent column overload. Identification of each fatty acid was made by comparing the retention times of peaks with an authentic standards mixture that was injected at intervals throughout chromatography.

The retention times of the methyl esters were normalized on C18:1n9 (which had the highest percent peak area) to adjust for slight shifts in retention time. Peaks representing less than 0.02% of the total flame ionization area for each replicate were not included in calculation of the percent fatty acid profile. Some peaks larger than 0.02% were unidentified, were could be localised and quantified by relative retention times in many but not all treatments. Each treatment nominally had 5 replicate fish, but only 3, 4, and 4 replicate extracted fats were available for analysis of the WM, WL and WI group respectively. All other treatments contain five replicates. Peak area data of the single injections of the duplicate methyl ester preparations were averaged, each mean thus representing a single replicate of a particular treatment for statistical analysis.

The fat quality of the seven eel groups was evaluated by the Atherogenic Index (AI) (Abrami *et al*., 1992). This index describes the relation of the most abundant saturated fatty acids (myristic acid, 14:0 and palmitic acid, 16:0) and the main polyunsaturated omega-3 fatty acids (EPA, 20:5 n-3 and DHA, 22:6 n-3).

AI = (14:0 + 16:0) / (20:5 n-3 + 22:6 n-3)

The lower the AI value, the better the nutritional status of the fat source.

2.4 Statistical analysis

Because the eels were PIT tagged, it was possible to follow the growth of each individual. The body weight (BW) and specific growth rate (SGR) were based on the values of the individual eels, and expressed as mean ± standard deviation (SD). The SD describes the dispersion of the individual eel values.

On the other hand, the values of biomass gain, feed intake, and consequently the feed conversion ratio were expressed as mean ± standard error of the mean

(SEM). In contrast to body weight and SGR, these parameters were calculated using the values of the tanks (n = 4 for each treatment), because they could not be calculated for individual eels. The SEM thus quantifies the error in calculating the mean of the population from the tank values.

Data were analysed for one-factor variance with Minitab statistical software 16.1.0 (Minitab® Statistical Software, State College, PA, USA). Before analysis, homogeneity of variance was confirmed using Bartlett test (Snedecor and Cochran, 1989). When a significant treatment effect was observed, individual means were compared with Tukey-Kramer HSD multiple comparison test.

Table 1 - Declared proximate composition, gross energy and ingredients of the two commercial pelleted diets.

	Eel diet	**Salmon diet**
Composition (%)		
Crude Protein	47	47 – 48
Fat	26	22 – 23
Carbohydrate	Not declared	13 - 16
Crude Fibre	1.2	Not declared
Moisture	8	7 – 9
Ash	10	8.5
Energy (MJ kg^{-1})		
Gross energy	22.2	22.2 – 22.7
Ingredients		
	Fish meal, fish oil, cereal grains, vitamins and minerals.	Fish meal, poultry protein meals, plant protein meals, wheat, fish oil, poultry oil, vitamins, minerals.
Dietary fat quality		
	Based mostly on marine sources.	Fish oil and alternatives are blended to maintain the consistently high level of omega-3 fatty acids.

Table 2 - A timeline of production and sampling events

Period	Day	Activity	Diet
Pre-trial acclimation period (15 days)	-15	Collection of the WI group for posterior chemical analyses.	No feeding
	Intervening days	Individual marking with PIT tag	No feeding
		Increasing water temperature gradually from 15 °C to 25.5 °C	Hoki roe
Trial period 1 (40 days)	0	Fish placed in tanks, sampling for physical measurements	No feeding
	1	Weaning started	Hoki roe + pelleted feeds (weaning stage)
	Intervening days	Discard dead fish	
	14	End of weaning	
	15		Pelleted feeds
	Intervening days	Discard dead fish	
	39	Non-growing and very slow growing eels were discarded	
	40	Sampling for physical measurements	No feeding
Trial period 2 (46 days)	41		Pelleted feeds
	Intervening days		
	86	Sampling for physical measurements. Collection of the EFM, EFL, SFM and SFL groups for posterior chemical analyses.	No feeding
Post-trial		Biochemical analyses of eel body composition	

Wild fish: WI, initial (90 to 120g). - Cultured fish: EFM, fed eel diet (220 to 300g); EFL, fed eel diet (400 to 600g); SFM, fed salmon diet (220 to 300g); SFL, fed salmon diet (400 to 600g).

3. Results

3.1 Growth parameters and feed efficiency

For each day of sampling (Days 0, 40 and 86), the body weights (BW) were not significantly different between fish fed the eel diet and the salmon diet (*Table 3*). The eel mortality was lower than 9% in both treatments (*Table 3*), and only occurred during the first 29 days. The fraction of discarded eels on Day 39 was slightly higher in eel-diet tanks (25.0%) than in salmon-diet tanks (19.6%).

There were no significant differences in specific growth rate (SGR) between the two dietary treatments (*Table 3*). The mean growth rate was slightly higher in the first period (Day 0 to Day 40) than in the second (Day 41 to Day 86). During this second period the fish were fed only with pelleted diet and the growth performance was the same for both dietary groups (SGR = 1.1 ± 0.4% day^{-1})
Analysis of feed conversion efficiency from Day 41 to the finish showed a numerically better FCR for the fish fed the eel diet (1.0 ± 0.02) than the salmon diet (1.1 ± 0.03), but not statistically significant (P = 0.06). There were no significant differences in the percentage of biomass gain between the treatments (P = 0.16). Eel-diet tanks showed around 63.0% and salmon-diet tanks around 65.8% biomass increment in 46 days. However, the proportion of food consumption in eel-diet tanks was significantly lower than in salmon-diet tanks (P = 0.02), they consumed 12.2% less feed (*Table 3*).

An approximate doubling of body weight coefficient of variation (CV, %) was observed from Day 0 to Day 40 (without analysing the eels discarded on Day 39) for both dietary treatments, but from Day 41 to Day 86 the CV decreased in the fish group fed an eel diet and slightly increased in the fish fed a salmon diet (*Table 3*).

3.2 Body proximate composition

The fat content of the shortfin eel body was markedly different between cultured and wild fish (*Table 4 & Figure 1*). The mean fat content of wild eels of any category was less than 7%, with no significant difference between the three body-size ranges (WI, WM and WL). In contrast, that fat content of the cultured eels was between 19.7 and 22.4%, very much higher than for wild eels (P <

0.001). The fat content of cultured eels was not statistically affected by diet or body weight range (P > 0.05 for all comparisons). For both diets the large-size eels had a higher fat content (about 22%) than the medium-size eels (about 20%).

The cultured shortfin eels had lower moisture content than the wild eels (P < 0.001), demonstrating an inverse relationship between moisture and body fat related to the body fat content (*Table 4 & Figure 1*). Protein and ash contents did not differ significantly between cultured and wild eels for any size range.

3.3 Fatty acid profiles

A total of 53 fatty acids above 0.02% of the total chromatographic profile were included in the initial analysis of percent fatty acids. All the larger peaks were identified, 28 in all, and made up at least 92.4% of the total fatty acid profile of the different eel groups analysed. These 28 fatty acids form the basis of *Table 6*. The remaining unidentified 25 fatty acids above 0.02% were spread throughout the chromatographic profile and they form the basis of *Table 5*.

The percents of total unidentified fatty acid for the different eel groups ranged between 3.8 and 7.6% (*Table 5*). Although, they were quantitatively unimportant in relation to the values of identified fatty acids, their differences among the groups were significant. These unidentified fatty acids were qualitatively and quantitatively more prominent in the wild eels, WI, WM and WL, than in the cultured treatments (*Table 5*). Moreover, wild eel groups showed a much higher variability (CV) in the percent of total unidentified fatty acids than cultured eels. The CV values of wild eels ranged between 17.7 and 73.5, whereas they were between 6.3 and 14.4 for cultured eels. The comparison of unidentified fatty acid profile among body weight ranges showed important qualitative and quantitative differences for both wild and cultured fish (*Table 5*).

The results of *Table 6* indicate that the percent of total saturates, monoenes and polyunsaturated fatty acid did not vary significantly for the different eel groups; they ranged from 29.2 to 31.4%, 34.7 to 43.2% and 20.5 to 28.3% respectively. The fatty acids that were found in highest proportion were C18:1

n-9 (oleic acid, ranging from 21.7 to 27.8%), C16:0 (palmitic acid, from 19.7% to 21.0%), C16:1 n-7 (palmitoleic acid, from 7.0 to 9.3%), C22:6 n-3 (DHA, from 3.2 to 11.4%) and C20:5 n-3 (EPA, from 2.1 to 7.1%). Also were found in relatively high percent C14:0 (myristic acid), C18:0 (stearic acid) and C18:1 n-7 (cis-vaccenic acid).

Analysis of the 28 identified fatty acids showed several differences between cultured and wild eels (*Table 6*). The tank-reared eels showed a higher proportion of C14:0 (myristic acid), C20:1 and C20:5 n-3 (EPA), and lower proportion of C17:0, C18:1 n-7, C20:4 n-6 (arachidonic acid) and C22:4 n-6 fatty acids than wild eels. In regard to the unidentified fatty acids groups, wild eels showed a much higher variability in the fatty acid values than cultured eels. The CV values of total saturates (1.9 to 5.5%), monoenes (12.5 to 36.7%) and PUFA (29.9 to 52.4%) for wild eels contrast strongly with the corresponding values of 0.9 to 3.0%, 1.3 to 8.3% and 0.8 to 10.6% for cultured eels.

The identified fatty acid profile of cultured eels was not significantly affected by body weight range. The comparison of EFM versus EFL, and SFM versus SFL groups did not show statistical differences for any fatty acid (*Table 6*). Regarding the wild fish, there were found significant differences in the fatty acid profile of WI in relation to the other two wild groups (WM and WL), but they were only restricted to three fatty acids that were present in less than 1% (*Table 6*).

The analysis of PUFA clearly showed significant differences in percent of arachidonic ($P < 0.001$), EPA (< 0.001) and DHA ($P= 0.023$) acids among the eel groups (*Table 6*). As a result of these differences, the $\sum$ n-3 / $\sum$ n-6 ratio presented values lower than 3.4 in wild eels (Wi, WM and WL), higher than 5.8 in fish cultured under the eel diet (EFM and EFL) and intermediate values (4.1 and 4.2) in fish cultured under the salmon diet (SFM and SFL) (*Table 6 & Figure 2a*). The Atherogenic Index (AI) of the initial wild eel group (WI) at least doubled the values of the other eel groups. Moreover, the contrast among groups indicated differences in the AI between wild eels (> 2.3) versus cultured eels

(1.4 to 2.0), and also between fish fed eel diet (< 1.5) and fish fed salmon diet (> 1.8) (*Table 6 & Figure 2b*).

Table 3 – Body weights, specific growth rate, biomass gain, food consumption, feed conversion ratio, fish discarded and mortality of yellow shortfin eel (*Anguilla australis*) fed two different commercial diets for 86 days.

	Eel diet	**Salmon diet**
Number of fish at Day 0	n = 56	n = 56
Body weight		
Day 0 Mean ± SD (g)	103.1 ± 10.7	104.8 ± 11.5
Coefficient of variation, CV %	10.4	11.0
Day 40 Mean ± SD (g)	175.3 ± 47.5	184.4 ± 39.7
Coefficient of variation, CV %	27.1	21.5
Day 86 Mean ± SD (g)	285.8 ± 70.9	306.0 ± 70.1
Coefficient of variation, CV %	24.8	22.9
Period 1 – Day 0 to Day 40		
Eel mortality % (number of fish)	8.9 (5)	7.1 (4)
Eels discarded [1] % (number of fish)	25.0 (14)	19.6 (11)
SGR (Specific Growth Rate) (mean ± SD, % day^{-1})	1.2 ± 0.6	1.3 ± 0.4
Period 2 – Day 41 to Day 86		
Eel mortality (%)	0	0
Eels discarded (%)	0	0
SGR (Specific Growth Rate) (mean ± SD, % day^{-1})	1.1 ± 0.4	1.1 ± 0.4
BG (Biomass gain) * (% of initial biomass)	63.0 ± 0.9	65.8 ± 1.5
FI (Feed intake) * (% of initial biomass)	63.5 ± 1.7 [a]	72.3 ± 2.0 [b]
FCR (Feed conversion ratio) *	1.0 ± 0.02	1.1 ± 0.03

[a,b] Values in the same row with different superscripts are significantly different at $P < 0.05$.

[1] Non-growing and very slow-growing eels were discarded on day 39.
* Each value is the mean ± SEM of 4 tanks.

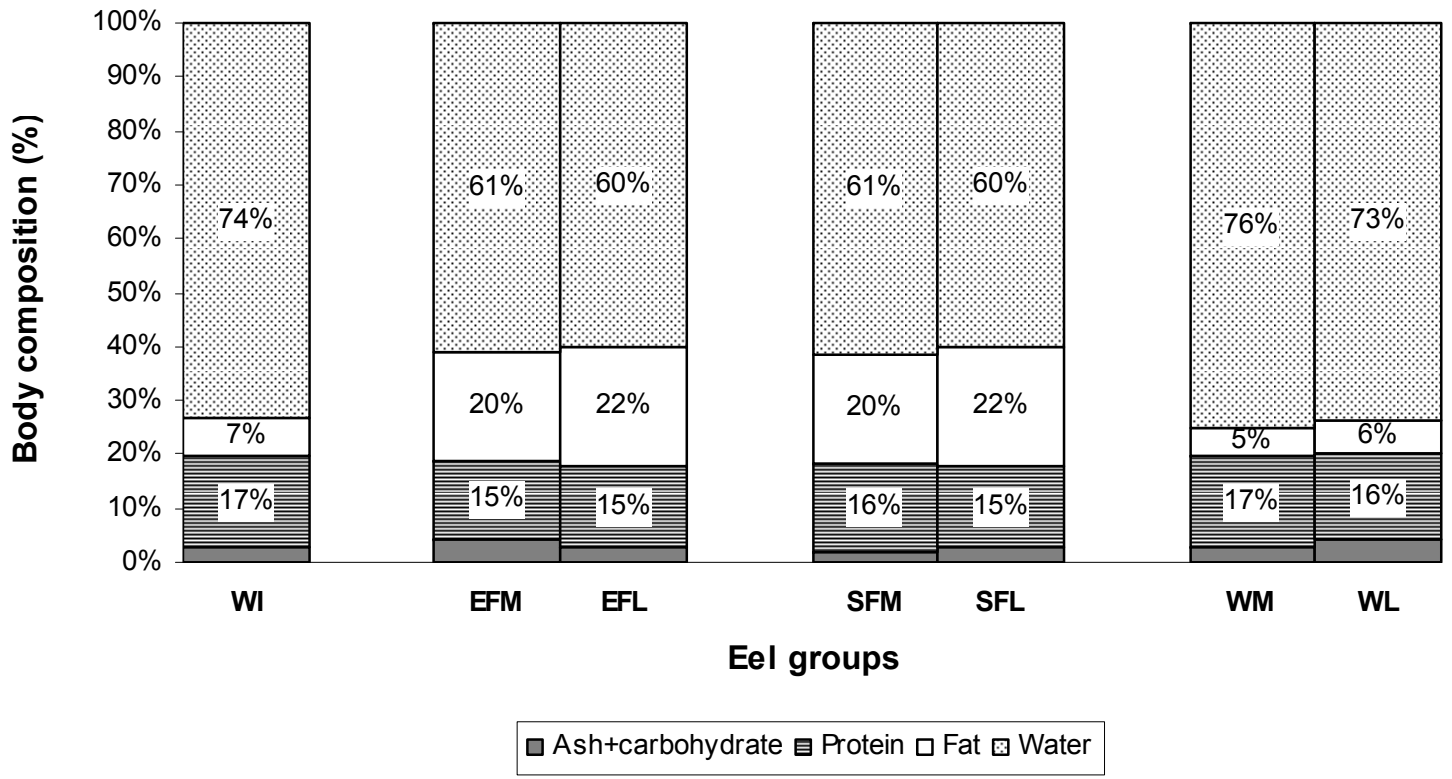
Table 4 – Body proximate composition on a wet weight basis in different body weight ranges of wild and cultured shortfin eels (*Anguilla australis*).

	Initial (wild)	Cultured eel fed eel diet		Cultured eel fed salmon diet		Wild eel	
	WI	**EFM**	**EFL**	**SFM**	**SFL**	**WM**	**WL**
Moisture (%)	73.7 ± 3.5 [a]	60.6 ± 2.9 [b]	59.8 ± 2.1 [b]	61.2 ± 2.4 [b]	59.9 ± 1.2 [b]	75.6 ± 5.6 [a]	73.4 ± 2.5 [a]
Crude protein (%)	16.6 ± 0.9	15.4 ± 0.6	14.8 ± 0.4	16.1 ± 1.4	15.3 ± 1.5	16.8 ± 1.4	16.3 ± 1.2
Fat (%)	6.9 ± 2.7 [a]	19.7 ± 3.2 [b]	22.4 ± 2.0 [b]	20.3 ± 2.4 [b]	22.2 ± 2.4 [b]	4.5 ± 5.6 [a]	6.3 ± 1.7 [a]
Ash (%)	1.5 ± 0.2	1.4 ± 0.1	1.4 ± 0.1	1.5 ± 0.1	1.4 ± 0.2	1.6 ± 0.1	1.5 ± 0.2

Wild fish: WI, initial (90 to 120g); WM, medium (220 to 300g); WL, large (400 to 600g). Cultured fish: EFM, fed eel diet (220 to 300g); EFL, fed eel diet (400 to 600g); SFM, fed salmon diet (220 to 300g); SFL, fed salmon diet (400 to 600g).

[a,b] Values in the same row with different superscripts are significantly different at $P < 0.05$. Data are means ± SD (n = 5).

Figure 1 – Body composition at different body-size range of the cultured and wild shortfin eel (*Anguilla australis*). The values of protein, fat and water are expressed as percent wet weight.



Wild fish: WI, initial (90 to 120g); WM, medium (220 to 300g); WL, large (400 to 600g). Cultured fish: EFM, fed eel diet (220 to 300g); EFL, fed eel diet (400 to 600g); SFM, fed salmon diet (220 to 300g); SFL, fed salmon diet (400 to 600g).

Table 5 – Unidentified fatty acid profile of body fat of different body weight ranges of wild and cultured shortfin eels (*Anguilla australis*). Data are mean percent of total fatty acids ± standard deviation for only unidentified fatty acids above 0.02% of the total chromatographic profile. The sum of fatty acids presented in this table plus those in *Table 6* add to 100%

	WI	**EFM**	**EFL**	**SFM**	**SFL**	**WM**	**WL**	Statistical effect (*P*)
Unidentified 1	1.01 ± 0.31[a]	0.43 ± 0.49[b]	0.27 ± 0.15[b]	0.36 ± 0.36[b]	0.47 ± 0.47[ab]	1.06 ± 0.15[a]	0.61 ± 0.38[ab]	0.020
C12 [1]								
Unidentified 2	Not detected[2]					0.07 ± 0.12		Not tested [3]
Unidentified 3						0.09 ± 0.16		
C14								
Unidentified 4	0.61 ± 0.28	0.08 ± 0.11	0.02 ± 0.05	0.05 ± 0.10		0.48 ± 0.52	0.53 ± 0.62	
Unidentified 5	0.05 ± 0.12					0.21 ± 0.36		
Unidentified 6						0.07 ± 0.12		
C15								
Unidentified 7						0.15 ± 0.17	0.00 ± 0.00	
Unidentified 8	0.30 ± 0.08					0.33 ± 0.29	0.24 ± 0.28	
Unidentified 9	0.13 ± 0.19					0.37 ± 0.34	0.27 ± 0.05	
Unidentified 10	0.66 ± 0.18[a]	0.36 ± 0.03[b]	0.32 ± 0.04[b]	0.32 ± 0.05[b]	0.31 ± 0.05[b]	0.64 ± 0.31[a]	0.38 ± 0.11[b]	0.000
C16								
Unidentified 11		0.04 ± 0.08				0.09 ± 0.16		
Unidentified 12	0.26 ± 0.07	0.05 ± 0.12				0.25 ± 0.24	0.13 ± 0.16	
Unidentified 13	0.81 ± 0.17[a]	0.39 ± 0.04[b]	0.30 ± 0.17[b]	0.35 ± 0.06[b]	0.27 ± 0.03[b]	0.72 ± 0.36[a]	0.80 ± 0.40[a]	0.000
Unidentified 14			0.08 ± 0.18			0.18 ± 0.16		

Unidentified 15	0.45 ± 0.12[a]		0.05 ± 0.12[b]			0.59 ± 0.70[a]	0.43 ± 0.37[a]	0.003
Unidentified 16	0.07 ± 0.17[a]	0.38 ± 0.21[b]	0.45 ± 0.11[b]	0.66 ± 0.03[b]	0.63 ± 0.12[b]	0.32 ± 0.12[ab]	0.23 ± 0.17[ab]	0.000
Unidentified 17	0.83 ± 0.47[a]	0.31 ± 0.10[b]	0.25 ± 0.15[b]	0.61 ± 0.04[ab]	0.50 ± 0.29[ab]	0.32 ± 0.47[b]	0.30 ± 0.23[b]	0.034
C17								
Unidentified 18		0.07 ± 0.17				0.09 ± 0.15		
Unidentified 19						0.11 ± 0.19		
Unidentified 20						0.19 ± 0.17		
Unidentified 21		0.63 ± 0.16	0.77 ± 0.05	0.89 ± 0.06	0.80 ± 0.20	0.09 ± 0.15	0.03 ± 0.05	
Unidentified 22		0.22 ± 0.06	0.21 ± 0.12	0.31 ± 0.08	0.34 ± 0.05	0.17 ± 0.30	0.14 ± 0.16	
Unidentified 23	0.15 ± 0.14[a]	0.31 ± 0.09[b]	0.37 ± 0.02[b]	0.40 ± 0.10[b]	0.45 ± 0.08[b]	0.32 ± 0.07[b]	0.31 ± 0.10[b]	0.001
C18								
C20								
Unidentified 24	0.49 ± 0.46	0.23 ± 0.09	0.31 ± 0.02	0.27 ± 0.01	0.25 ± 0.01	0.53 ± 0.47	0.55 ± 0.68	0.384
Unidentified 25		0.30 ± 0.08	0.34 ± 0.04	0.38 ± 0.05	0.36 ± 0.09	0.18 ± 0.06	0.16 ± 0.18	
∑ Unidentified	5.87 ± 1.04	3.82 ± 0.54	3.75 ± 025	4.60 ±0.29	4.37 ± 0.63	7.63 ± 5.61	5.11 ± 1.63	0.077

Wild fish: WI, initial (90 to 120g); WM, medium (220 to 300g); WL, large (400 to 600g). Cultured fish: EFM, fed eel diet (220 to 300g); EFL, fed eel diet (400 to 600g); SFM, fed salmon diet (220 to 300g); SFL, fed salmon diet (400 to 600g).

[a,b] Values in the same row with different superscripts are significantly different at $P < 0.05$

[1] On the basis of identified fatty acids, the Cn positions were distributed through the ascending order of retention times peaks. These Cn values are included as a rough guide to the approximate molecular mass the unidentified fatty acids. Note that unidentified fatty acids between Cn and Cn+1, could be all Cn, all Cn+1 or a mix of Cn and Cn+1.

[2] Blanks mean fatty acid not detected in that treatment.

[3] Blanks in this column mean not tested because fatty acid not detected in one or more treatments.

Table 6 – Identified fatty acid profile of body fat of different body weight ranges of wild and cultured shortfin eels (*Anguilla australis*). Data are mean percent of total fatty acids ± standard deviation for only identified fatty acids above 0.02% of the total chromatographic profile. The sum of fatty acids presented in this table plus *Table 5* adds to 100%

	WI	EFM	EFL	SFM	SFL	WM	WL	Statistical effect (*P*)
C12:0	0.25 ± 0.02	Not detected [1]				0.13 ± 0.23	0.13 ± 0.15	Not tested [2]
C14:0 Myr	4.09 ± 0.82^{a}	5.54 ± 0.14^{b}	5.64 ± 0.20^{b}	5.39 ± 0.22^{b}	5.21 ± 0.51^{b}	4.20 ± 0.40^{a}	4.51 ± 0.90^{ab}	<0.001
C15:0	0.56 ± 0.16^{a}	0.60 ± 0.03^{a}	0.58 ± 0.02^{a}	0.41 ± 0.06^{b}	0.35 ± 0.03^{b}	0.77 ± 0.34^{a}	0.41 ± 0.22^{ab}	0.005
C16:0 Pam	20.30 ± 1.08	19.94 ± 0.69	19.70 ± 0.15	20.60 ± 0.66	20.78 ± 0.33	19.08 ± 3.31	20.99 ± 0.64	0.277
C17:0	1.11 ± 0.32^{a}	0.56 ± 0.04^{b}	0.44 ± 0.05^{b}	0.43 ± 0.11^{b}	0.30 ± 0.03^{b}	0.99 ± 0.46^{ab}	0.69 ± 0.05^{b}	<0.001
C18:0	4.13 ± 0.68^{a}	2.86 ± 0.11^{b}	2.87 ± 0.26^{b}	3.30 ± 0.26^{b}	3.48 ± 0.09^{ab}	4.96 ± 1.06^{a}	4.63 ± 0.28^{a}	<0.001
C20:0	0.02 ± 0.05	0.06 ± 0.13				0.11 ± 0.12		
∑ saturates	30.45 ± 1.16^{ab}	29.56 ± 0.70^{ab}	29.23 ± 0.26^{a}	30.12 ± 0.92^{ab}	30.12 ± 0.38^{ab}	30.25 ± 1.67^{ab}	31.37 ± 0.58^{b}	0.029
C14:1	0.25 ± 0.16	0.02 ± 0.05						
C16:1 n-7	9.29 ± 1.75	7.80 ± 0.31	7.44 ± 0.31	8.25 ± 0.34	7.78 ± 0.20	7.00 ± 2.42	9.30 ± 2.06	0.083
C17:1	0.09 ± 0.21^{a}	0.50 ± 0.13^{b}	0.43 ± 0.12^{b}	0.29 ± 0.03^{ab}	0.28 ± 0.03^{ab}	0.78 ± 0.22^{b}	0.30 ± 0.36^{ab}	0.001
C18:1 n-9 Ole	27.77 ± 3.62	24.42 ± 1.06	24.65 ± 0.67	25.36 ± 1.81	27.68 ± 3.62	21.67 ± 10.72	27.18 ± 6.36	0.434
C18:1	4.96 ± 0.20^{a}	3.41 ± 0.15^{b}	3.18 ± 0.14^{b}	3.42 ± 0.29^{b}	3.12 ± 0.07^{b}	4.49 ± 0.56^{a}	4.64 ± 0.90^{a}	<0.001
C20:1	0.59 ± 0.17^{a}	2.75 ± 0.18^{b}	3.01 ± 0.10^{b}	1.53 ± 0.06^{ab}	1.66 ± 0.03^{ab}	0.55 ± 0.24^{a}	0.67 ± 0.13^{a}	<0.001
C24:1	0.05 ± 0.11	0.04 ± 0.10	0.02 ± 0.05			0.21 ± 0.36	0.10 ± 0.14	
∑ monoenes	43.20 ± 5.38	38.96 ± 1.07	38.72 ± 0.51	38.84 ± 1.52	40.52 ± 3.36	34.69 ± 12.72	42.19 ± 9.15	0.464
C18:2 n-6 Lin	3.34 ± 1.38	2.93 ± 0.29	2.81 ± 0.05	3.28 ± 0.58	3.63 ± 0.65	3.81 ± 4.07	1.36 ± 0.29	0.227
C18:3 n-6	0.07 ± 0.16			0.05 ± 0.07	0.04 ± 0.09	0.30 ± 0.27	0.26 ± 0.30	

C18:3 n-3	1.74 ± 0.77	0.75 ± 0.11	0.58 ± 0.07	0.66 ± 0.14	0.51 ± 0.02	3.08 ± 3.93	0.68 ± 0.16	0.078
C18:4		0.69 ± 0.07	0.74 ± 0.06	0.67 ± 0.05	0.63 ± 0.13	0.60 ± 0.56	0.24 ± 0.27	
C20:2	0.54 ± 0.05^{a}	0.26 ± 0.02^{ab}	0.24 ± 0.01^{ab}	0.18 ± 0.09^{b}	0.10 ± 0.11^{b}	0.40 ± 0.36^{a}	0.47 ± 0.27^{a}	0.003
C20:3 n-6	0.39 ± 0.23			0.25 ± 0.02	0.17 ± 0.11	0.47 ± 0.57	0.25 ± 0.29	
C20:4 n-6 Ach	2.19 ± 1.29^{a}	0.94 ± 0.11^{b}	0.72 ± 0.10^{b}	1.17 ± 0.19^{b}	0.86 ± 0.09^{b}	3.03 ± 1.59^{a}	2.34 ± 0.61^{a}	<0.001
C20:3 n-3	0.18 ± 0.17					0.27 ± 0.30	0.11 ± 0.13	
C20:4 n-3	0.24 ± 0.15^{a}	0.92 ± 0.05^{b}	1.02 ± 0.06^{b}	0.74 ± 0.06^{ab}	0.78 ± 0.15^{b}	0.84 ± 0.69^{b}	0.43 ± 0.38^{a}	0.003
C20:5 n-3 EPA	2.09 ± 1.47^{a}	5.94 ± 0.53^{b}	6.14 ± 0.25^{b}	7.09 ± 0.47^{b}	6.72 ± 1.30^{b}	3.88 ± 0.53^{a}	3.97 ± 2.43^{a}	<0.001
C22:4 n-6	0.44 ± 0.25^{a}	0.05 ± 0.10^{b}	0.02 ± 0.05^{b}	0.13 ± 0.14^{b}	0.05 ± 0.07^{b}	0.75 ± 0.38^{a}	0.54 ± 0.17^{a}	<0.001
C22:3 n-3	0.43 ± 0.45	0.42 ± 0.05	0.33 ± 0.04	0.29 ± 0.09	0.15 ± 0.11	0.44 ± 0.35	0.34 ± 0.40	0.355
C22:5 n-3	1.77 ± 1.26	3.99 ± 0.23	4.26 ± 0.21	4.28 ± 0.41	3.57 ± 2.07	3.06 ± 0.53	3.23 ± 1.98	0.152
C22:6 n-3 DHA	3.22 ± 2.89^{a}	10.76 ± 0.76^{b}	11.42 ± 0.23^{b}	7.63 ± 0.50^{ab}	5.98 ± 3.37^{ab}	6.49 ± 2.96^{ab}	7.10 ± 7.32^{ab}	0.023
∑ PUFA	20.48 ± 6.12	27.66 ± 1.84	28.30 ± 0.24	26.43 ±1.15	24.99 ± 2.66	27.42 ± 8.53	21.32 ± 11.18	0.193
∑PUFA/∑saturates	0.67	0.94	0.97	0.88	083	0.90	0.68	
∑ n-6	7.13 ± 1.27^{ab}	3.92 ± 0.34^{ab}	3.56 ± 0.16^{a}	4.89 ± 0.39^{ab}	4.76 ± 0.78^{ab}	8.36 ± 6.32^{b}	4.75 ± 1.34^{ab}	0.019
∑ n-3	12.82 ± 5.59	22.79 ± 1.55	23.75 ± 0.34	20.70 ± 1.33	19.50 ± 3.23	18.07 ± 1.42	15.87 ± 12.45	0.034
∑ n- 3 / ∑ n-6	1.79	5.81	6.67	4.23	4.10	2.16	3.34	
AI[3]	4.59	1.53	1.44	1.77	2.05	2.25	2.30	

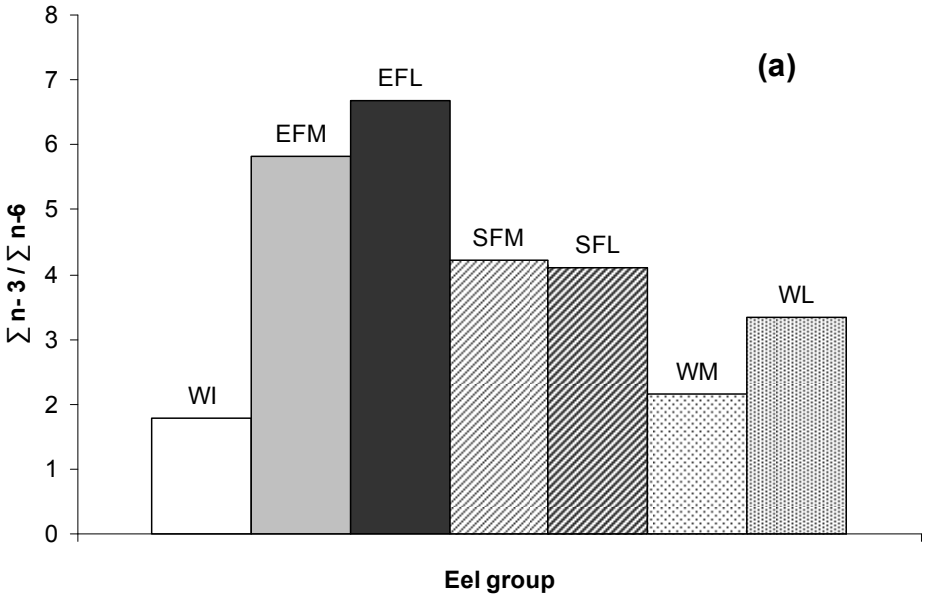
Wild fish: WI, initial (90 to 120g); WM, medium (220 to 300g); WL, large (400 to 600g). Cultured fish: EFM, fed eel diet (220 to 300g); EFL, fed eel diet (400 to 600g); SFM, fed salmon diet (220 to 300g); SFL, fed salmon diet (400 to 600g).

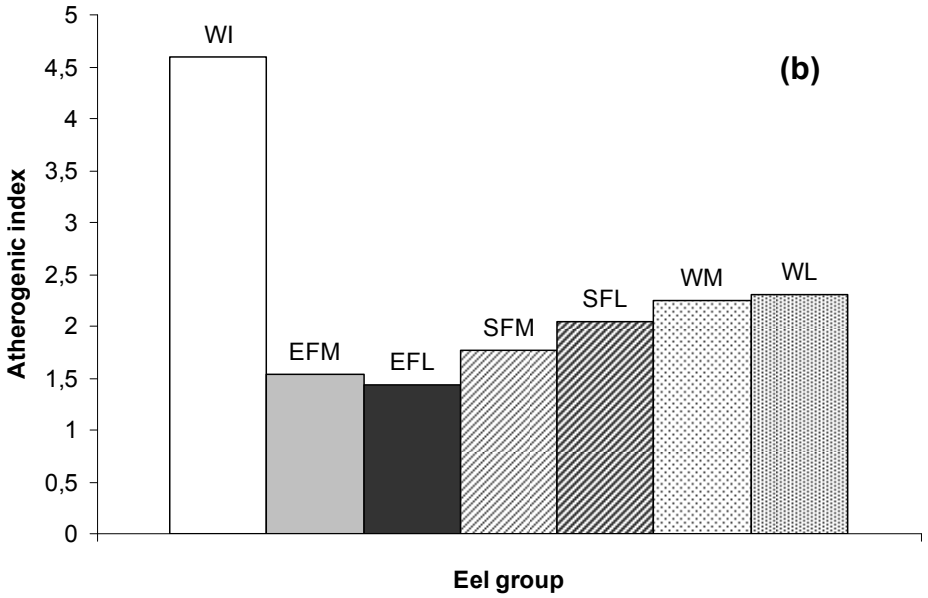
^{a,b} Values in the same row with different superscripts are significantly different at $P < 0.05$

Myr, myristic acid; Pam, palmitic acid; Ole, oleic acid; Lin, linoleic acid; Ach, arachidonic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid

[1] Blanks mean fatty acid not detected in that treatment. [2] Blanks in this column mean not tested because fatty acid not detected in one or more treatments. [3] Atherogenic Index = (C14:0 + C16:0) / (C20:5 n-3 + C22:6 n-3) (Abrami *et al*., 1992)

Figure 2 - Index of fat quality of different body weight ranges of wild and cultured shortfin eels (*Anguilla australis*). (a) Ratio of percent total omega 3 (n-3) and total omega 6 (n-6) fatty acids, (b) Atherogenic Index: ratio of sum of myristic and palmitic acids to sum of EPA and DHA.





Wild fish: WI, initial (90 to 120g); WM, medium (220 to 300g); WL, large (400 to 600g).

Cultured fish: EFM, fed eel diet (220 to 300g); EFL, fed eel diet (400 to 600g); SFM, fed salmon diet (220 to 300g); SFL, fed salmon diet (400 to 600g).

4. Discussion

4.1 Growth parameters and feed efficiency

The specific growth rate observed for both dietary treatments in the present experiment (SGR= 1.1% day^{-1}) compares favourably with growth rates reported for eel species of similar size range, fed pellet diets and reared indoors in recirculation-system tanks. Laboratory studies on European eel showed a growth rate between 0.4 and 1.2% day^{-1} (usually around 0.8% day^{-1}) for eel specimens bigger than 30 g (Heinsbroek, 1991; Kushnirov & Degani, 1991; Suarez *et al*., 2002; Heinsbroek *et al*., 2007; Heisnbroek *et al*., 2008; Karipoglou & Nathanailides, 2009). On a commercial scale, Mas-Alvarez & Barrera (1989) reported SGR values of around 1.1% day^{-1} for glass eel and elvers, and 0.8% day^{-1} for fish bigger than 12 g for European eels reared at 25 °C in a recirculation system.

Available information about growth rate of cultured yellow shortfin eel is restricted to a few experiments carried out in outdoor ponds, or indoor tanks where the fish were fed moist paste diets. Studies on shortfin reared in ponds recorded a mean SGR of 0.9% day^{-1} or lower with highly variable individual growth (Tomiyama *et al*., 1979; Waught, 1980; Chisnall & Martin, 2002). Trials on wild shortfin eel (mean weight 262 g) reared in a semi-recirculating aquaculture system and weaned on a synthetic wetmix food reported a biomass gain of 12% in a 43-day period (SGR 0.3% day^{-1}) (Martin & Chisnall, 2004).

A comparative analysis of feed conversion ratio (FCR), biomass gain (BG) and feed intake (FI) between the eel and salmon diets suggests a better feed efficiency for the eel diet. Nevertheless, both diets resulted in good performance. The values of FCR = 1.0 for the eel-diet tanks and FCR = 1.1 for the salmon-diet tanks are promising from an economic point of view. They compare favourably with other studies on eel species. Studies on European and Japanese eels bigger than 30 g (cultured in indoor RAS and fed pelleted diets) reported values of FCR from 0.9 to 3.3, generally between 1.3 and 1.9 (Heinsbroek, 1991; Suarez *et al*., 2002; Gooley & Gavine, 2003; Karipoglou & Nathanailides, 2009).

In the present experiment, approximately 30% of the shortfin eels did not adapt well to the rearing conditions. They died or were discarded because of negative or very slow growth rates. Several studies on eel species have shown similar or higher proportion lost during the acclimation and weaning phase of glass eels (Wickins, 1983; Appelbaum & Birkan, 1992; Heinsbroek & Kreuger, 1992; Ingram, 2002; R. Barrera, VALAQUA S.A., Puçol, Spain, *personal communication*, 2009). In relation to yellow shortfin eel, the experimental results of Martin & Chisnal (2004) agree with those of the present study. Those researchers successfully weaned around 70% of the original fish stock onto a wetmix diet. The fraction of farmed eels that die or do not grow well is still a high proportion in modern eel farming, which causes important economic losses (R. Barrera, *personal communication,* 2009). This is an aspect of culturing eels that needs further investigation.

The increment in the coefficient of variation (CV) from Day 0 to Day 40 may be explained by the different grades of adaptability of the individual eels to the artificial feed (weaning stage) and the new culture environment. These differences are reflected in differences of individual growth rate, and consequently in variation of fish size, which increased with time, thus raising the CV. In the second period of the experiment, where only eels well adapted to the rearing tanks were maintained, the CV decreased in eel-diet tanks and increased slightly in the salmon-diet tanks. That indicates a relatively uniform individual growth rate in both dietary groups during the 46 days of the *Period 2*.

The SGR and FCR values achieved in the present study, with the yellow eel phase, are very encouraging and indicate that from a biological point of view the shortfin eel has good potential as a cultured species. Both pelleted feeds, with the same protein content (47%) and gross energy (22.2 MJ kg^{-1}), resulted in good performance. The overall performance of the eel diet was slightly superior to the salmon diet. Although the eels fed the salmon diet attained a final mean weight a little higher than the eels fed the eel diet, the group of eel-diet tanks converted the food into biomass better and grew slightly more uniformly than the salmon-diet tanks group. It must be noted that the values of SGR and FCR

obtained in the present experiment were under a laboratory setting with very careful handling. A commercial small-scale pilot study should be carried out.

4.2 Body proximate composition

The proximate composition of cultured fish is governed by endogenous and exogenous factors. The endogenous factors are genetically controlled and are associated to the size and life-cycle stage of the fish, whereas the exogenous factors are related to the environment and the diet (Shearer, 1994; Morris, 2001). It is recognised that the protein content and ash are endogenously controlled (life cycle and size-dependent), whereas the fat content is affected by both endogenous and exogenous factors and the whole body moisture content is inversely related to the fat content (Shearer, 1994). This implies that through fish culture the protein content and ash cannot be changed easily. In contrast, the fat content and moisture of the fish body can be modified by changes in diet. As hypothesised, in the present study it was possible to grow a fatty shortfin-eel product of about 20 to 22% fat starting from wild yellow eels with an initial fat content below 7% by feeding high energy pelleted feeds.

The values for fat content of wild shortfin eels observed in this study are in agreement with other reports on this species (Hopkirk *et al*., 1975; Wills & Hopkirk, 1975; Sumner & Hopkirk, 1976; Sumner *et al.*, 1984). These previous studies on wild eels indicate values of fat between 2 and 15% (generally below 10%), which are dependent on fish-size, section of the fish sampled, and the time of the year they were captured. Wills & Hopkirk (1975) reported a large variation in fat content along the length of wild yellow shortfin eels (mean 340 g), with the fat increasing from 2% in the pectoral-fin section to 14% in the tail section. The research of Sumner & Hopkirk (1976) on the whole body fat content of wild shortfin eels showed values of 10% for fish of 200 g and 15% for fish of 500 g; the research also indicated that the fat content of eels of similar size (around 300 g) captured in October (post-hibernation) was 8%, whereas for eels captured in January (summer) the content was 10%. Sumner *et al.* (1984) compared the fat content of yellow shortfin eels from Australia and New Zealand, found that New Zealand eels had on average considerably less fat in

their body (8.7%) than Australian eels (12.6%), and also indicated a general value of 5% fat for a New Zealand fish size of about 200 g.

Analysis of fat content of yellow shortfin eels captured in the wild and cultured for a period of time, is limited to the report of Tomiyama *et al.* (1979). These researchers fed eels (mean initial weight of 135 g) using high oil diet (20%) and low oil diets (10%) formulated in a moist paste. The initial body fat of the wild eels captured in Lake Ellesmere (New Zealand) in late January was around 10%, and at the end of the 34-day experiment, they were able to harvest eels with a mean body fat of 21.7 and 18.0%, for the high and low fat diets respectively.

The results of the present study suggest no differences in the contents of crude protein, fat, moisture and ash/carbohydrate of fish fed the eel diet or the salmon diet. The diets were claimed to be iso-nitrogenous (47% of protein) and iso-energetic (22.2 MJ kg^{-1}) but they differed in the contents of dietary fat and carbohydrate. The fact that both diets allow attainment of the same concentration of fat in the eel body, in spite of differences in dietary lipid, can be explained by the use of dietary carbohydrate for synthesis and storage of body fat. Degani & Gallagher (1995) and Suarez *et al.* (2002) found that eels have the ability to utilise a relative high percentage of carbohydrates to induce storage of body fat. The present study indicates that it is possible to obtain a similar fatty-eel product of about 20% of total fat using a diet with of fat content of 22% instead of 26% but increasing dietary carbohydrate to compensate.

In both dietary treatments, the large-size eels (EFL and SFL) showed a numerically fat content higher than the medium-size eels (EFM and SFM). This is in agreement with numerous studies on eel species, which state that there is a direct relationship between eel size and total fat content. Bigger fish have more fat (Gallagher *et al*., 1984; Böetius & Böetius, 1985; Degani *et al.,* 1988; Lie *et al.*, 1990; Degani & Gallagher, 1995; Garcia-Gallego & Akharbach, 1998; Lie, 2001; Heinsbroek *et al.,* 2007).

4.3 Fatty acids

Fish lipids are rich in long-chain n-3 polyunsaturated fatty acids, which are especially important in human nutrition because of their role in prevention of cardiovascular and other associated degenerative diseases (Bang & Dyerberg, 1985; Kinsella, 1987; Simopoulos, 1991; Vazquez & Sanchez-Muñiz, 1994; Horrocks & Yeo, 1999; Sargent *et al*., 2001, Alasalvar *et al*., 2002). Among these n-3 series, EPA and DHA are considered the most beneficial fatty acids for human health. They play a protective role against arterial thrombosis and atherosclerotic cardiovascular disease (Kromhout *et al*., 1985; Leaf, 1989; Singer *et al.,* 1992). EPA and DHA can be synthesised in humans from precursor linolenic acid (18:3) but the process is inefficient. A diet containing these fatty acids is the better source (Alasalvar *et al*., 2002).

From the perspective of consumers' health, the nutritional quality of fish fat can be defined by the ratio of the amount of saturated, monoenes and polyunsaturated (PUFA) fatty acids present in the fish tissue. From this perspective, a good quality fish product should have high proportion of PUFA and a low proportion of saturated fatty acids. The Atherogenic index (AI) (Abrami *et al*., 1992) is a good indicator of fat quality. It presents the ratio between the sum of the most abundant among the saturated fatty acids (myristic and palmitic acids) and the sum corresponding to the main PUFA n-3 (EPA and DHA). Therefore, good dietary fat should have a low Atherogenic Index.

Besides the proportion of PUFA, the ratio between n-3 and n-6 fatty acids is also important. Both n-3 and n-6 fatty acids are precursors of eicosanoids, which are a family of biologically active compounds. Ideally, the human body should have a near balanced intake of these fatty acids, because the eicosanoids derived from n-3 fatty acids have opposing metabolic properties to those derived from n-6 fatty acids. The n-3 fatty acids have anti-inflammatory, anti-thrombotic, anti-arrhythmic, hypolipidemic, and vasodilatory properties, and they play an important role in the modulation and prevention of human diseases, particularly coronary heart disease, type-2 diabetes, and renal disease. On the other hand, n-6 fatty acids promote platelet aggregation and

vasoconstriction, as well as increasing blood viscosity and decreasing bleeding time.

In today's average Western diet, the ratio between n-3 to n-6 fatty acids is extremely unbalanced toward n-6, ranging from 1:15 to 1:20 instead of the traditional, and optimum, range of 1:1 as is the case with wild animals (Simopoulus, 2008). This means that the eicosanoids produced from the metabolism of n-6 fatty acids are formed in greater proportions than those derived from the n-3 fatty acids. The result is an increase in cardiovascular and inflammatory disorders (Sargent *et al*., 2001). Consequently, people should be encouraged to eat more foods rich in n-3 fatty acids in order to make the ratio more balanced. The n-3 to n-6 ratio can be improved by increasing our intake of n-3 fatty acids from foods such as oily fish. A beneficial fish product should content a relatively high n-3 to n-6 ratio (Ahlgren *et al*., 1994, Sargent *et al*., 2001).

The fatty acid profiles of the wild eel groups analysed in this study were in agreement with other studies on wild shortfin eels. The n-3 to n-6 ratio (Henderson & Tocher, 1987) and the Atherogenic Index (AI) (Abrami *et al*., 1992), calculated from tables of fatty acid composition presented in previous investigations on yellow wild *A. australis* (Wills & Hopkirk, 1975; Sumner & Hopkirk, 1976; Sumner *et al*., 1984; De Silva *et al*., 2002), were similar to those in this study. The n-3 to n-6 ratios were typical of freshwater fish, with values < 3.5 in all the studies. The Atherogenic Index values were always > 2, and also there seems to be a tendency for small fish to have a higher AI than large fish. Moreover, the wild Australian shortfin eel had less unsaturated fat (lower n-3 to n-6 ratio and higher AI) than the wild New Zealand shortfin eel. Sumner *et al*. (1984) explained these differences in the fatty acid profile as a consequence of lower water temperatures in New Zealand compared with Australia, such that the lower the temperature the higher the degree of unsaturation.

It has been recognised that the fatty acid composition of fish can be affected by diet (Watanabe *et al*., 1983; Olsen & Skjervold, 1995; Bell *et al*., 2002; Mourente & Bell, 2006), food deprivation (Dave *et al.,* 1976; De Silva *et al*., 1997) and environmental factors (Satoh *et al*., 1984; Bell *et al*., 1986). Among

these factors, diet has been confirmed in several studies as the main reason for the differences in the fatty acid profile between cultured and wild fish species (Otwell & Rickards, 1981/1982; Krajnovic-Ozretic *et al*., 1994; Chen *et al*., 1995; Garcia-Gallego & Akharbach, 1998; Grigorakis *et al*., 2002; Alalsalvar *et al.*, 2002, among others).

The results of this study showed important biological and nutritional differences between the fatty acid profile of wild and cultured eels. Although the 25 unidentified fatty acids were quantitatively unimportant, their differences among the groups were biologically significant. The higher quantitative and qualitative values of unknown fatty acids in wild eels may be associated to diet. The diet of wild eels was very likely to be much more varied in terms of wild species consumed, and this is probably responsible for the more complex pattern of fatty acids that make up the fat fraction. From a nutritional point of view, the analysis of the 28 identified fatty acids (*Table 6*), based on the n-3 to n-6 ratio, and the Atherogenic Index, indicates that the fat quality of the shortfin eel reared in the present experiment was appreciably superior, in terms of human diet, to the shortfin eels caught in the wild. Likewise, the fat quality of fish fed eel diet was higher than of those fed salmon diet, suggesting a better dietary fat in the former. It is interesting to note that the fatty acid composition of cultured eels was not significantly affected by body weight range. This is in agreement with the results of Garcia-Gallego & Akharbach (1998) who stated that European eels cultured for the same time but with different weights did not vary significantly in their fatty acid profile.

Fatty acid studies on other eel species have shown differences between wild and cultured fish, but with diverse results. A better final quality of cultured fish was found by Ozaki *et al*. (2008) and Oku *et al*. (2009) with Japanese eel, and by Abrami *et al*. (1992) wih European eel. On the other hand, Otwell & Rickards (1981/1982) and Garcia-Gallego & Akharbach (1998) researching American and European eel respectively, found a poorer quality of body fat of cultured in relation to wild fish. These disparities of results might be associated to the dietary fat quality. Cultured eel tissue with a lower fat quality in these studies may reflect the inclusion in the pellet diets of fat sources other than fish oil,

presumably fat from animals other than fish as well as vegetable oils. Therefore, a careful selection of the dietary lipid must be made to obtain a fish product that addresses the perceived and real health benefits that contribute to consumer demand.

In conclusion, the results of the present study are encouraging about the prospect of farming shortfin eels (*Anguilla australis*) and obtaining a market-oriented eel product in New Zealand. The values of growth rate and feed efficiency obtained in the feeding trial are comparable to the values observed in the very well developed European and Japanese cultured eel industries. Through aquaculture it was possible to increase the fat content and fat quality (higher proportion of polyunsaturated omega-3 fatty acids) of the final New Zealand eel product. Therefore, the requirement of the global market for a high-fat product that it is at the same time perceived as 'healthy' can clearly be achieved. Moreover, farm production of any biological product results in lower product variability. Low variability is a prerequisite to successful branding, which in turn can lead to market premiums and a loyal customer base. At this time the particular flavour outcomes of the combination shortfin eel plus eel diet or salmon diet are unknown, but there is no reason to suspect that the flavour, when smoked or otherwise, would be adverse. Flavour studies, however, should be a future objective when market prospects are explored in some future research.

Chapter 3

Effect of size grading on the growth performance of shortfin eel (*Anguilla australis*) during its yellow stage

1. Introduction

Variation in individual growth rate within a fish stock is a significant obstacle to profitable management of a commercial fish culture (Jobling, 1985; Barki *et al*., 2000). Among the cultured fish species, eels are known to have one of the highest variations in individual growth rates (Koops & Kuhlman, 1979; Knights, 1982; Seymour, 1984; Appelbaum & Birkan, 1992; Kamstra, 1993). In order to minimise the heterogeneity of size, it is common practice to grade the cultured species several times during their production cycle. Grading refers to the process of sorting the fish in different size-classes; fish in a heterogeneous population are regrouped into more homogeneous groups according to size similarities (Conte, 2004).

Size grading facilitates the feeding and harvesting operation in the fish farm, by enabling each graded group of fish to be fed with the optimum ration and pellet size, and by avoiding aggressive behaviour such as bullying, tail and fin nipping and sometimes cannibalism (Wallace & Kolbeinshavn, 1988; Shepherd & Bromage, 1992). Furthermore, it is generally believed that size grading improves the growth rate of small individuals, and consequently increases the total biomass output of the cultured species (Lee, 1988; Baardvik & Jobling, 1990; Sunde *et al.,* 1998; Seppa *et al*., 1999; Barki *et al.,* 2000; Lambert & Dutil, 2001; Zakés *at al*, 2004; Martins *et al*, 2006).

The logic behind the concept that size grading increases the total biomass production lies in studies that have identified the establishment of social hierarchies as the main factor responsible for the growth variation observed in cultured fish (Metcalfe, 1986; Metcalfe *et al*., 1989; Johnsson, 1997; Martins *et al*., 2005; Wallat *et al.*, 2005). Larger fish are usually considered the dominant fish, which have a suppressive effect on the feed intake and growth of the subordinate (smaller) fish (Cutts *et al*., 1998). Therefore, it is expected that size grading will minimise the stress imposed by the larger individuals over small individuals, resulting in a higher feed intake by the smaller specimens, and consequently a higher growth rate and total biomass output (Gunnes, 1976; Brzeski & Doyle, 1995; Seppa *et al*., 1999).

Nevertheless, studies testing the assumption that grading has a beneficial effect on the growth of small individuals are contradictory. A number of studies have shown a positive effect of size grading on growth, such as in Atlantic halibut (*Hippoglossus hippoglossus*) (Imsland *et al.,* 2009), in Arctic charr (*Salvelinus alpinus*) (Seppa *et al*., 1999), in salmonids (Gunnes, 1976), and in tilapia (*Oreochromis niloticus*) (Brzeski & Doyle, 1995). However, several other studies do not support the notion of a positive effect on growth, for example in catfishes (*Ictalurus punctatus* and *Clarias gariepinus*) (Carmichael, 1994; Martins *et al*., 2005), in Arctic charr (*Salvelinus alpinus*) (Baardvik & Jobling, 1990; Wallace & Kolbeinshavn, 1988); in flatfishes (*Scophthalmus maximus* and *Hippoglossus hippoglossus)* (Sunde *et al*., 1998; Stefansson *et al.*, 2000), and in percid (*Sander lucioperca* and *Perca flavescens*) (Zakés *et al*., 2004; Wallat *et al*., 2005).

Studies on eels about the relation between grading and growth have focused on the European eel (*Anguilla anguilla*). The development of hierarchies and aggressive behaviour among tank-mates has been well established for this species (Peters *et al*., 1980; Wickins, 1985, 1987; Knights, 1987). Several authors have concluded that size-grading increases the biomass of European eels during their glass-eel and elver stages (Seymour, 1984; Knights, 1987; Yahyaoui, 1988; Appelbaum & Birkan, 1992). However, studies on the effect of grading on larger eels are practically nonexistent. The only publication known is the study of Kamstra (1993), who worked with marked European eels over 26 g and found that size grading did not have a significant effect on total biomass output.

The aim of the present study was to evaluate the effect of size grading on individual growth performance during the yellow stage of shortfin eel (*Anguilla australis*). The hypothesis that small-size eels can improve their growth rate in the absence of large-size eels has been tested. PIT tagging technology, whose suitability for the marking of eel was confirmed in *Annexe 3,* allowed tracking the growth rate of each specimen throughout the experiment.

2. Materials and methods

2.1 Fish and maintenance

Shortfin eels used in the present study were collected from Lake Waikare, North Island, New Zealand in October 2008, and transported to AUT University's Aquaculture Laboratory. On arrival, the eels were transferred to rearing tanks that were connected to freshwater recirculation modules (*Annexe 2*).

Fish were held for six weeks until the start of the experiment. During this period, the eels were weaned to artificial feed and individually marked with PIT tags as explained in the *Chapter 2* and *Annexe 3*. From the arrival to the laboratory until the termination of the experiment, the fish were reared under the same environmental conditions as described in *Chapter 2*.

2.2 Experimental Procedures

Six weeks after arrival, the eels were individually measured (total weight (BW) and length (TL)) and manually graded into 3 experimental groups by weight (mean ± SD, minimum to maximum weight):

(1) *Graded-S*, a relatively homogeneous group of 21 small eels (98.5 ± 18.1, 70 to 135 g)

(2) *Graded-L*, a relatively homogeneous group of 32 large eels (182.2 ± 28.6, 136 to 250 g)

(3) *Ungraded*, a relatively heterogeneous group containing a mixture of 19 small and 31 large eels (150.0 ± 49.0, 70 to 250 g).

Eel were distributed in nine tanks such that each experimental group was split uniformly within three tanks. Stocking density for these tanks ranged from 12 to 15 kg m^{-3}. Different volumes of water were used in the tanks in order to keep stocking density between these values. The *Ungraded* group, comprised of small and large eel specimens being reared together in the same tank, was analysed as two separate sub-groups: (a) *Ungraded-S*, comprised of the small individuals, and (b) *Ungraded-L*, comprised of the large individuals. At the start of the experiment, the mean values of *Ungraded-S* were not significantly different from *Graded-S*, nor were *Ungraded-L* significantly different from

Graded-L. Therefore, it was possible to compare the growth performance during the experiment of:

(1) small fish with and without the social interaction of large fish, by comparing the responses of *Ungraded-S* and *Graded-S*;

(2) large fish with and without the social interaction of small fish, by comparing the responses of *Ungraded-L* and *Graded-L*.

The eels were kept in the rearing tanks for 67 days, and fed with pelleted feed to apparent satiation twice daily from Monday to Friday, and once a day on weekends. The feeding protocol for each pellet ration was as indicated in *Chapter 2*. Cleaning and removal of dead or dying fish were also as for previous chapter.

2.3 Sampling schedule and growth parameters

At Days 0, 26, 46 and 67, the eels were anaesthetised, and individual weight (BW), total length (TL) and general external condition were recorded. Fish were not fed the day before and after handling.

The following parameters were calculated:

SGR (Specific growth rate, % day^{-1}) = 100 (ln BW_f – ln BW_i) / days

CV (Body weight coefficient of variation, %) = 100 (SD / mean body weight)

K (Fish condition factor) (Fulton, 1904) = 100 Body weight / (Total Length (cm))^3

Survival rate (%) = 100 (Initial fish stock – deaths of fish) / Initial fish stock)

BG (Biomass gain, percent of initial biomass) = 100 (B_f –B_i) B_i

FI (Feed intake, percent of initial biomass) = 100 (Tank total feed weight consumed (g) / B_i)

FCR (Feed conversion ratio) = Tank total feed weight consumed (g) / (B_f – B_i)

Where BW_i and BW_f are the initial and final body weight of each individual (g); B_i and B_f the initial and final tank stocked biomass (g); SD = standard deviation.

The fish condition factor (K) is a coefficient that expresses the relative robustness, or degree of well-being of a fish (Williams, 2000).

It was not possible to separately record the feed intake (FI) by *Ungraded-S* and *Ungraded-L* fish because they were reared together in the same tank. Therefore, the FI and consequently the FCR were calculated collectively for the *Ungraded* eels. However, for graded eels was possible to calculate the FI and FCR separately between small (*Graded-S*) and large (*Graded-L*) fish.

2.4 Statistical analyses

The mean values of BW and SGR were expressed as mean ± standard deviation (SD), based on the values of individual eels. The mean values of BG, FI and FCR were expressed as mean ± standard error of the mean (SEM), based on the values of the tanks (n = 3 for each treatment). Data were analysed for one-factor variance with Minitab statistical software 16.1.0 (Minitab® Statistical Software, State College, PA, USA) as described in *Chapter 2.*

3. Results

For each day of sampling (Days 0, 26, 46 and 67), the body weight was not significantly different between *Graded-S* and *Ungraded-S*, or between *Graded-L* and *Ungraded-L* (*Table 1*). Over the entire 67-day experiment, there was no significant difference in specific growth rate (SGR) between the graded and ungraded eels, but there was a significant difference between small and large eels (*Table 1*). The SGR values for large eels (0.9 ± 0.3% day^{-1}) were higher than for small eels (0.4 ± 0.7% day^{-1}). All large fish had a positive SGR (*Figure 1a)*, but small fish had a high percentage of null or negative growth rate; 38.1% of *Graded-S* and 36.8% of *Ungraded-S* fish did not grow (*Figure 1b).*

There were no significant differences in body weight coefficient of variation (CV) between the *Graded-S* and *Ungraded-S* group, or between the *Graded-L* and *Ungraded-L* group during the trial (*Figure 2*). At the start of the experiment, the CV was not significantly different between any of the groups, but over the rearing period the values increased in small eels, but were static in large eels (*Figure 2*).

The fish condition factor (K) was not significantly different between the *Graded-S* and *Ungraded-S* at any point, nor was it different between the *Graded-L* and *Ungraded-L* (*Table 1*). However, large eels were in a better condition than small eels throughout the experiment (*Table 1*). Likewise, the proportion of eels with an improvement in their final condition (Day 67) in relation to their initial condition (Day 0) was higher in large fish (*Graded-L*, 66.7% and *Ungraded-L*, 76.7%) than in small fish (*Graded-S*, 47.1% and *Ungraded-S*, 55.5%) (*Figures 3a and 3b*).

The survival rate of the *Graded-S* (90.5%) and the *Ungraded-S* (94.7%) eels, was not statistically different over a 67-day period. All large eels survived.

At the end of the experiment, the percentage of feed intake in relation to the initial biomass was lower for the graded fish (*Graded-S* plus *Graded-L*, 72.5 ± 5.9%) than in the ungraded fish (*Ungraded-S* plus *Ungraded-L*, 82.3 ± 3.7%). The feed conversion ratio (FCR) was the same for the graded and ungraded

groups (1.1 ± 0.1) (*Table 1*). Among the graded eels, it was found that the *Graded-S* group had a significantly lower feed intake (54.9 ± 14.1%) than the *Graded-L* group (78.9 ± 2.9%). Additionally, FCR of *Graded-S* (1.5 ± 0.1) was significantly higher than *Graded-L* (1.0 ± 0.1) eels (*Table 1*).

Table 1 - Comparison of body weight, condition factor, survival, specific growth rate, biomass gain, feed intake and feed conversion ratio between graded and ungraded shortfin eels (*Anguilla australis*).

	Graded eels		**Ungraded eels**	
	Graded-S	***Graded-L***	***Ungraded-S***	***Ungraded-L***
	n = 21	n = 32	n = 19	n = 31
Social interaction	Small eels interacting with small eels	Large eels interacting with large eels	Small eels interacting with large eels	Large eels interacting with small eels
Sampling days				
Body weight (BW, mean ± SD, g)				
Day 0	98 ± 18 [a]	182 ± 29 [b]	97 ± 17 [a]	183 ± 30 [b]
Day 26	114 ± 40 [a]	246 ± 39 [b]	112 ± 42 [a]	247 ± 40 [b]
Day 46	133 ± 71 [a]	298 ± 50 [b]	127 ± 66 [a]	304 ± 55 [b]
Day 67	151 ± 88 [a]	321 ± 59 [b]	149 ± 85 [a]	337 ± 69 [b]
Condition factor (K, mean ± SD)				
Day 0	0.16 ± 0.06 [a]	0.26 ± 0.06 [b]	0.15 ± 0.05 [a]	0.27 ± 0.06 [b]
Day 26	0.18 ± 0.08 [a]	0.30 ± 0.09 [b]	0.16 ± 0.06 [a]	0.31 ± 0.09 [b]
Day 46	0.19 ± 0.13 [a]	0.32 ± 0.11 [b]	0.16 ± 0.08 [a]	0.33 ± 0.09 [b]
Day 67	0.20 ± 0.15 [a]	0.32 ± 0.12 [b]	0.18 ± 0.09 [a]	0.33 ± 0.09 [b]
Whole trial – 67 days				
Specific Growth rate (SGR,mean ± SD, % day^{-1})	0.4 ± 0.7 [a]	0.9 ± 0.3 [b]	0.4 ± 0.7 [a]	0.9 ± 0.3 [b]
Survival (% ,(number of fish))	90.5 (19)	100 (32)	94.7 (18)	100 (31)
Biomass gain* (BG, % of initial biomass)	38.2 ± 11.5 [a]	76.0 ± 7.7 [b]	46.3 ± 6.4 [a]	85.1 ± 10.5 [b]
Feed intake* (FI, % of initial biomass)	54.9 ± 14.1 [a]	78.9 ± 2.9 [b]	NC	NC
	72.5 ± 5.9		82.3 ± 3.7	
Feed conversion ratio * (FCR)	1.5 ± 0.1 [a]	1.0 ± 0.1 [b]	NC	NC
	1.1 ± 0.1		1.1 ± 0.1	

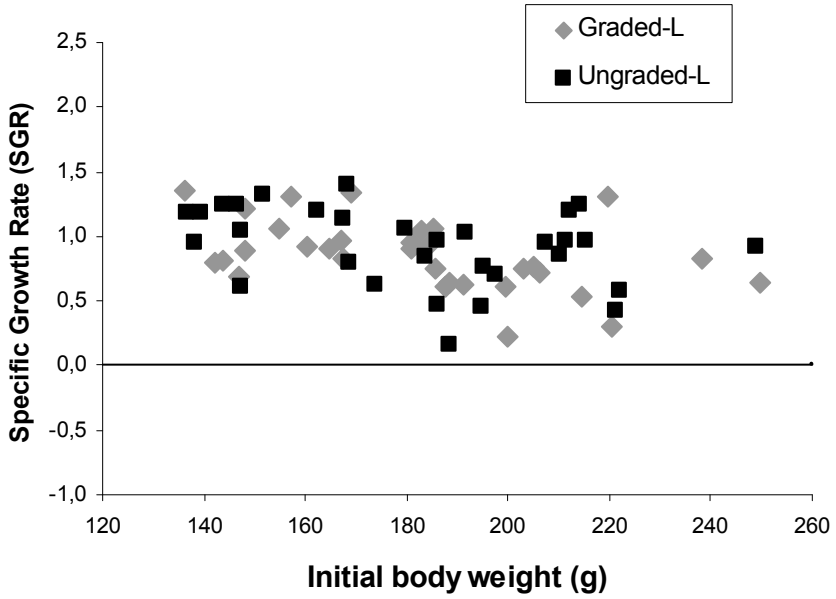
[a,b] Values in the same row with different superscripts are significantly different at $P < 0.05$.

[1] NC: Not calculable. It was not possible to separately record the FI (and consequently, FCR) by *Ungraded-S* and *Ungraded-L* fish because they were reared together in the same tanks.

***** Each value is the mean ± SEM of 3 tanks.

Figure 1 - Relationship between initial body weight and specific growth rate (SGR) of shortfin eels (*Anguilla australis*) grown for 67 days. Each dot represents a specimen.

(a) Large eels



(b) Small eels

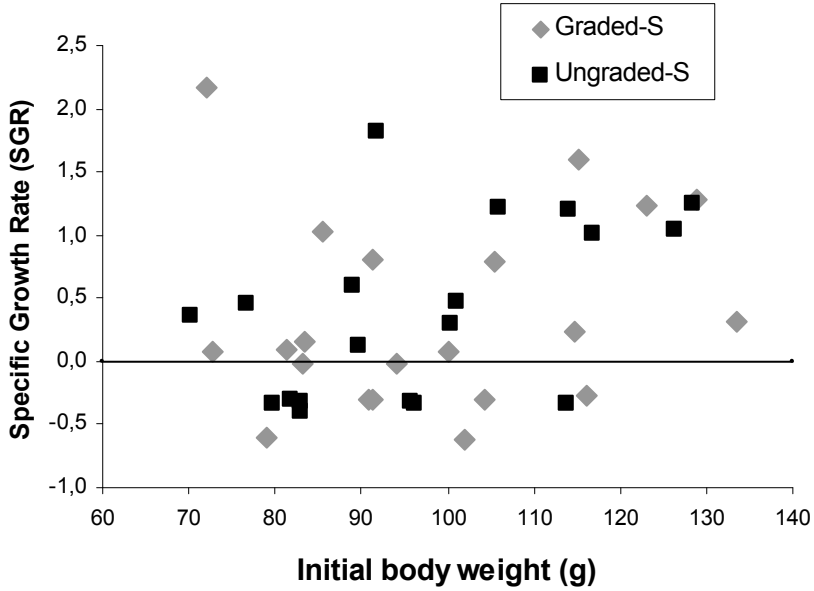


Figure 2 - Body weight coefficient of variation (CV) of large (*Graded-L* and *Ungraded-L*) and small (*Graded-S* and *Ungraded-S*) shortfin eel (*Anguilla australis*) at 0, 26, 45 and 67 days of rearing. Each dot represents the CV value of all the specimens for each group analysed together. Higher CV values indicate a higher heterogeneity of fish size within the group.

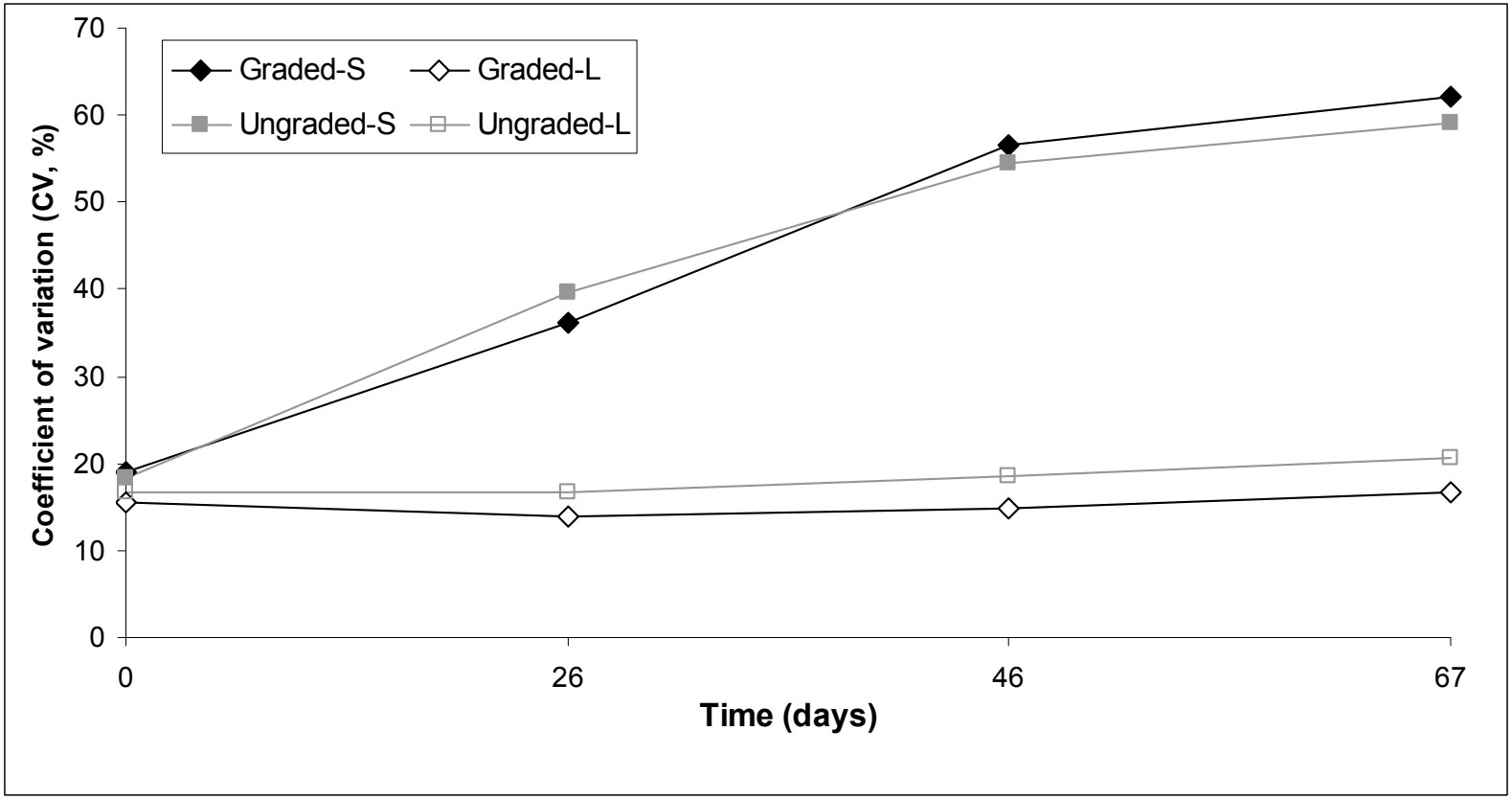
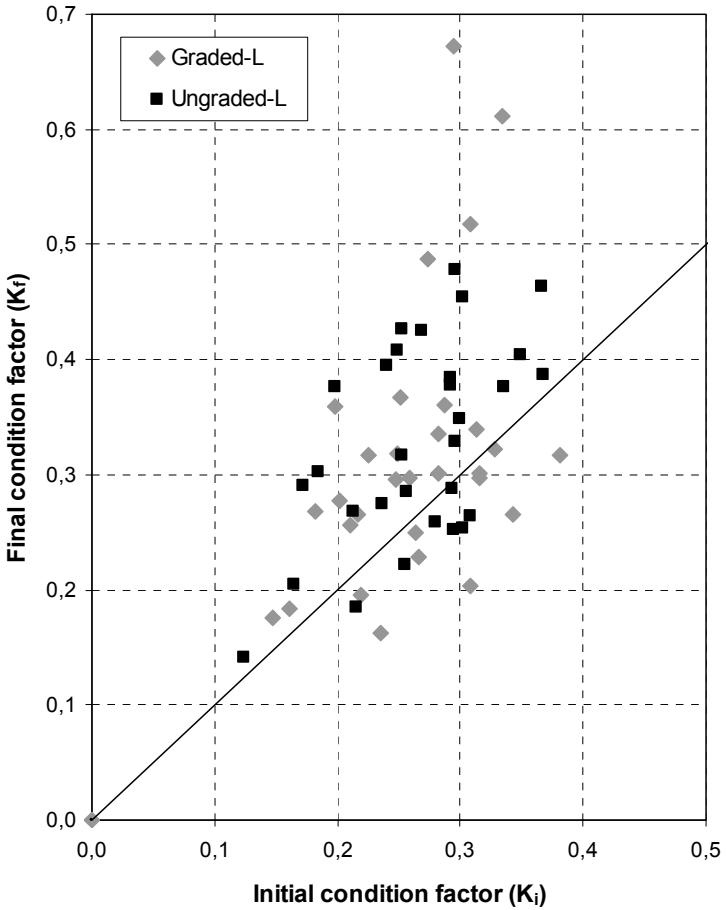
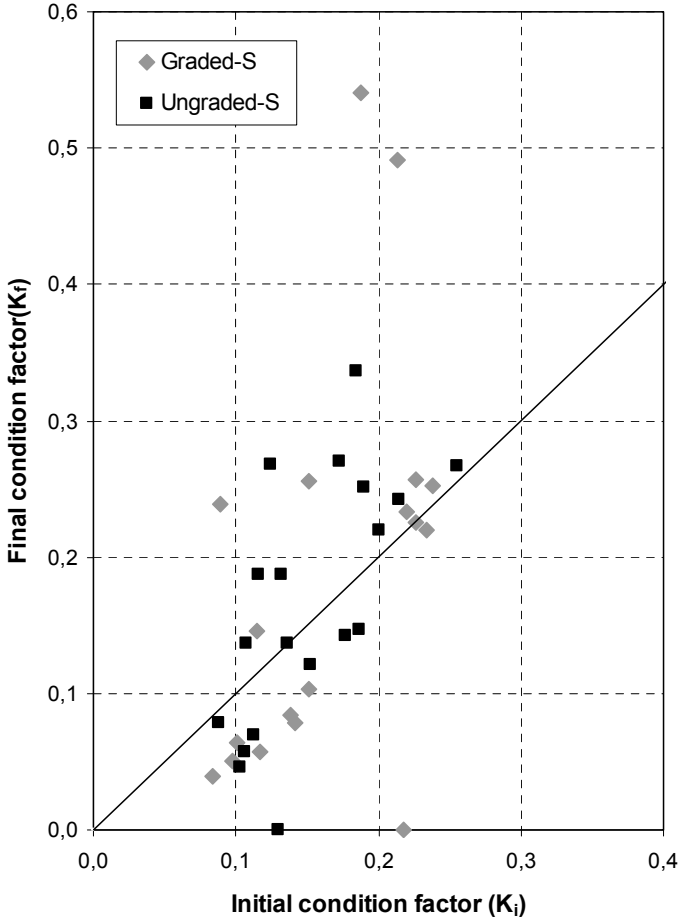


Figure 3 - Relationship between initial (K_i) and final (K_f) condition factor of specimens of shortfin eels (*Anguilla australis*) grown for 67 days. Each dot represents a specimen. Fish specimens above the diagonal line have improved their condition factor at the end of the experiment.

(a) Large eels



(b) Small eels



4. Discussion

Stocking densities used in this laboratory study (12 to 15 kg m^{-3}, semi-intensive culture) are considerably lower than those in a commercial RAS (≥ 100 kg m^{-3}, high-density culture). Prior studies on eels suggest that in high-density culture, fish size variability decreases, rather than increases as might be intuitively expected, due to aggression being more evenly distributed throughout the population because smaller individuals are less easily distinguished and thus victimised by larger dominants (Wickins, 1987; Knights, 1987; Jellyman, 2003). Therefore, it is expected that if size grading were to have a beneficial effect on eel growth performance it would be more clearly observed in a semi-intensive culture, where the hierarchical effect is more obvious, than in a high-density culture.

The results of this experiment did not show any significant effect on the growth performance, feed efficiency or survival of the yellow stage shortfin eel by size grading. It was not possible to improve individual growth rate, or to reduce the coefficient of variation of the small eels through the absence of large eels in the same tank. This is in agreement with the Kamstra (1993) study, which showed that grading did not have a significant effect on total biomass output or size-frequency distribution of cultured European eel (*Anguilla anguilla*).

These findings suggest that the wide variability in individual growth performance of the shortfin eels observed in the AUT Laboratory is not primordially a consequence of social interaction (hierarchical position) among tank-mates. This is in line with many studies that suggest that high growth heterogeneity in eels (Wickins, 1987; Knights, 1987; Kamstra, 1993; Garcia-Gallego & Akharbech, 1998) and other fish species (Doyle & Talbot, 1986; Jobling & Reinsnes, 1987; Cutts *et al*., 1998; Sunde *et al*., 1998; Lambert & Dutil, 2001; Martins *et al.,* 2005, 2006) is not necessarily linked with the establishment of social hierarchies. Other factors, such as genetic background, differences in metabolism, utilisation of food resources and/or different ability to adapt to intensive culture may, be responsible for the highly variable growth. By rearing individually confined European eels (*Anguilla anguilla*), Wickins (1985)

confirmed that highly variable growth rates can occur in this species without physical interaction among the individuals, and without competition for food.

However, there was a marked growth performance difference between large and small eels, independently of whether they belonged to the graded or ungraded group. The large eels had a much better growth performance than small eels, had a significantly higher specific growth rate (SGR), condition factor (K), and percentage biomass gain (BG) than small eels. The body weight coefficients of variation (CV) of large eels were unchanged during the experiment, but the CV of small eels increased in the same period. This means that the small eels grew at markedly different rates. Analysis of feed intake (FI) and feed conversion ratio (FCR) indicates that the large graded eels not only consumed more feed (as a percentage of initial biomass) but also had a significantly better conversion of this consumed feed to biomass gain than small graded eels.

The present study also indicates that the differences in individual growth rate are a reflection of feeding capacity. The slow-growing eel specimens exhibited lower feed consumption and a poorer feed conversion ratio than fast-growing eels. These differences in feeding capacity seem to be associated with different degrees of individual adaptation to the consumption of artificial feed and to the rearing conditions, rather than only with a competition for feed among the specimens. This appears to be the reason why the grading process, by itself, was unable to reverse the individual poor growth of small-size eels.

In conclusion, this study indicates that size-grading does not improve growth performance during the yellow phase of shortfin eel. Small eels did not exhibit increased growth rates when isolated from large eels. Others options should be explored in order to answer the question of how slow-growing eels could be helped to grow faster. Further research focusing on feeding stimulants to increase the appetite of eels is suggested.

Chapter 4

Effects of feeding stimulants on feed consumption, growth and survival of different developmental stages of the European eel (*Anguilla anguilla*)

1. Introduction

It is well established that the financial success of a fish farm is intimately related to the feed consumption of the cultured species (Seymour, 1989; Jeff, 2003; El-Shebly *et al*., 2007; Heinsbroek *et al*., 2007). In most fish culture operations, maximum profitability is reached at the highest fish growth rates (Heinsbroek *et al.*, 2008), which is generally connected to the highest feed consumption rate and the lowest feed conversion rates (Vahl, 1979; Forbes, 2000; De Oliveira & Cyrino, 2004). There are several procedures used by aquaculturists to increase feed consumption. One is the inclusion of feeding stimulants into the diets.

Feeding stimulants are specific compounds or ingredients added to the feed to enhance the diet palatability and, consequently, its acceptance by the cultured fish. As a result of improved diet acceptability, the fish can adapt earlier to an artificial dry diet during the weaning period, and attain a higher overall feed consumption and growth rate (Tandler *et al*., 1982; Nakajima *et al*., 1990; Kolkovski *et al.,* 1997; De Oliveira & Cyrino, 2004; Gaber, 2005). Moreover, the use of feeding stimulants promotes quicker food intake, minimising the time that the feed remains in water, thus preventing deterioration of the water quality (Yilmaz, 2005; Shankar *et al*., 2008).

Neurophysiologic and behavioural studies on eels indicate that feeding stimulants have the potential to enhance productivity in commercial eel culture. Studies on the neurophysiology of eels show that they have high olfactory and gustatory sensitivity (Marui & Caprio, 1992; Hara, 1994), and several behavioural studies have indicated a positive response of eels to different feeding stimulants (Carrieri *et al.*, 1986; Sola & Tosi, 1993; Sola *et al.*, 1993; Knights, 1996; Sola & Tongiorgi, 1998).

Laboratory studies on the effect of stimulants on feed intake and/or growth rate have shown positive results with eels. Kamstra & Heinsbroek (1991) and Heinsbroek & Kreuger (1992) established that cod roe extract, bovine spleen extract or a mixture of L-amino acids (alanine, glycine, proline and histidine) improved acceptance and growth rate of a formulated trout fry diet in European glass eels (*Anguilla anguilla*). Other feeding stimulant studies on European eel

(*e.g.* a mixture of L-amino acids, chicken spleen, and chicken blood) added to moist-paste diets also showed a positive increase in growth rate (Mackie & Mitchell, 1983; Degani & Levanon, 1986; Ajuzie & Appelbaum, 1993). Experiments with Japanese eel, *A. japonica*, concluded that paste diets supplemented with L-amino acids yield better performance than plain diets (Takeda *et al.*, 1984; Takii *et al*., 1984; Takeda & Takii, 1992). However, all these studies were based on a moist-paste diet or a dry diet not specifically formulated for eels.

Currently in Europe, eel farmers have access to pelleted feeds formulated specifically for the European eel species; nevertheless, they still face the problem of the limited feed acceptance by this species (R. Barrera, VALAQUA S.A., Puçol, Spain, *personal communication*, 2009). Laboratory studies on glass eel and elvers of shortfin eel have also shown a similar problem, with a high variability in growth rate and mortality (Gooley *et al*., 1999; Gooley & Ingram, 2002; Kearney, 2009). Some eels do not become accustomed to the pelleted feed, so they lose weight or grow too slowly. The use of feeding stimulants may facilitate the acceptance of these artificial diets. At the same time, it is necessary to evaluate not only their effect but also the minimum effective level. The cost of inclusion must be minimised without compromising the potential benefits from increases in feed intake.

The aim of the present study was to evaluate the effects of feeding stimulants at different concentrations on the feed consumption, growth rate and survival of cultured European eels (*A. anguilla*) during the glass eel and elver stages. Although the study was carried out on European eels, because of logistical reasons, the results were expected to be a good reference point for a future study on shortfin eels.

2. Materials and methods

2.1 Fish and maintenance

Glass eels and elvers of the European eel used in the present study were obtained from a commercial fish farm *Base Viva* located in Sant Pere Pescador, Spain. These eels were originally collected as glass eels from the Daró River, Catalonia, Spain during the 2008 to 2009 fishing season, and maintained in outdoor flow-through 500-L tanks at *Base Viva*. In March 2009, 2 kg of glass eels that were fed only with *Artemia* nauplii, and 4 kg of elvers already weaned onto an artificial pelleted diet (Microbaq® 8, Dibaq Acuicultura, Fuentepelayo, Spain), were transported by road (3.5 hours) to the Institut de Recerca i Tecnologia Agroalimentaries (IRTA) research facilities in Sant Carles de la Ràpita (IRTA-SCR), Spain. On arrival, glass eels and elvers were stocked separately in two 1,500-L holding tanks that were connected to a IRTAMAR™ 5,000-L freshwater recirculation unit (Carbó *et al*., 2002).

Fish were maintained under a 12 hours light: 12 hours dark regime and each tank was covered with a black plastic cloth to reduce light intensity (80.1 ± 10.5 lux at water surface; Lx-101 Lux Meter, Lutron Electronic Enterprise, Taipei, Taiwan). The recirculation unit was provided with constant aeration and a flow of water at 40 L min $^{-1}$. Water temperature, conductivity, pH (pH meter 507, Crison Instruments SA, Barcelona, Spain) and oxygen (OXI330, Crison Instruments SA) were kept at 22 to 23˚C, 2100 ± 200 µS cm^{-1}, 7.5 ± 0.5 and 8 to 9 mg L^{-1} (92 to 100% saturation), respectively. The concentrations of total ammonia (NH_3/NH_4^+, 0.1 ± 0.04 mg L^{-1}), nitrite (NO_2^-, 0.1 ± 0.09 mg L^{-1}) and nitrate (NO_3^-, 1.4 ± 0.5 mg L^{-1}) were tested weekly by a HACH® DR /870 colorimeter using HACH Standard Colorimetric Test Kits (HACH, Loveland, USA).

Glass eels were fed with a mixture of *Artemia* metanauplii and cod (*Gadus morhua*) roe to apparent satiation twice a day, whereas elvers were fed (4% dry weight per fresh body weight day^{-1}) with the commercial pelleted feed Microbaq® 8. The proximal biochemical composition of the pelleted feed was as follows: 50% protein, 20% fat, 0.5% fibre, 10% ash and 5% moisture (data provided by the feed manufacturer). Fish were held for six weeks prior to the

start of the experiment. During this period of acclimation, tanks were inspected daily and dead fish and debris removed. All animal experimental procedures were conducted in compliance with the experimental research protocol approved by the Committee of Ethic and Animal Experimentation of the IRTA (reference number 621303898-3898-4-8), which follows the international principles of replacement, reduction and refinement for the use of animals in research.

2.2 Experimental diets and procedures

2.2.1 Experimental diets

Six diets were prepared at Aquativ France (Elven, France) to contain two types of potentially stimulatory feeding stimulants at different concentrations *(Table 1*). One type of stimulant was based on processed marine proteins (MBFS) and the other on yeast proteins (YBFS) (*Table 2*). Feeding stimulants as powder were applied by top-coating the pelleted feed Microbaq® 8 by spraying with 2% fish oil. The concentrations achieved were 2, 4 and 6%. Top-coating was done in a 7 kg Forberg mixer (Forberg International, Larvik, Norway) with a speed of 20 rpm. Fish oil was applied for 30 s, the feeding stimulants in powder for 60 s, and the retention time was 60 s.

2.2.2 Experiment 1 - Elvers

A total of 1,134 elvers were selected for this experiment and distributed uniformly among the 21 35-L cylindrical 150 µm mesh baskets located in three 1,500-L holding tanks that were connected to a recirculation system (Carbó *et al.,* 2002). Each basket was stocked with 54 elvers (1.5 ± 0.3 g, mean ± SD) at a density of 2 fish L^{-1}. In addition, six cylindrical PVC tubes (100 mm long, 15 mm inner diameter) were placed in each basket to provide refuge and protection from aggressive behaviour and cannibalism (Rodríguez *et al*., 2009).

Elvers were maintained under the same environmental conditions as in the acclimation tanks, and fed to apparent satiation twice a day with different diets for 60 days. Seven different dietary treatments were evaluated: the commercial pelleted feed Microbaq® 8 as a control diet, and the MBFS and YBFS diets at 2, 4 and 6%. Each treatment was randomly assigned to three experimental

baskets. Feeds provided to the elvers were deposited in feeding stations (trays; 10 x 10 cm). In order to assure the minimum nutrient leaching from the feeding stimulants, the uneaten feed was removed from the trays after 45 minutes, and kept at 4 ˚C for later calculation. If the first ration was totally consumed, a second ration was provided. During the course of the experiment, the baskets were cleaned by siphoning three times a week, and checked daily for dead or moribund fish. If any were found, they were removed and recorded as a death.

2.2.3 Experiment 2 - Glass eels

A total of 1170 glass eels, were selected for the experiment and distributed uniformly among 9 cylindrical 150 µm mesh baskets located in one 1,500-L holding tank that were connected to a recirculation system (Carbó *et al*., 2002). Each basket was stocked with 130 glass eels (250 ± 100 mg) at an initial density of 4 glass eels L^{-1}. The fish were provided with shelter by a rectangular mesh basket (100 x 60 x 30 mm) suspended in the middle of the water column of each basket. The fish were kept under the same environmental conditions as in the acclimation tanks.

Based on the experimental results obtained from *Experiment 1*, three different diets were evaluated to test the effects of feeding stimulant on glass eel weaning: the commercial pelleted feed Microbaq® 8 (control diet), and the two feeding stimulants added to the control diet at the inclusion level of 6% (6% MBFS and 6% YBFS) (*Table 1*). During the first 15 days of the 30-day experiment, the fish of each tank were weaned gradually from cod roe to one of the three feeds. After that they were fed only with the corresponding extruded pellets, twice per day to apparent satiation. Each treatment was randomly assigned to three experimental baskets. The feeding procedures, cleaning and removing of dead or dying fish were as for *Experiment 1.*

2.3 Sampling, data collection and biochemical analyses

2.3.1 Sampling schedule and growth parameters

In *Experiment 1*, the wet body weight (BW, g) of each elver was individually measured on Day 0, 30 and 60 of the experiment. Prior to their handling, fish

were anaesthetised with tricaine methanesulfonate (MS222; Sigma, Barcelona, Spain) at a final concentration of 50 mg L^{-1} (Chiba *et al*., 2006). Fish were not fed the day before and after handling. Mortalities were recorded daily. For analytical purposes, 15 (1.5 ± 0.3 g) elvers were sampled from the acclimation tank at Day 0, and 10 elvers were sampled from each basket at D 60 (30 fish per treatment). Specimens were sampled early in the morning before feed was offered and sacrificed by an overdose of MS222. The 15 elvers from Day 0 were kept frozen at -20 °C for later proximate composition analysis. The 10 elvers collected from each basket (30 per experimental condition) at Day 60 were split in 3 pools: (1) 3 fish for proximate composition were kept at -20 °C, (2) 2 fish were dissected and the liver and intestine were fixed in 4% formaldehyde, dehydrated in a graded series of ethanol for later histological analysis, (3) 5 fish were kept frozen at -80 °C for digestive enzymes analysis.

In *Experiment 2*, BW (mg) of each glass eel was determined at the start (Day 0) and at the end of the experiment (Day 30). Similar to *Experiment 1*, fish were anesthetised prior their handling for BW measurement. At the start of the experiment, 36 glass eels (0.35 ± 0.10 g) were taken from the acclimation tank, sacrificed by an overdose of MS222, and kept for later body composition analysis at -20ºC. At the end of the weaning period (Day 15) and at the end of the experiment trial (Day 30), 26 glass eels were randomly taken from each basket (78 for each experimental condition); 12 fish for proximate composition, 10 for digestive enzyme analyses and 4 for histology. The glass eels were sampled early in the morning before feed was offered, sacrificed and kept for analysis as previously indicated. Mortalities were recorded daily in each basket.

For both experiments, data obtained were analysed for fish growth and feed utilization, and the following indices were used:

SGR (Specific growth rate, % day^{-1}) = 100 (ln BW_f – ln BW_i) / days

CV (Body weight coefficient of variation, %) = 100 (SD/ mean body weight)

Survival (%) = 100 (Initial basket stock numbers – deaths of fish) / Initial basket stock numbers

BG (Biomass gain, percent of initial biomass) = 100 (B_f –B_i) B_i

FI (Feed intake, percent of initial biomass) = 100 (Basket total feed weight consumed (g) / B_i)

FCR (Feed conversion ratio) = Basket total feed weight consumed (g) / (B_f –B_i)

Where BW_i and BW_f are the initial and final mean body weight (mg or g) per experimental basket; B_i and B_f the initial and final basket stocked biomass (g); SD = standard deviation

2.3.2 *Feed and body composition analyses*

Chemical analyses of diets for moisture, protein, fat, ash and carbohydrate were performed according to AOAC (1990) methods. Body composition was determined individually for elvers (Day 0, *n* = 15; Day 60, *n* = 9 by treatment), and as a pool of 4 fish for glass eels (Day 0, *n* = 9; Days 15 and 30, *n* = 9 by treatment). Specimens for body analysis were ground, and small aliquots were dried (120 ºC, 24 h) to estimate water content. The total fat content was quantified gravimetrically after extraction in chloroform/methanol (2:1) and evaporation of the solvent under a stream of nitrogen followed by vacuum desiccation overnight (Folch *et al*., 1957). Protein and carbohydrate contents were determined according to Lowry *et al*. (1951) and DuBois *et al*. (1956), respectively. All chemical analyses were done in triplicate.

2.3.3 Digestive activities

The fish were individually dissected to separate pancreatic and intestinal segments, under a dissecting microscope on a chilled glass plate maintained at 0 ºC as previously described in Gisbert *et al*., 2009. The pancreatic segment was homogenised (Ultra-Turrax T25 basic, IKA©-Werke, Staufen, Germany) in 5 volumes (v/w) of ice-cold Milli-Q water, centrifuged at 3300 gravities for 3 min at 4 °C, sonicated for 1 min and the supernatant frozen at -20ºC for subsequent enzyme quantification (trypsin, lipase and α-amylase). For determination of intestinal brush border membrane enzymes (leucine aminopeptidase and alkaline phosphatase), dissected samples were homogenised in cold 50 mM mannitol, 2 mM Tris–HCl buffer (pH 7.0). Intestinal brush border membranes were purified according to Crane *et al*. (1979) and stored at -20º C.

Trypsin (E.C. 3.4.21.4) activity was assayed at 25 °C using BAPNA (N-α-benzoyl-dl-arginine p-nitroanilide) as the substrate. One unit of trypsin per ml (U) was defined as 1 µmol BAPNA hydrolysed per min per ml of enzyme extract at 407 nm (Holm *et al*., 1988). Bile salt-activated lipase (E.C. 3.1.1) activity was assayed for 30 min at 30 °C using pnitrophenyl myristate as substrate dissolved in 0.25 mM Tris–HCl, pH 9.0, 0.25 mM2-methoxyethanol and 5 mM sodium cholate buffer. Lipase activity (U/ml) was defined as the µmol of substrate hydrolysed per min per ml of enzyme extract (Ijima *et al*., 1998). α-Amylase (E.C. 3.2.1.1) was measured according to Métais & Bieth (1968), using 0.3% soluble starch dissolved in Na_2HPO_4 buffer (pH 7.4) as substrate. α-Amylase activity (U) was defined as the mg of starch hydrolysed during 30 min per ml of tissue homogenate at 37 °C at 580 nm.

Intestinal alkaline phosphatase (E.C. 3.1.3.1) was quantified at 37 °C using 4-nitrophenyl phosphate (PNPP). One unit (U) was defined as 1 µg PNPP released per min per ml of homogenate at 407 nm (Bessey *et al*., 1946). Leucine aminopeptidase (E.C.3.4.11.2) was determined at 25 °C according to Maroux *et al*. (1973), using L-leucine p-nitroanilide as substrate (in 0.1 mM DMSO). One unit of enzyme activity (U) was defined as 1 µg nitroanilide released per min per ml of homogenate at 410 nm.

Soluble protein from crude enzyme extracts was quantified as described by Bradford (1976), using bovine serum albumin as standard. Enzyme activity was expressed as specific activity (activity units per milligram of protein, U mg protein^{-1}). For each basket, all enzymatic assays were run individually for elvers (n = 5; *Experiment 1*) and from five pools of 2 glass eels each (*Experiment 2*).

2.3.4 Histology of the liver and intestine

For histological purposes, six elvers and twelve glass eels from each experimental condition in each experiment were fixed in 4% formaldehyde, dehydrated in a graded series of ethanol, embedded in paraffin, and cut in serial transversal or sagittal sections (3–5 µm thick) as previously described in Rodríguez *et al*. (2005b). Sections were stained by Harris' haematoxylin and

eosin for general histomorphological observations, while periodic acid-Schiff (PAS) was used to detect glycogen deposits in the liver (Pearse, 1985).

2.4 *Statistical analyses*

The mean values of body weight (BW) were expressed as mean ± SD. The calculation was based on the values of the individual body weight of all the eels belonging to the same treatment (fish from the 3 baskets/replicates per treatment analysed together) and consequently, the SD describes the dispersion of the individual eel values. The mean values of specific growth rate (SGR), survival, biomass gain (BG), feed intake (FI), and consequently the food conversion ratio (FCR) were expressed as mean ± standard error of the mean (SEM). In contrast to body weight, these parameters were calculated using the values of the baskets (n = 3 for each treatment); they cannot be calculated for individual eels. The SEM quantifies the error in calculating the mean of the population from the basket values.

Data were analysed for one-factor variance with Minitab statistical software 16.1.0 (Minitab® Statistical Software, State College, PA, USA). Before analysis, homogeneity of variance was confirmed using the Bartlett test (Snedecor & Cochran, 1989). When a significant treatment effect was observed, individual means were compared with Tukey-Kramer HSD multiple comparison test.

Table 1 - Proximate composition on a percent dry weight basis of experimental diets containing different levels of feeding stimulants.

		YBFS diets			MBFS diets		
		Yeast-based feeding stimulant at			Marine-based feeding stimulant at		
	Control	2%	4 %	6%	2 %	4 %	6 %
Moisture	7.4 ± 0.1	7.7 ± 0.1	7.5 ± 0.1	7.6 ± 0.1	7.3 ± 0.1	7.5± 0.1	7.3 ± 0.1
Protein	49.8 ± 0.2 [a]	51.2 ± 0.5 [b]	52.4 ± 0.3 [c]	53.3 ± 0.4 [d]	50.8 ± 0.2 [b]	52.4 ± 0.1 [c]	53.4 ± 0.2 [d]
Fat	22.2 ± 0.4	21.8 ± 0.3	22.3 ± 0.5	22.2 ± 0.3	21.9 ± 0.3	22.3 ± 0.3	22.1 ± 0.2
Carbohydrate	11.8 ± 1.7	12.8 ± 0.2	12.1 ± 0.3	12.0 ± 0.7	11.5 ± 0.4	11.9 ± 0.2	10.9 ± 1.1
Ash	10.0 ± 0.2 [a]	10.4 ± 0.3 [b]	11.1 ± 0.2 [c]	11.4 ± 0.2 [d]	10.3 ± 0.2 [b]	10.5 ± 0.3 [c]	11.4 ± 0.1 [d]
Gross energy (kJ g^{-1})	22.60	22.95	23.31	23.47	22.67	23.27	23.22

[a,b] Values in the same row with different superscripts are significantly different at $P < 0.05$.

Each value is the mean ± SD of triplicate analysis (n = 3).

Gross energy was calculated based on known energetic values of protein, fat and carbohydrate (NRC, 1983).

Table 2 - Proximate composition, amino acid profile, soluble protein and percent molecular weight protein profile of the two feeding stimulants.
Data provided by the manufacturer of the feeding stimulants.

	YBFS Yeast-based feeding stimulant	**MBFS** Marine-based feeding stimulant
Proximate analysis (%)		
Moisture	4.9	4.8
Crude protein	48.2	54.0
Crude fat	1.8	7.9
Ash	23.6	24.4
Amino acid profile (%)		
Arginine	2.50	3.32
Lysine	1.81	2.36
Methionine	0.28	0.83
Cysteine	0.80	0.58
Threonine	2.00	2.17
Phenylalanine	1.98	2.39
Tyrosine	0.85	1.45
Isoleucine	1.99	1.97
Leucine	3.18	3.33
Histidine	0.59	0.96
Valine	2.94	2.68
Alanine	2.45	2.88
Serine	3.43	3.47
Glutamic acid	4.98	6.29
Proline	3.15	3.65
Glycine	2.84	3.43
Aspartic acid	3.47	4.30
Soluble protein ratios (%)		
Soluble protein / total protein	67	89
Soluble protein / total stimulant	32	48
Insoluble protein / total stimulant	16	6
Molecular weight (MW) distribution of protein compounds (%)		
MW < 500 Da	59	74
500 Da < MW < 1 000 Da	8	9
1 000 Da < MW < 5 000 Da	12	10
5 000 Da < MW < 10 000 Da	2	1
10 000 Da < MW < 20 000 Da	2	1
20 000 Da < MW < 30 000 Da	2	1
MW > 30 000 Da	16	5

3. Results

3.1 Growth, survival, feed intake and biochemical composition

At the beginning of *Experiment 1*, elvers from different experimental groups were homogeneous in BW_i (1.5 ± 0.3 g, $P > 0.05$). No statistically significant differences among dietary treatments were observed at Day 30 ($P > 0.05$). However, at the end of the experimental period (Day 60), BW_f was higher in those groups fed the diet containing 6% marine-based and yeast feeding stimulants. Elvers fed 6% MBFS showed a significantly higher BW_f, than animals fed the control diet; they were 13.5% heavier. Likewise, eels fed 6% YBFS were 5.7% heavier than the control group (*Table 3*). Size dispersion in BW_f of elvers was not significantly affected by the different dietary treatments (*Fig. 1a*). However, the BW_f dispersion in the group fed 6% MBFS tended to be lower than that observed with the other diets (coefficient of variation 35.1 vs. 38.9 to 43.2%; $P = 0.06$), showing a more symmetrical distribution in size and no individual eel with a BW_f greatly higher than the other eels in that group (i.e. absence of outlier values) *(Fig. 1a*).

The incorporation of MBFS and YBFS at a level of 2 and 4% did not affect elvers SGR values calculated for the overall experimental period. Nevertheless, data on SGR were higher in elvers fed diets containing 6% marine-based and yeast feeding stimulants. In particular, elvers fed 6% MBFS and YBFS diets grew 11.9 ($P = 0.001$) and 5.6% ($P = 0.051$) faster than those animals fed the control feed, respectively (*Table 3*). Biomass gain (BG) was also affected by the incorporation of different types of feeding stimulants and level of their inclusion.

The increase in BG in those groups compared with the control was 18.7 and 6.9%, respectively ($P < 0.05$; *Table 3*). Within the limits of one stimulant, BG values from fish fed 6% MBFS diet were 21.6 and 24.4% higher than those recorded in elvers fed the 2 and 4% MBFS diets, respectively. For YBFS in diets, the BG in animals fed the diet containing 6% YBFS was 11.4 and 12.9% higher than that recorded in elvers fed the 2 and 4% YBFS diets, respectively. There were no significant differences in survival among treatments, ranging from 92.6 to 96.9% ($P > 0.05$; *Table 3*).

No significant differences were observed in FCR between elvers fed different diets ($P > 0.05$; *Table 3*). However, statistically significant differences were observed between groups when the feed intake (FI) was considered ($P < 0.05$; *Table 3*). In particular, FI values in elvers fed the diet containing 6% MBFS were 12.8% higher than the control group, whereas these values were 16.5 and 13.8% higher than those recorded in elvers fed diets with the same feeding stimulant but at a lower concentration (2 and 4%, respectively).

Table 4 shows the proximate biochemical composition of elvers at the beginning of the trial and after 60-day experimental period. At Day 60, results showed that there were no significant differences in moisture, protein, fat and carbohydrate levels in the elvers' bodies due to diet.

In *Experiment 2*, glass eels from different dietary treatments were similar in BW_i (250 ± 100 mg) and homogeneous in size distribution ($P > 0.05$; *Table 5*). At the end of the trial period (30 days), the observation of glass eel guts under the binocular microscope revealed the effective ingestion of the diets in all groups and confirmed their successful weaning into the three tested feeds (control, 6% MBFS and 6% YBFS). No significant differences in BW_f, SGR and survival were detected among different treatments ($P > 0.05$; *Table 5; Fig. 1b*). Final body weight distribution was similar among different groups; 84% of glass eels had BW_f values ranging from 200 to 800 mg (*Fig. 2*), although those groups fed diets containing both feeding stimulants showed more outliers in comparison to the control diet (*Fig. 1b*).

The body proximate composition of glass eels weaned onto the three tested diets is shown in *Table 6*. At Day 30, animals fed the control diet showed higher values in corporal protein content in comparison to the other groups ($P = 0.042$), whereas no differences in fat content were detected between dietary treatments ($P > 0.05$). Those eels fed the 6% MBFS presented the highest values of moisture and carbohydrate content ($P < 0.05$).

3.2 Digestive enzymes and histological organisation of the liver and intestine

At the end of *Experiment 1* on elvers, specific activity values of trypsin were similar among groups ($P > 0.05$; *Fig. 3a*) with values ranging from 3.6 to 4.9 mU mg^{-1} protein, although values tended to be higher with increasing levels of each feeding stimulant. For amylase in elvers, no statistically significant differences were detected among treatments ($P > 0.05$; *Fig. 3b*) with specific mean values ranging from 0.108 to 0.145 U mg^{-1} proteins. Lipase specific activity was significantly affected by the inclusion of different feeding stimulants in diets ($P = 0.008$; *Fig 3c*). The highest activity values were observed in elvers fed 6% MBFS (0.143 ± 0.067 U mg^{-1} protein), whereas those animals fed YBFS diets showed the lowest activity values in lipase (0.095 ± 0.004 U mg^{-1} protein) irrespective of the level of inclusion of the feeding stimulant. In addition, elvers fed 2 to 4% MBFS and control diets showed intermediate values in lipase specific activity (0.116 ± 0.0025, 0.105 ± 0.011 and 0.109 ± 0.002 U mg^{-1} protein; respectively). At the end of the trial, the activity of the intestinal brush border enzymes analysed (alkaline phosphatase and leucine aminopeptidase) was similar among elvers fed diets containing different feeding stimulants at different levels of inclusion in comparison to the control group (Fig. 3d, e).

Data from pancreatic (trypsin, amylase and lipase) and intestinal brush border (alkaline phosphatase and leucine aminopeptidase) enzymes from glass eels weaned onto the experimental diets (*Experiment 2*) is shown in *Figure 4*. After 15 days, glass eels fed 6% MBFS showed the highest values in trypsin specific activity in comparison to the other groups ($P < 0.05$). At the end of the experiment period (Day 30), trypsin activity values were similar between glass eels fed the control and 6% MBFS diets ($P > 0.05$), whereas animals fed 6% YBFS diet had the lowest trypsin activities ($P < 0.05$; Fig. 4a). No significant differences were recorded in amylase specific activity along the trial period (15 and 30 days; Fig. 4b). Lipase specific activity results showed no statistically significant differences between glass eels fed the different diets after 15 days of weaning. However, at the end the trial (Day 30), glass eels fed 6% MBFS showed the highest values in lipase specific activity in comparison to the other groups ($P < 0.05$; Fig. 4c). Regarding intestinal brush border enzymes, alkaline phosphatase specific activities in glass eels fed 6% MBFS and YBFS diets at

Day 15 were higher than those recorded in the control group ($P < 0.05$). At Day 30, alkaline phosphatase activity values were significantly higher in the group fed 6% MBFS diet in comparison with the group fed 6% YBFS. The control group showed a value slightly higher than 6% YBFS ($P < 0.05$; Fig. 4d). At Day 15, leucine aminopeptidase specific activities were similar among glass eels fed different diets ($P > 0.05$). However, once the animals were completely weaned onto the experimental feeds at day 30, glass eels fed the 6% MBFS diet showed higher specific activities in comparison with control group, where those animals fed the 6% YBFS diet had intermediate enzyme values ($P < 0.05$; Fig. 4e).

Histologically, the hepatic tissue of elvers and glass eels appeared as a compact tissue formed by basophilic polyhedral hepatocytes characterised by spherical nuclei with euchromatin and prominent nucleolus, and a large eosinophilic cytoplasm with glycogen (PAS-positive) and fat inclusions. Although some variation was observed in the level of fat accumulation in the liver of elvers among individuals fed the same diet, the fish fed the control diet showed low level of lipidic inclusion (*Fig. 5a*). In contrast the 6% MBFS and YBFS diets showed a higher deposition of fat, as indicated by the displacement of the hepatocytes' nuclei towards the periphery of the cells and enlarged colourless cytoplasmatic inclusions (Fig. 5b). Contrary to the results with elvers, no differences in the organisation and accumulation of fat in the liver of glass eels were observed between the experimental groups.

The histological organisation of the intestine in elvers and glass eels was uniform throughout its length with only changes in the level of folding of the intestinal epithelium, which was more prominent in the anterior than in the posterior regions. In brief, the intestinal wall was formed by the serosa, muscularis, submucosa, mucosa and epithelium lined by enterocytes, rodlet and abundant goblet cells. In general, the level of fat accumulation in enterocytes was very low, and fat was only observed in perivisceral deposits. At the end of both experiments, no histological alterations were observed among elvers and glass eels fed diets containing different feeding stimulants (Fig. 5c, d).

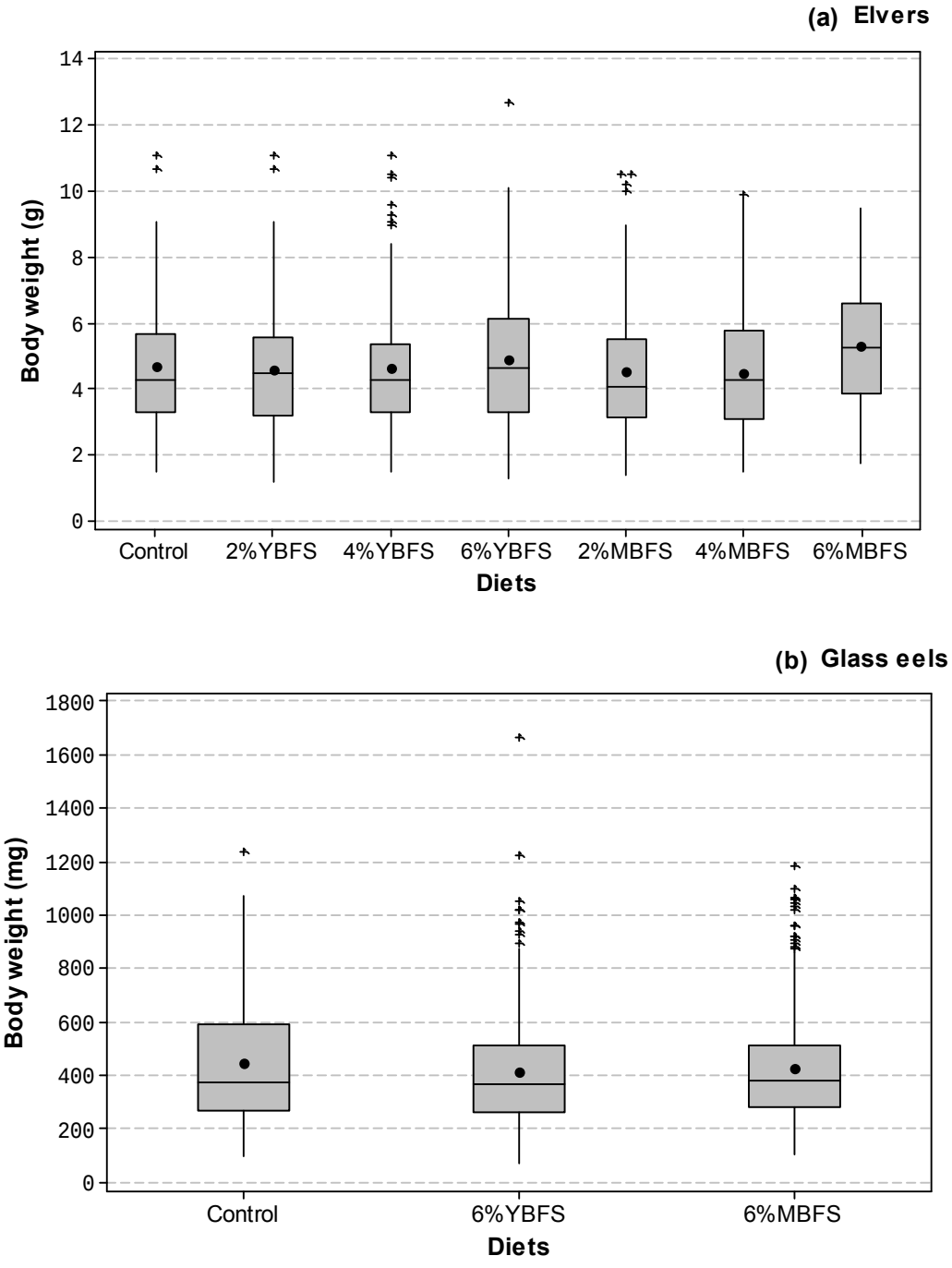
Table 3 - Growth performance, feed intake, diet utilization efficiencies and survival rates of European eel (*Anguilla anguilla*) elvers fed a commercial pelleted diet (control) and two different feeding stimulants at increasing levels of 2, 4, 6% for 60 days *(Experiment 1).*

	Control	YBFS diets Yeast-based feeding stimulant at			MBFS diets Marine-based feeding stimulant at		
		2%	4%	6%	2%	4%	6%
Initial body weight (BW_i) * (g)	1.52 ± 0.34	1.53 ± 0.32	1.51 ± 0.31	1.50 ± 0.32	1.52 ± 0.34	1.51 ± 0.33	1.51 ± 0.33
Final body weight (BW_f)* (g)	4.62 ± 0.04 ^{a}	4.62 ± 0.13 ^{a}	4.66 ± 0.05 ^{a}	4.90 ± 0.11 ^{ab}	4.55 ± 0.09 ^{a}	4.51 ± 0.04 ^{a}	5.34 ± 0.05 ^{b}
Biomass gain (BG) ** (%)	199.8 ± 6.0 ^{a}	189.1 ± 6.1 ^{a}	186.0 ± 3.8 ^{a}	213.5 ± 6.9 ^{ab}	186.0 ±14.9 ^{a}	179.3 ± 6.0 ^{a}	237.2 ± 3.8 ^{b}
Specific growth rate (SGR) ** (% day^{-1})	1.86 ± 0.03 ^{a}	1.84 ± 0.06 ^{a}	1.89 ± 0.03 ^{a}	1.97 ±0.03 ^{ab}	1.83 ± 0.06 ^{a}	1.83 ± 0.01 ^{a}	2.11 ± 0.01 ^{b}
Feed Intake (FI) ** (as % of initial biomass)	215.4 ±0.8 ^{a}	203.0 ± 2.6 ^{a}	207.9 ± 2.4 ^{a}	221.2 ±10.8 ^{ab}	202.9 ± 6.3 ^{a}	209.4 ± 4.9 ^{a}	243.0 ± 2.2 ^{b}
Feed conversion ration (FCR) **	1.08 ± 0.03	1.08 ± 0.05	1.12 ± 0.03	1.04 ± 0.08	1.10 ± 0.06	1.17 ± 0.05	1.02 ± 0.02
Survival rate ** (%)	96.9 ± 1.6	95.7 ± 2.2	92.6 ± 1.1	96.3 ± 1.1	95.1 ± 2.2	93.2 ± 1.2	95.1 ± 1.6

^{a,b} Values in the same row with different superscripts are significantly different at $P < 0.05$.

* Values are means ± SD of the individual eel weight per treatment. ** Values are means ± SEM of three groups (baskets) per treatment.

Figure 1- Box and whisker plot graph of the final wet body weight of elvers (a, *Experiment 1*) and glass eels (b, *Experiment 2*) of European eel *(A. anguilla)* fed diets containing different types and levels of feeding stimulants.



The bottom and top lines of the box are the lower (Q_1) and the upper (Q_3) quartiles respectively. The line inside represent the median (Q_2), whereas the black dot represents the mean. The ends of the whiskers are the lowest datum still within 1.5 IQR of the lower quartile (Q_1) and the highest datum still within 1.5 IQR of the upper quartile (Q_3). Outliers' values are represented by a plus sign. IQR = $Q_3 - Q_1$

Table 4 - Body proximate composition on a wet weight basis of European eel (*Anguilla anguilla*) elvers before starting the experiment (Day 0) and at the end (Day 60), fed a commercial pelleted diet (control) and two different feeding stimulants at concentrations of 2, 4 and 6% *(Experiment 1)*.

	Day 0	Day 60						
			YBFS diets			MBFS diets		
	Initial	Control	Yeast-based feeding stimulant at			Marine-based feeding stimulant at		
			2%	4%	6%	2%	4%	6%
Moisture (%)	68.6 ± 3.8	69.0 ± 1.2	69.2 ± 2.1	69.8 ± 0.5	69.0 ± 1.3	68.5 ± 1.7	68.4 ± 1.8	69.9 ± 1.7
Crude protein (%)	11.7 ± 0.2	17.0 ± 1.0	16.0 ± 1.2	16.1 ± 1.0	15.9 ± 1.4	16.6 ± 1.1	16.3 ± 1.0	16.1 ± 1.3
Crude fat (%)	10.7 ± 2.2	10.5 ± 2.1	11.0 ± 1.4	9.1 ± 1.3	9.4 ± 2.4	10.5 ± 2.0	10.3 ± 2.3	10.8 ± 2.4
Carbohydrate (%)	0.6 ± 0.1	0.7 ± 0.1	0.7 ± 0.1	0.6 ± 0.1	0.7 ± 0.1	0.7 ± 0.1	0.7 ± 0.1	0.8 ± 0.2

Values are means ± SD. Means in the same row with different superscripts are significantly different at $P < 0.05$.

Table 5 - Growth performance and survival rates of European glass eels (*Anguilla anguilla*) weaned to a commercial pelleted diet (control) and two different feeding stimulants at concentrations of 6% for 30 days *(Experiment 2)*

		YBFS diet	MBFS diet
	Control	Yeast-based feeding stimulant at 6%	Marine-based feeding stimulant at 6%
Initial body weight (BW_i) * (mg)	245 ± 101	246 ± 100	245 ± 96
Final body weight (BW_f)* (mg)	446 ± 225	416 ± 214	431 ± 208
Specific growth rate (SGR) ** (% day^{-1})	2.00 ± 0.28	1.75 ± 0.14	1.88 ± 0.18
Survival rate ** (%)	86.9 ± 4.4	89.2 ± 5.9	94.6 ± 3.9

* Values are means ± SD of the individual eel weight per treatment. ** Values are means ± SEM of three groups (baskets) per treatment. Means in the same row with different superscripts are significantly different at $P < 0.05$.

Figure 2 - Final size class frequency distribution of glass eels of European eel *(A. anguilla)* reared for 30 days (*Experiment 2*). Weaned onto (a) a commercial pelleted diet (control), (b) a diet containing YBFS at 6%, and (c) a diet containing MBFS at 6% .

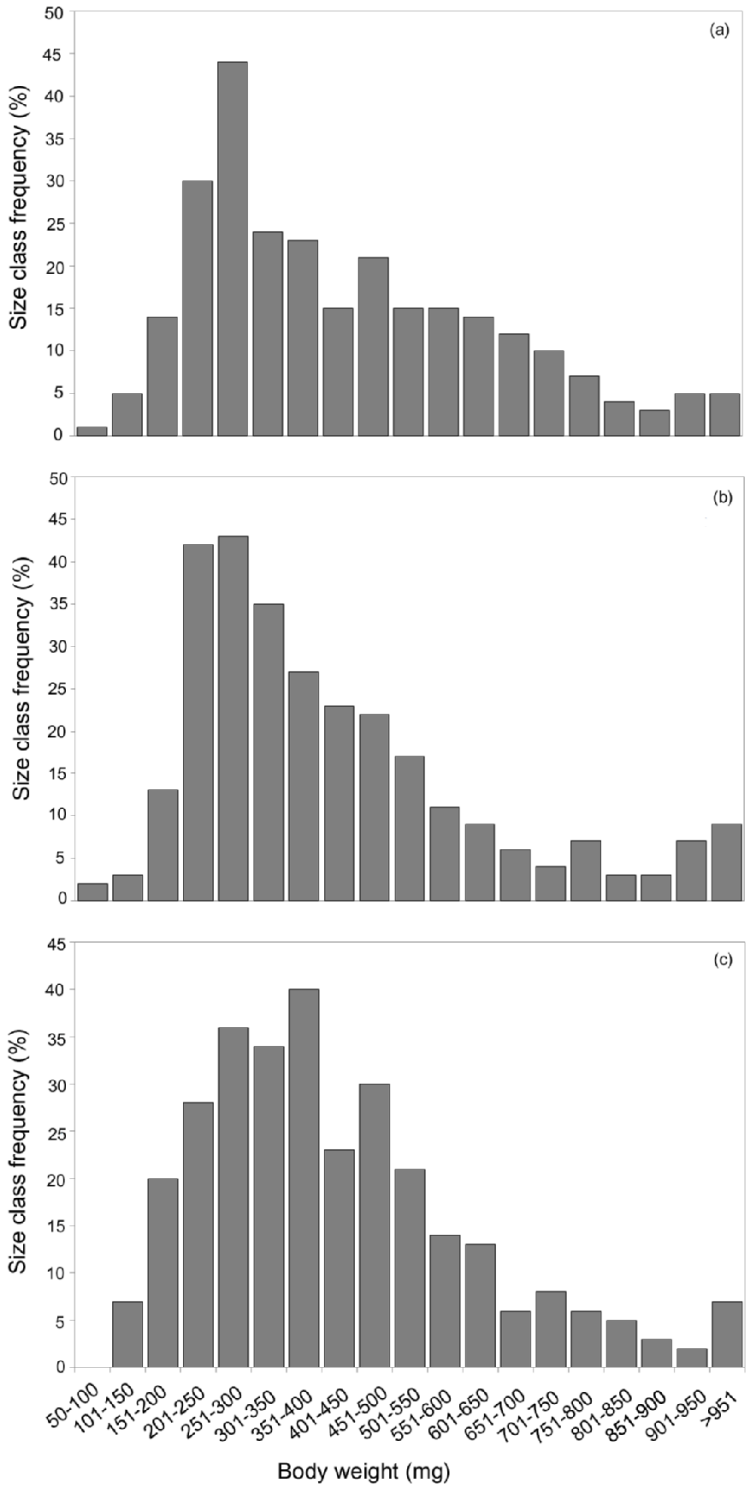


Table 6 - Body proximate composition on a wet weight basis of European glass eels (*Anguilla anguilla*) before starting the weaning (Day 0), at the end of weaning (Day 15) and the end of the experiment (Day 30) fed two diets containing 6% yeast-based and marine-based feeding stimulants *(Experiment 2).*

	Day 0	Day 15			Day 30		
	Initial	Control	YBFS diet Yeast-based stimulant at 6%	MBFS diet Marine-based stimulant at 6%	Control	YBFS diet Yeast-based stimulant at 6%	MBFS diet Marine-based stimulant at 6%
Moisture (%)	73.7 ± 1.3	73.4 ± 2.0 ^{ab}	70.6 ± 0.2 ^{ab}	70.6 ± 2.5 ^{ab}	72.5 ± 1.3 ^{ab}	71.0 ± 2.7 ^{b}	74.0 ± 1.0 ^{a}
Crude protein (%)	11.9 ± 1.1	12.6 ± 0.2 ^{ab}	14.3 ± 0.9 ^{ac}	12.7 ± 0.6 ^{ab}	14.0 ± 1.2 ^{a}	12.5 ± 1.1 ^{bc}	11.2 ± 0.7 ^{b}
Crude fat (%)	8.6 ± 2.9	7.9 ± 2.0	9.9 ± 1.4	10.7 ± 2.5	8.5 ± 1.8	7.9 ± 2.1	8.0 ± 1.6
Carbohydrate (%)	0.8 ± 0.1	0.8 ± 0.2 ^{ab}	0.8 ± 0.1 ^{ab}	0.7 ± 0.1 ^{ab}	0.6 ± 0.1 ^{a}	0.6 ± 0.1 ^{a}	0.9 ± 0.2 ^{b}

Values are means ± SD. Means in the same row with different superscripts are significantly different at $P < 0.05$

Figure 3 – Specific activities values of digestive enzymes in elvers of European eel (*A. anguilla*) fed different experimental diets containing different levels of feeding stimulants at the end of *Experiment 1* (Day 60). Where overall significant differences were observed, multiple range tests have been applied at the P <0.05 level, and indicated with letters.

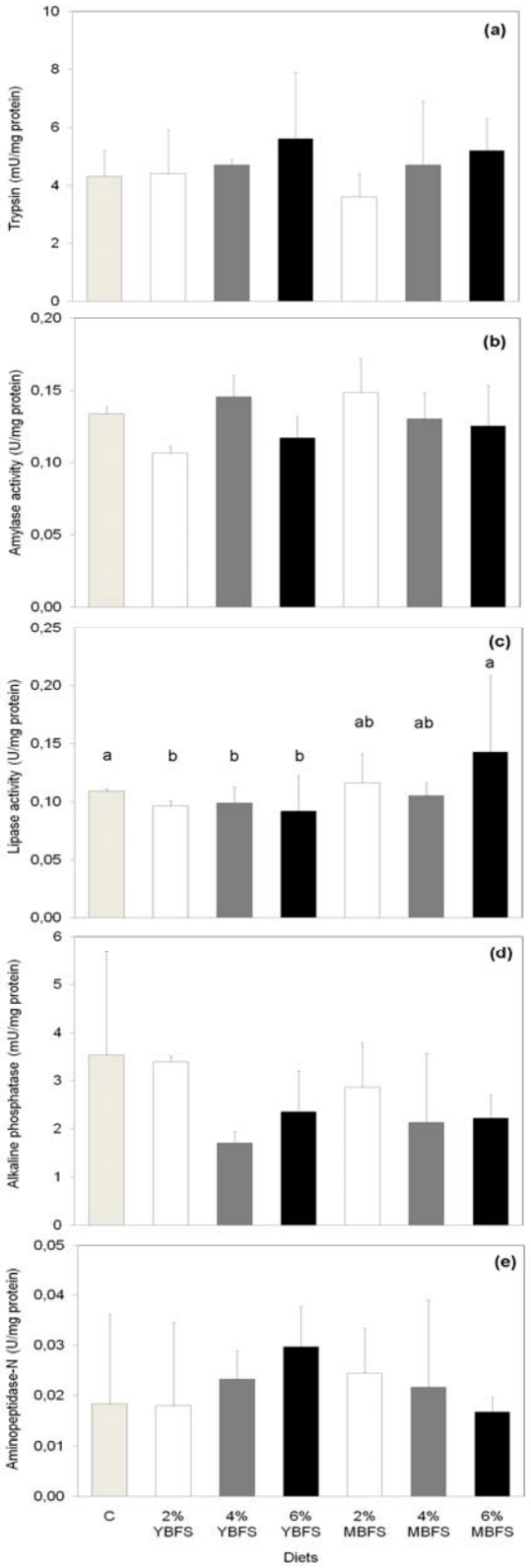


Figure 4- Specific activities values of digestive enzymes in glass eels of European eel (*A. anguilla*) fed to different dietary treatment at the end of the weaning period (Day 15), and at the end of *Experiment 2* (Day 30). Where overall significant differences were observed, multiple range tests have been applied at the P <0.05 level and indicated with letters.

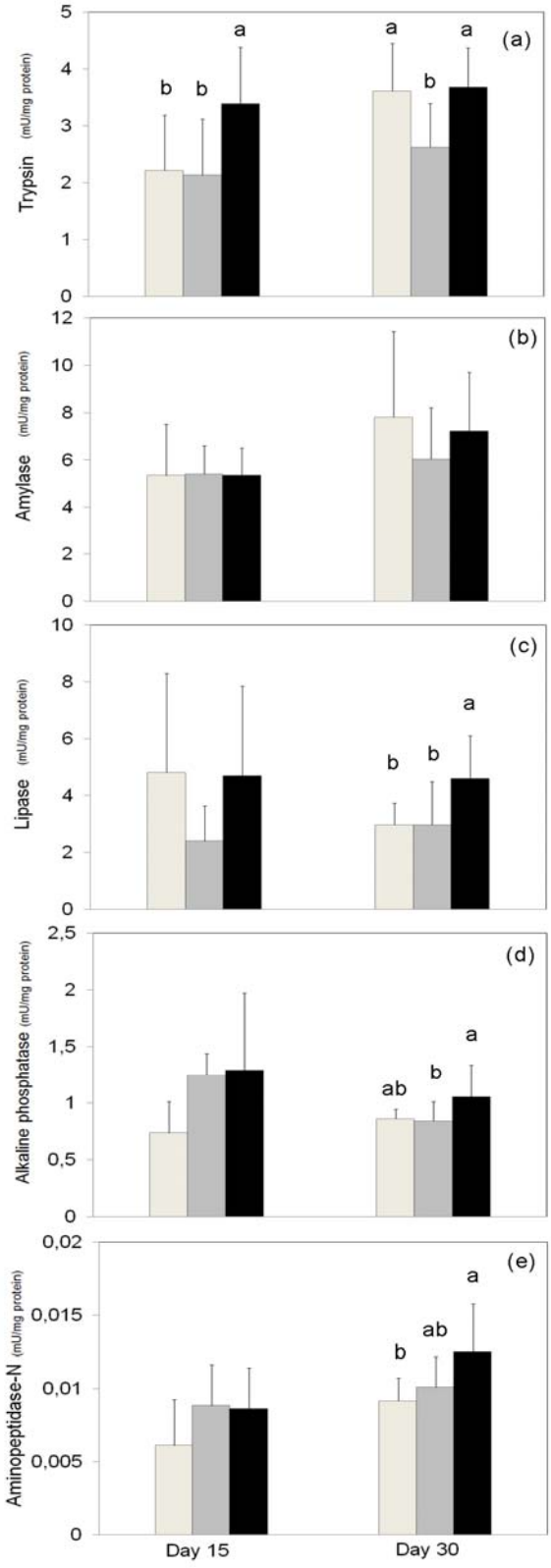
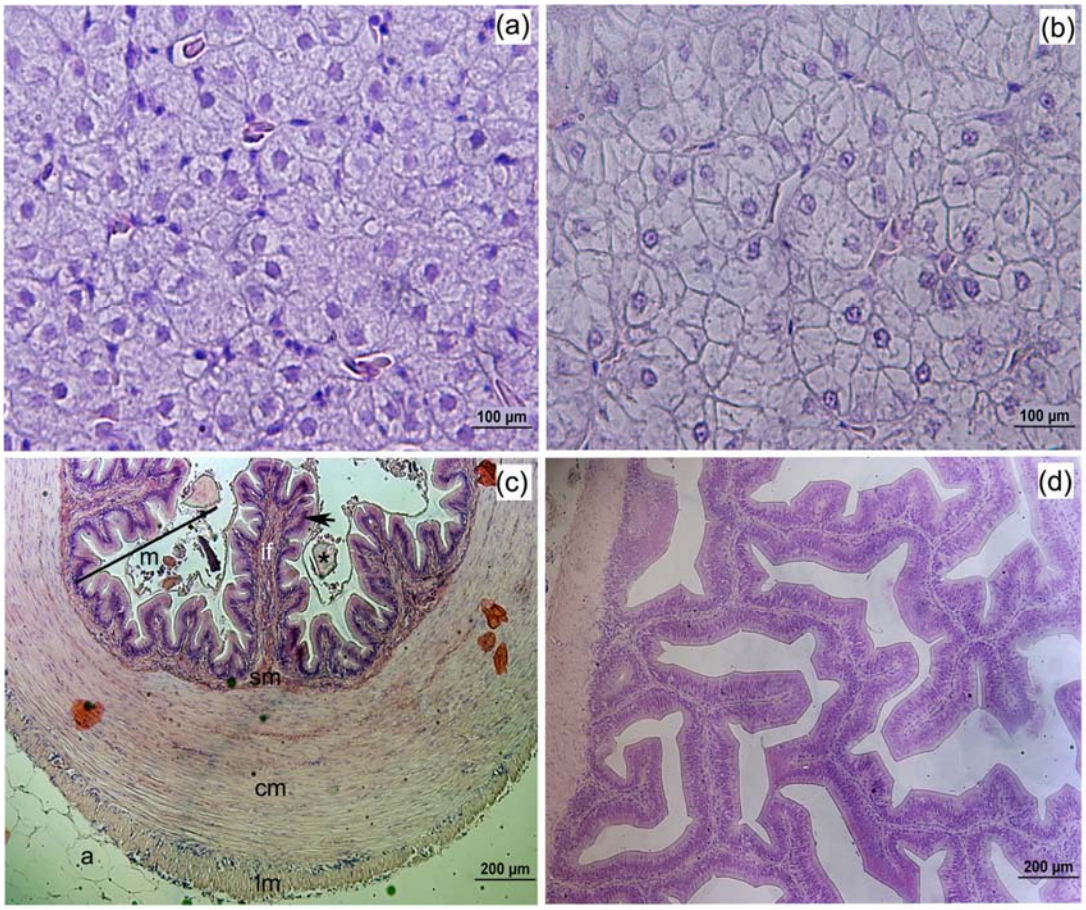


Figure 5 **-** Histological organization of the liver and intestine in elvers of European eel (*A. anguilla*).



Detail of the liver of an elver fed the control diet showing the absence of lipidic cytoplasmatic inclusions within hepatocytes (a). Detail of the liver of an elver fed the 6% MBFS diet; note the displacement of nuclei to the periphery of the hepatocytes and the presence of large lipid inclusions (haematoxylin-eosin stained) occupying most part of the cytoplasm of the hepatocytes (b).

Transversal (c) and sagittal (d) sections of the intestine in elvers fed the 6% MBFS diet, showing the normal organization of the intestinal mucosa and the absence of remarkable lipid vacuoles in the enterocytes and submucosa.

Abbreviations: a, adipocytes (mesenteric fat deposits); arrow, columnar simple epithelium; asterisk, feed particle; cm; circular muscular layer; lm, longitudinal muscular layer; if, intestinal fold; m, mucosa; sm, submucosa.

4. Discussion

In the present study, tested diets containing different types and levels of feeding stimulants were not isoproteic due to the chosen system for incorporating the feed stimulants on the manufactured control diet (50% protein). However, considering that the optimum level of dietary protein for juvenile eels was estimated near to 45% for *A. japonica* (Nose & Arai, 1973, Okorie *et al*., 2007) and 47% for *A. rostrata* (Tibbets et al., 2000), the difference in total protein content observed among tested diets might not have affected the overall fish performance in this study, as previous results indicated that increasing dietary protein above 45-47% did not result in a significant benefit for growth improvement and protein accumulation in juvenile eels. Consequently, differences observed among different diets might be attributed to the type and level of inclusion of feed stimulants (FS) rather than the total dietary protein content.

The results from many studies on various fish species demonstrate the efficacy of including feed stimulants (FS) in practical diets for improving feed intake and growth performance. Although a diversity of FS have been identified from numerous experiments on fish species, most belong to a small group of chemicals of low molecular weight (<10,000 Da): (1) free amino acids, (2) nucleotides and nucleosides, and (3) quaternary ammonium bases (Takeda & Takii, 1992; Gómes *et al.,* 1997; Papatryphon & Soares, 2000; Houlihan *et al.,* 2001; among others).

Several studies have shown that the spectra of stimulatory free amino acids are highly species-specific, and the list of palatable amino acids is different for different fish species (Kasumyan & Døving, 2003). Neurophysiologic studies of fish taste response to amino acids suggests that two general gustatory responses to amino acids occur in fish species; those species that respond to many different types of amino acids (wide response range group) and those species that are highly selective and only respond to a few amino acids (limited response range group). The Japanese and European eel species belong to this second group, as they respond to a relatively narrow range of amino acids (see review in Marui & Caprio, 1992). In particular, among the amino acids with most

stimulatory effects on eels are L-glycine, L-alanine, L-arginine, L-histidine, L-proline and L-leucine (Yoshii *et al.*, 1979; Mackie & Mitchell, 1983; Takeda *et al*., 1984; Takii *et al*., 1984; Hara & Zielinski, 1989; Marui & Caprio, 1992; Hara, 1994; Knights, 1996).

In the present study, the tested FS were formulated with proteins from different raw materials (marine and yeast sources) that mainly differed in their amino acid profile, as well as in the molecular weight fractions of the soluble protein. Considering the peptide nomenclature proposed by the International Union of Pure and Applied Chemistry (IUPAC), our FS were composed by a high proportion (74 and 59% for MBFS and YBFS, respectively) of free amino acids and di-and tripeptides (MW <500 Da), whereas the YBFS contained a higher proportion of large polypeptides (MW >30,000 Da) than the MBFS (16 versus 5%).

The results from the dose-response nutritional trials with elvers (*Experiment 1*) of *A. anguilla* showed that the incorporation of FS into a pelleted compound diet had a beneficial effect on overall fish performance, although the results observed were different depending on the type and inclusion level of the tested FS. In particular, the use of both FS at 2 and 4% did not result in any advantage in terms of elver growth, survival and size dispersion, nor had any effect on the functionality and organisation of the digestive system compared with the control group. However, at higher levels of inclusion (6%) the MBFS had a positive effect on elvers' performance. In this sense, elvers fed 6% MBFS diet increased feed intake, which resulted in higher mean BW_f, specific growth rate and biomass gain than elvers fed the other dietary groups. Elvers fed the 6% YBFS diet showed intermediate values for the above-mentioned parameters in relation to the 6% MBFS and control groups. The results of Mackie & Mitchell (1983) on European eel elvers agree with those of our trial, showing an increment on feed intake with fish fed a paste diet supplemented with a mixture of neutral and acidic L-amino acids.

In none of these cases did the inclusion of FS affect the proximate biochemical body composition of elvers and their efficiency in converting feed into increased

body mass as measured by FCR, which confirmed that both FSs enhanced feed ingesta rather than improving the nutritional value of the feeds. However, although FS did not affect the proximate biochemical composition of elvers fed different diets, animals fed 6% MBFS and YBFS diets showed higher fat deposition levels in the liver, which confirmed the better growth performance of fish fed these diets. No histopathological change in the organisation of the liver and intestine was observed as a consequence of the dietary treatments.

In addition, elvers fed different diets showed similar overall digestive capabilities. The levels of activities were not different for any of the pancreatic (trypsin, amylase and lipase) and intestinal brush border (alkaline phosphatase and leucine aminopeptidase) enzymes among animals fed the same FS. However, lipase specific activity in animals fed the 6% MBFS diet was significantly higher than in elvers fed YBFS diets, but similar to that recorded in elvers fed the control diet. These results differed from those reported in Japanese eel (Takii *et al*., 1986b) where FS had a more pronounced effect on feed utilisation than on feed intake. The former authors suggested that the chemical cues originating from the dietary FS enhanced the cephalic reflex response regulating feed intake, promoting the digestive function of the animal as reported in mammals (Giduck *et al*., 1987). In the present study, the absence of significant differences in the digestive function (activity of pancreatic and intestinal enzymes) in European elvers fed different diets might be attributed to the sampling protocol, as elvers were sampled early in the morning before feeding. Consequently, enzyme activities in our study reflected the effects of FS on the level of maturation of the digestive system (Zambonino-Infante *et al*., 2008) but not their impact on the improvement of digestive function as Takii *et al*. (1986b) reported for elvers of Japanese eel.

Contrary to the results found in elvers, no differences in terms of growth performance, survival and size distribution were detected in glass eels (*Experiment 2)* fed 6% MBFS, 6% YBFS and control diets at the end of the trial period. A few studies have reported that the addition of feeding stimulant in European glass eel improved the weaning process, feed intake, growth performance and survival of glass eels (Kamstra & Heinsbroek, 1991;

Heinsbroek & Kreuger, 1992; Ajuzie & Appelbaum, 1993). However, Kamstra & Heinsbroek (1991) indicated that the effects of dietary FS were only evident in glass eels fed the experimental diets at low feeding levels (< 5% body weight day^{-1}), at higher feeding rates no effect of the FS was detected in terms of the overall growth performance of glass eels. Our results were in agreement with those reported by the former researchers, since the *ad-libitum* administration of feeds might have masked the effects of FS on growth performance. Although at Day 30 most of the glass eels were eating the experimental compound diets as indicated by the fullness of their guts and the unimodal size distribution, a longer experimental period might have demonstrated more clearly the effects of FS on fish performance, and consequently these results should be considered as preliminary.

Although no significant effects in terms of growth and histological organisation of the liver and intestine were observed among diets, FS affected the level of maturation of the digestive function in glass eels fed 6% MBFS. These results were supported by the higher activities of trypsin and lipase from the pancreas, and enterocyte's brush border enzymes like alkaline phosphatase and leucine aminopeptidase (Zambonino-Infante *et al*., 2008). Considering the molecular size of the soluble protein in both FS tested, the MBFS contained a higher proportion of short peptides (MW<1,000 Da) in comparison to the YBFS (40 vs. 22%, respectively). Thus, it seems plausible that the molecular size of the soluble protein compounds included in FS might have affected the level of maturation and functionality of the digestive system, as the inclusion of short peptides in compound diets has already been proved advantageous in fish larvae in terms of nutrient assimilation and larval performance (Zambonino-Infante *et al.,* 1997).

Under present experimental conditions, the reasons why the MBFS had a better performance than the YBFS might be associated to the differences in the concentration of several amino acids and their ratio between both FS. In this sense, the amino acids in the MBFS that exceeded 15% of their content in the YBFS were arginine, lysine, methionine, phenylalanine, tyrosine, histidine, alanine, proline, glutamic acid, glycine and aspartic acid. Among them, alanine,

arginine, glycine, histidine and proline are described as a group of amino acids with a most prominent stimulatory effect in eel species (Mackie & Mitchell, 1983; Takeda *et al*., 1984; Takii *et al.,* 1984; Marui & Caprio, 1992; Knights, 1996).

Size heterogeneity is a common feature and problem in eel farming (Kamstra & Heinsbroek, 1991; Heinsbroek & Kreuger, 1992; Tesch, 2003) that directly affects the performance of the rearing process. This involves a substantial amount of work in size-grading activities. Under present experimental conditions, feeding elvers with diets containing 6% MBFS and YBFS resulted in a more symmetrical distribution of body size. Although oral taste preferences are considered a species-specific trait, it is noteworthy that taste preferences at the individual level might dramatically vary among conspecifics (see review in Kasumyan & Døving, 2003). In this sense, the reduction in elvers' size dispersion was likely due to the inclusion of FS in diets, which promoted feed intake over a greater number of reared animals than the control group. In practical terms, this would represent a reduction in size-grading tasks in eel fish farms and consequently, an improvement of the rearing process.

Studies on shortfin eel in Australia have indicated a high variability in the growth rate of glass eels during their critical period of weaning to artificial feed. Australian researchers found an SGR of glass eel ranging from -2.1% day^{-1} to 3.6% day^{-1}, and also a highly variable individual growth rates (Gooley *et al*., 1999; Gooley & Ingram, 2002). These disparities in growth rate were attributed mainly to the wide range of the seed-stock and genetic variability within the seed, which have a different capacity of adaptation to the culturing environment. In New Zealand, Kearney (2009) also found a highly variable growth rates for the glass form of shortfin eel. He stated that the development of culturing strategies to increase the number of fast-growers eels and to reduce the high variability in individual growth rate remain key issues facing an eel aquaculture industry.

The present study revealed that incorporating FS into a pelleted diet had a beneficial effect on the overall performance of European glass eels and elvers. It would therefore be useful to continue on this stimulants research track to

improve the growth rate of slow-growing fish and size homogeneity of the shortfin eel.

Chapter 5

General Discussion

1. Outcomes of this thesis

(1) The development of a New Zealand eel product aligned with international market specifications on fat content.

Objective 1 of this thesis was to examine the quality of the final eel product, in terms of body proximate composition, with a focus on the fat content and fatty acid profile, of wild yellow shortfin eel cultured in a RAS. The results reported in *Chapter 2* confirmed that it is possible to raise a fatty shortfin-eel product with about 20 to 22% total fat starting from wild yellow eels with an initial fat content below 7%.

Likewise, the study indicated that it is possible to obtain a similar final fat content by feeding the eels a commercial diet with a declared fat content of 22% instead of 26%, but increasing dietary carbohydrate to compensate. From the standpoint of consumers' perception of 'healthy' foods, the fat quality of the shortfin eel cultured in the present study was appreciably superior to that of eels caught in the wild. Fatty acid analysis of cultured eels indicated a higher proportion of omega-3 fatty acids (EPA and DHA), higher n-3 to n-6 ratio, and a lower Atherogenic Index than for wild eels. At the same time, the fat 'health' quality of eels fed a commercial declared eel diet was slightly higher than of eels fed a commercial declared salmon diet, suggesting a better dietary fat profile in the former diet, where the aim is to produce a high omega-3 product.

Thus, the results of *Chapter 2* confirmed the prospect of adding value to the wild shortfin eel by increasing the quantity and quality of body fat through aquaculture. However, a careful selection of the dietary fat must be made to obtain a fish product that addresses consumer perceptions and demands. Inclusion in the pellet diets of high quantities of fat sources other than fish oil, like fat from animals other than fish as well as vegetable oils, could produce a cultured eel tissue of perceived lower fat quality. At the same time, the flavour profile of a 'less healthy' fat may be better than that of a 'healthy' high omega-3 fat, and this is an issue that should be addressed in future flavour studies.

(2) The achievement of good values of mean growth rate (SGR= 1.1% day^{-1}) and feed conversion ratio (FCR ≤ 1.1) for rearing yellow shortfin eels, which indicates a good potential for culturing.

Objective 2 of this thesis was to examine the growth rate, feed efficiency and fish size variability of wild yellow shortfin eel cultured in a RAS. From a biological point of view, the results showed in *Chapter 2* about growth rate and feed efficiency are very promising for culturing this species. The SGR and FCR observed in both dietary treatments, commercial eel and salmon pelleted feeds, compare favourably with the European and Japanese eel species of a similar size range. The salmon feed has the advantage of being readily available in New Zealand.

Both pelleted diets, with the same declared protein content (47%) and gross energy (22.2 MJ kg^{-1}), resulted in good performance. However, it must be noted that the values of SGR and FCR reported in the present experiment were obtained under a laboratory situation with careful handling. A commercial small-scale pilot study should be done.

(3) The rejection of a hypothesis that size grading can improve the proportion of fast-growers and reduce the high variation in individual growth rate of yellow shortfin eels.

Objective 3 of this thesis was to examine the effect of size grading on the growth rate of wild yellow shortfin eel cultured in a RAS. Results reported in *Chapter 3* show that size grading in this production system has no significant effect on the growth performance or survival of the yellow shortfin eel.

It was not possible to improve the individual growth rate, or to reduce the size variation within the small eel group by having no large eels in the same tank. These findings suggest that the wide variability in the individual growth rate performance of the eels is not primordially a consequence of social interaction (hierarchical position) among tank-mates. Moreover, *Chapter 3* clearly shows a

marked difference in growth performance between large and small eels, independently of whether they were interacting with fish of similar size or not. The large-eel group showed a better specific growth rate, condition factor, feed consumption, feed conversion ratio, and more uniform individual growth rate among tank-mates (lower CV) than the small-eel group. Other factors, such as genetic background, differences in metabolism, utilisation of food resources and/or different ability to adapt to intensive culture may be responsible for the highly variable growth.

(4) The confirmation of a hypothesis that feeding stimulants can improve the proportion of fast-growers and reduce the high variation in individual growth rate of juvenile eels.

Objective 4 of this thesis was to examine the effect of feeding stimulants on growth rate of juvenile European eels cultured in a RAS. *Chapter 4* shows that the incorporation of feeding stimulants into a pelleted diet had a beneficial effect on the overall growth performance of European elvers and glass eels.

However, the results also depended on the eel's stage of development, as well as on the type and inclusion level of the stimulants. In elvers, the inclusion of MBFS and YBFS at a concentration of 6% was useful in terms of feed intake, growth performance, and homogeneity of size distribution. In glass eels, the 6% MBFS diet promoted the maturation of digestive function during the weaning process as indicated by a higher enzymatic activity in the pancreas and intestinal brush border, but no effects on growth performance and size distribution were observed between treatments. By the end of the 30-day trial most of the glass eels were eating the stimulant diets. However, a longer experimental period might have more clearly demonstrated the effects of FS on glass eel performance, and consequently these current results should be considered as preliminary.

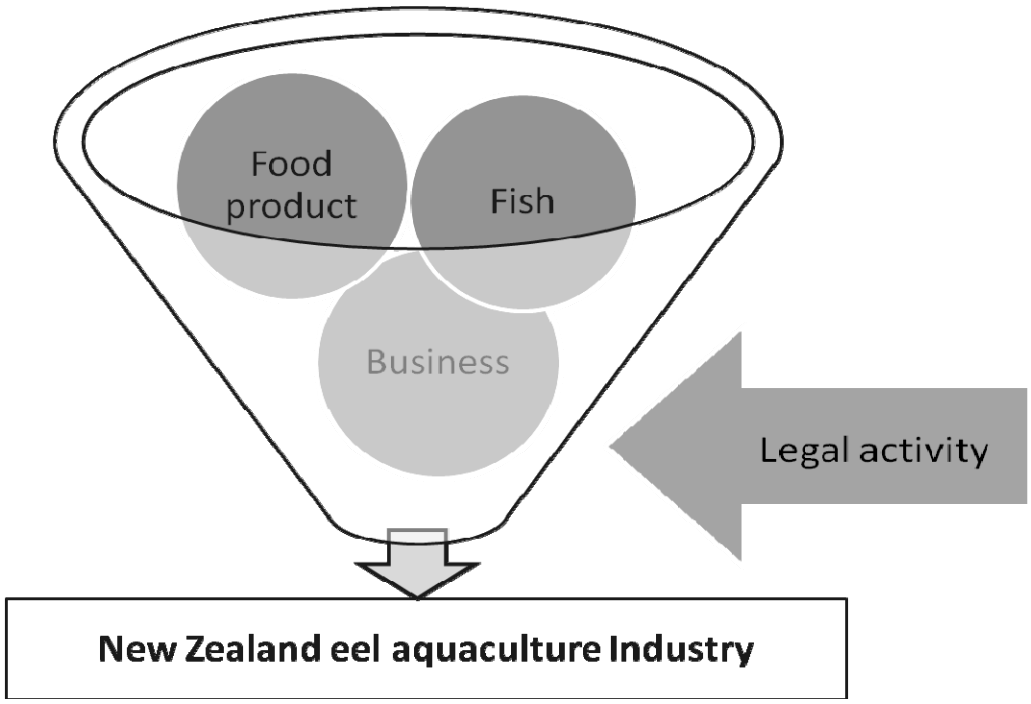
The results obtained on the effects of feeding stimulants in the juvenile stage of European eels are encouraging, and indicate that this culturing strategy may

help improve fish production and reduce the eel-farm operational work. Future research on the use of feeding stimulants in diets for cultured shortfin eels is worth doing. Moreover, on the basis of results obtained in *Chapter 4* and other eel studies, the use of a MBFS with high proportions of alanine, arginine, glycine, histidine and proline is recommended.

2. Viability of developing an eel aquaculture industry in New Zealand

Significant advances in our knowledge of shortfin eel culture have been made during the last ten years, but there are still numerous aspects that need to be assessed before the viability of developing a New Zealand eel aquaculture industry can be adequately determined.

In order to establish the potential of a farmed eel industry, New Zealand eels must be studied not only from a biological perspective ("*as a fish*"), but also as food and its market ("*as a food product*"), and as a profitable business enterprise ("*as a business*"). In addition, it will be necessary for central and local government to create the right regulatory environment ("*as a legal activity*").



Shortfin eel "*as a fish"*

Most of the studies on shortfin eel culture have been focused on the biology of the animal. In the last decade, New Zealand and Australia researchers have conducted many biological studies related to wild eel stock, transportation protocols, weaning, husbandry techniques and disease control of glass eel and elvers of shortfin eel. This research thesis provides new, complementary information on the rearing of the yellow stage of the shortfin eel, an area that has been explored by only a few studies previously.

Overall, these studies indicate that from a biological point of view the shortfin eel has good potential to be a cultured species. The values of growth rate and feed efficiency obtained in the rearing trials at a laboratory scale for the different fish stages are comparable to those observed in the European and Japanese eels, two well farmed species.

Considerable experience has been gained in the handling and husbandry of shortfin eels, but there are still several biological aspects that need further research. Important issues that must be addressed are:

- The production of glass eel artificially in the laboratory.
  Eel farming is currently a capture-based aquaculture activity. The main bottleneck affecting the global eel aquaculture industry is the inability to produce glass eels at the laboratory. Researchers around the world have been working on spawning and reproduction of different eel species. Some encouraging results have been obtained but the goal remains elusive.

- The availability of wild glass eel stock for culturing eels.
  Hatchery scale production of glass eels may be decades away and thus the eel farms will continue to rely on wild glass eel seed stock (Jellyman, 2010). Research to determine the timing and location of harvestable glass eel stocks throughout New Zealand waters have been planned. It is hoped that this programme by the National Institute of Water & Atmospheric Research (NIWA) will define quantities and sites where

glass eels can be harvested without affecting the New Zealand wild eel population.

- The high proportion of slow-growers or no-growers in the cultured eel environment.
  The high number of cultured eels that do not feed well leads to a large variation in individual fish size and mortality. The size-grading and feeding-stimulant chapters of this thesis have provided a better understanding of some aspects of this problem. However, this is a major issue that faces eel farmers around the world and has not yet been solved.

- The culture of shortfin eel in a commercial small-scale pilot study.
  Laboratory studies have yielded valuable experience in the handling and husbandry of shortfin eels, but prior to developing an eel farm, it will be necessary to evaluate the rearing performance of this species using parameters required for successful commercial eel culture (e.g. fish biomass, stocking density, feeding regimes).

Shortfin eel "*as a food product*"

To be successful, eel aquaculture must consider not only feed conversion efficiencies and growth rate, but also specific product quality attributes. Even though a diet may be developed with the right properties for rapid growth and food conversion, this may not result in an eel product that is appealing to the market.

By the use of commercial diets, this thesis showed that it is possible to develop a cultured New Zealand shortfin eel with a body fat content of 20 to 22% which is nominally appealing to the international market. However, further research is needed regarding the organoleptic quality (flavour, texture and colour) of these new cultured eel products.

The presence of an assured export market will remarkably increase the chance of success of eel farming in New Zealand. Considerable market research and

acceptability trials will be needed to confirm the potential of a cultured eel product from New Zealand in the high-priced Asian and European markets.

Shortfin eel "*as a business"*

Preliminary biological studies on shortfin eel indicate that there is a potential for the culture of this species, but an obvious requirement is that it must be economically viable. Studies in this area are critical before setting up any eel farm.

Bio-economic simulation modelling can be a valuable tool in determining whether a proposed commercial operation might show financial promise. Using spreadsheet-based models, the biological and financial inputs into an operation can be described and an estimate of financial output generated. Further, sensitivity analysis can be used to show which of the inputs has the greatest effect on the financial outcome (Mussely, 2010).

These models can help to define the feasibility of an eel farm and best culture strategy in aspects such as: the fish stage to start culturing from (glass eel, elvers or yellow eels), the market to aim for (local, Asian, European), culture techniques (outdoor ponds, indoor ponds, RAS), production scale, the feeds utilised, and the different eel products commercialised.

Shortfin eel "*as a legal activity"*

Currently there are legislatives barriers to the culture of glass eel in New Zealand. It is illegal to harvest glass eels because they are below the minimum size limit of 220 g for commercial purposes. At the same time, there is a moratorium on new permits for commercial eel fisheries in New Zealand. Thus, at present it is possible to farm yellow eels over 220 g, but they have to be sourced from a commercial fisher or a licensed fish dealer.

The New Zealand Government has expressed its determination to create the right regulatory environment for aquaculture to flourish, and it is looking to

remove the regulatory roadblocks to help this happen (McNee, 2010). Presently, the Ministry of Fisheries is evaluating ongoing mechanisms (within the context of the Quota Management System) for harvesting glass eel for aquaculture that do not undermine quota rights or compromise the sustainability of the wild stock.

In conclusion, the information provided by this thesis, in conjunction with previous mainly biological studies, indicate that shortfin eel has good a potential as a culture species, but there are yet several aspects to be overcome before a new eel culture industry can be a reality in New Zealand.

ANNEXES

Annexe 1

Eel farm: *Valenciana de Acuicultura S.A.*

Commercial recirculation aquaculture system

Valenciana de Acuicultura (VALAQUA S.A.) is a pioneer company in the sector of modern technology for intensive fish culture in Europe. This company, founded in 1984, was one of first fish farms to develop recirculation aquaculture system (RAS) on a commercial scale. It is the biggest eel farm in Spain, and also it is ranked among the biggest in Europe, with an annual production of around 450 tonne of European eel (*Anguilla anguilla*). *Valenciana de Acuicultura* has commercialised its know-how on recirculation aquaculture system in different European and Asian countries.

Valenciana de Acuicultura S.A.
(Puçol, Valencia, Spain)
www.valaqua.com

This eel farm has a total of 134 concrete rearing tanks, grouped in three categories: circular, square and race-way tanks with the following features:

Feature	Circular tanks	Squar tanks	Race-way tanks
High self-cleaning ability	Yes	Yes	No
High uniformity of water quality	Yes	Yes	No
High quality of oxygen distribution	Yes	Yes	No
Optimum use of space	No	Yes	Yes
High fish handling capability	No	No	Yes

Circular rearing tanks

Square rearing tanks

Race-way rearing tanks

Pelleted eel feed

Glass eels fed cod (*Gadus morhua*) roe

Yellow eel fed pelleted feed by a self-feeder

Emptying a squared tank for size grading.

Size-grading machine with plastic containers to collect large, medium and small eels.

Size grading of eels in progress

Annexe 2

AUT University's Aquaculture Laboratory
Recirculation Aquaculture System (RAS)

The New Zealand eel species cultured in this thesis were reared in a recirculation aquaculture system (RAS) built by the author alone. Three recirculation modules were built; each made up by three circular 160-L tanks and one media box biofilter. The construction of the RAS underwent several iterations until a suitable system for culturing the eels was achieved. The final version performed well, but required a high level of maintenance. Every day, the filter pad was cleaned, and 15% of the water was renovated for each recirculation module. Every three weeks, pipes and valves were cleaned.

The final version (v3) of each recirculation module comprised:

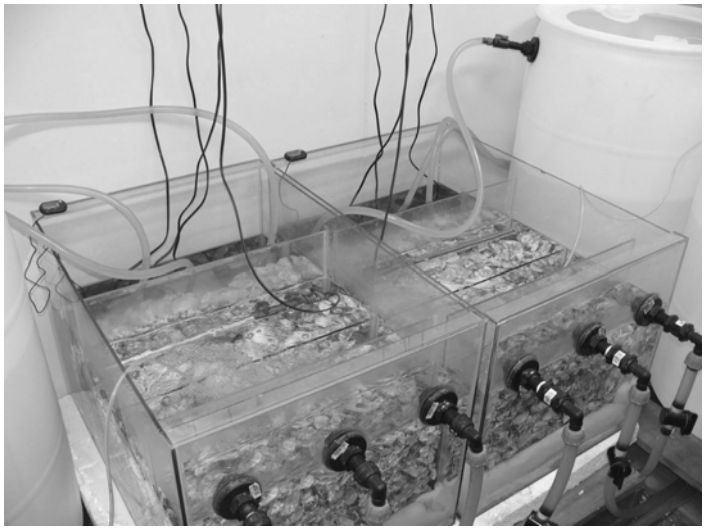
- three rearing circular tanks of 160 L each.
- one media box biofilter (Hilder, 1993) constructed of glass. For the mechanical filter, a washable white filter sponge, a wool pad, was used in the first section of the box. For the biological filter the media used was oyster shells.
- a 300 W heater (Eheim Jager, Germany).
- an 55 W ultraviolet light sterilisation unit (Tropical Marine Centre, UK).
- miscellaneous pump, pipes and valves to recirculate the water through the system.

The daily feeding and rearing maintenance were carried out by the author.

RAS first version (v1)

Plastic box biofilter

The plastic container suffered deformation and the internal partitions could not control water circulation. Water did not flow uniformly through the shell media.

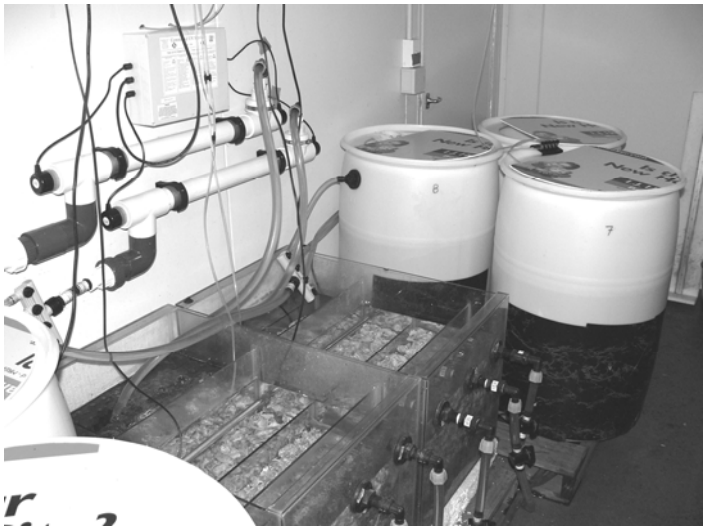


RAS second version (v2)

Glass box biofilter
The rigid structure controlled water circulation well. The flow of water was uniform through the shell media.

RAS second version (v2)

System without the ultraviolet light unit.



RAS final version (v3)

System with ultraviolet light unit installed.

Feeding shortfin eels

Immediately after the pelleted feed was placed in the tray.

Feeding shortfin eels

Ten minutes after the pelleted feed was placed in the tray.

Cultured shortfin eels

Annexe 3

Evaluation of the PIT tagging as a method for individual marking of yellow shortfin eel (*Anguilla australis*)

1. Introduction

There are several marking and tagging methods available for individual fish identification. The selection of the best method depends of the species, fish size, and type and duration of the experiment. The selected method should not affect the behaviour, physiology, growth and survivorship of the fish, and in addition the mark on the individual should be permanent or at least persist for the duration of the study (Gibbons & Andrew, 2004).

PIT tags (passive integrated transponder) are among the best candidates for tagging fish, because of their small size and mass, their long functional life (> 10 years), the availability of millions of individual codes, and because the animal does not have to be sacrificed for the tag number to be read (Baras *et al*., 2000; Acolas *et al*., 2007; Jellyman *et al*., 2007). A PIT tag is an electronic microchip encased in biocompatible glass with a unique alphanumeric code that can be read by a scanner (Gibbons & Andrews, 2004). It is inserted by surgical incision under the animal's skin, usually into muscle or the abdominal body cavity.

There are few controlled laboratory studies that have evaluated the performance of PIT tags with eel species, and they did not test the effect of the tag in the growth rate. The applicability of using transponders to tag European eel (*Anguilla anguilla*) during their silver eel stage was tested in the laboratory by Winter *et al.* (2005), who focused on tag loss and mortality of eels over 680 g. Zimmerman & Welsh (2008) compared the retention rate of PIT tags placed in three different tagging locations (dorsal musculature behind the head, dorsal musculature near the dorsal fin origin, and the abdominal cavity) in American eel (*Anguilla rostrata*).

To date there have been no reports of studies under controlled laboratory conditions to evaluate PIT tag retention, survival and growth rate, for New Zealand shortfin eel. The objectives of this study were to determine whether there is a significant difference in growth rates and survival between PIT-tagged and a control group of untagged fish, and to evaluate PIT-tag retention for the yellow stage of the shortfin eel.

2. Materials and methods

2.1 Origin of fish and their maintenance

Shortfin eels used in the present study were collected with fyke net from Lake Waikare, North Island, New Zealand in June 2008, and transported by road to AUT University's Aquaculture Laboratory. On arrival, the eels were transferred to the freshwater recirculation modules described in *Annexe 2.*

From the arrival to the laboratory until the termination of the experiment, the eels were maintained under the environmental conditions described in *Chapter 2*.

2.2 Tagging protocol

The eels were fasted for 24 h before tagging, so the digestive tract would be clear, and consequently create more space in the abdominal cavity of the fish. This reduces the probability of eel injury during the implantation (Mahapatra *et al*., 2001). Eels were each anaesthetised with benzocaine (100 mg L^{-1}), weighed, total length measured, and tagged with coded PIT-tag in the abdominal body cavity. The tags from Zoodiac (Hallprint, Hindmarsh Valley, Australia) were 11 mm long and 2.86 mm in diameter and weighed 110 mg (in air).

The tagging procedure was as follows: the fish were placed ventral side up and a 6 mm incision was made with a scalpel approximately 5 cm ahead of the vent and slightly off-centre of the mid-ventral line (*Figure 1a*); then the tag was inserted through the incision and smoothly pushed forward into the body cavity. The incision was left open (not closed by stitches or adhesives) as is common with PIT tags (Prentice *et al.,* 1990; Baras & Jeandrain, 1998, Zydlewski *et. al*., 2001; Jepsen *et al*., 2002). Because of the size and shape of the fish, it was decided to insert the tag in the body ventral cavity of the eel by a small incision made with a scalpel instead of a PIT-tag injector.

Scalpel blades were changed frequently to avoid tearing of the tissue, and all incisions were treated with two drops of a natural antibacterial medication (API Betta Fix, 37 mL, Chalfont, PA, USA) to prevent infection, before releasing the

eel in the tanks (HallPrint, 2008). On average, each surgical implant was completed within 1 minute.

2.3 Experimental procedures

A total of 99 shortfin eels (101.7 ± 12.1 g) were selected for the experiment. After 10 days of acclimation and feeding only with hoki roe, all the eels were anaesthetised, sampled, and 72 shortfin eels tagged (Day 0). The fish were distributed among nine circular tanks. Each tank was stocked with eight tagged and three untagged eels at a density of 7 kg m^{-3}.

During the first 10 days, the eels were fed with hoki (*Macruronus novaezelandiae)* roe once a day. After that, the fish were weaned to a diet of commercial pelleted feed. The artificial diet progressively replaced hoki roe such that after 3 weeks only pelleted feed was provided. The proximal biochemical composition of the feed was as follows: 47% protein, 26% fat, 1.2% fibre, 10% ash and 8% moisture (data provided by the feed manufacturer). The eel were fed with dry pellets twice daily from Monday to Friday, and once a day on weekends to apparent satiation. Each ration of pellets provided to the eels was deposited in feeding stations (trays) as shown in *Annexe 2*, and after approximately 40 minutes uneaten food was removed. During the course of the experiment, the tanks were checked daily for dead or dying fish. If any were found, they were removed from the tank and recorded as a mortality.

2.4 Sampling schedule and growth parameters

The body weight (BW, g) and total length (TL, cm) were recorded in the first three sampling events, Day 0 (tagging day), 22 and 42.

Eel were checked on Day 22 for the state of the tag incision. The cut was considered as healed when the external layers of the body wall had closed up, with only scar tissue visible (Baras & Westerloppe, 1999).

PIT tag retention was checked until Day 108. Likewise, if the PIT-tag of any specimens could not be read, the fish skin was checked carefully in search of any wound, mark, or cicatrisation which may explain the loss of the tag through

the body wall. At the end of the experiment, the specimens for which tags could not be detected by the reader were dissected and examined visually and tactilely to confirm that the PIT tags were not in the fish.

The following parameters were calculated:

SGR (Specific growth rate, % day^{-1}) = 100 (ln BW_f – ln BW_i) / days

Survival (%) = 100 (Initial fish stock – deaths of fish) / Initial fish stock)

Tag retention (%) = 100 (Initial tagged fish number – tag loss number) / Initial tagged fish number

Where BW_i and BW_f are the initial and final body weight of each individual (g).

2.5 *Statistical analysis*

Data were analysed for one-factor variance with Minitab statistical software 16.1.0 (Minitab® Statistical Software, State College, PA, USA). Before analysis, homogeneity of variance was confirmed using Bartlett test (Snedecor and Cochran, 1989). When a significant treatment effect was observed, individual means were compared with Tukey-Kramer HSD multiple comparison test.

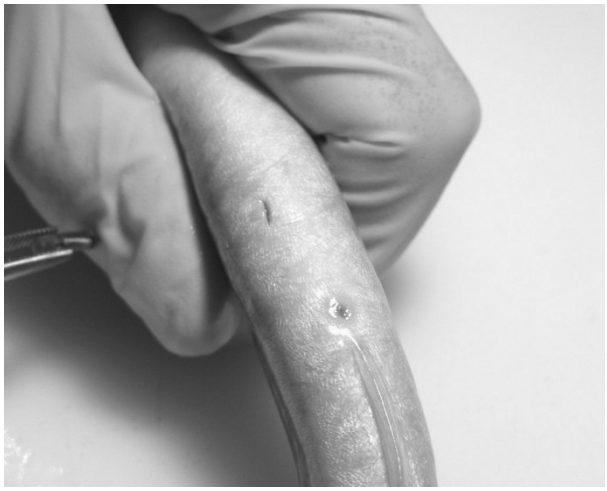
3. Results

For Days 0, 22 and 42 the body weight (BW) and total length (TL) of shortfin eel were not significantly different between control and PIT-tagged eels (*Table 1*). The specific growth rate (SGR) on Day 22 was significantly different between PIT-tagged and untagged eels (P = 0.045); untagged eels performed better *(Table 1*). However, there was no significant difference in SGR from Day 23 to 42 (P = 0.479) or for the 42-day period (P = 0.11).

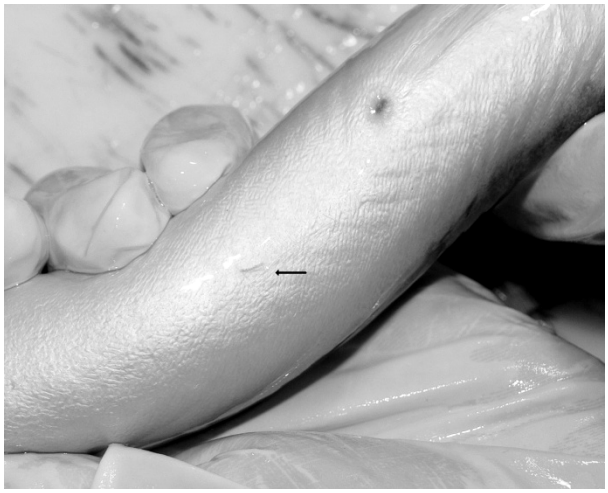
The survival rate between the tagged and the untagged fish were no significantly different 42-day period. At Day 108, tagged eels had a survival rate of 94.4% (*Table 1*).

All specimens showed the tagging incision completely closed with only a small scar tissue visible when they were checked in the first sampling event after tagging (Day 22). PIT tag retention showed values of 95.6% (3 tag losses) at the end of the experiment on Day 108 (*Table 1*). Two tag losses occurred after the wound was completely closed. In one of these cases, expulsion through the body wall was confirmed by the observation of a tag-size protuberance in the fish abdomen during one inspection event, and damaged skin in the abdomen and no tag in the subsequent inspection (*Figure 1c).* Dissection of eels whose tag could not be read, confirmed tag loss rather than tag malfunction.

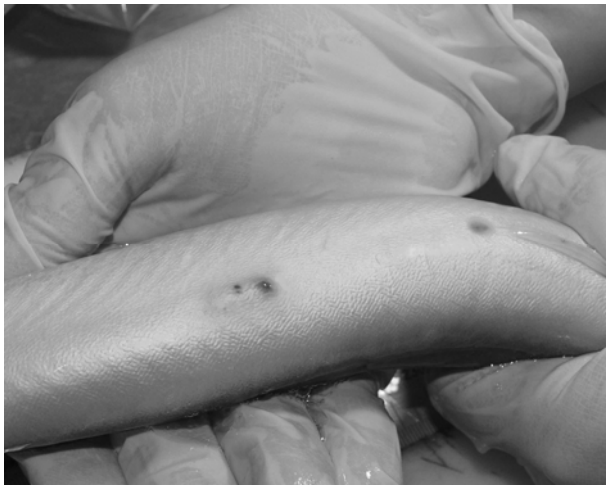
Figure 1 – PIT-tagging of shortfin eel (*Anguilla australis*).



(a) Incision made with a scalpel to insert the 11 mm PIT-tag.



(b) Tagging incision completely closed with only a small scar tissue.



(c) Skin damaged by the expulsion of the PIT-tag through the body wall.

Table 1 - Body weight, total length, SGR, survival and tag retention of tagged and untagged shortfin eels (*A. australis).*

	Tagged eels (n=72)	**Untagged eels (n=27)**
Body weight (BW, mean ± SD, g)		
Day 0	102 ± 13	100 ± 10
Day 22	121 ± 31	130 ± 32
Day 42	158 ± 52	175 ± 56
Total length (TL, mean ± SD, cm)		
Day 0	39 ± 2	39 ± 2
Day 22	39 ± 2	40 ± 2
Day 42	41 ± 3	42 ± 3
Specific Growth Rate (SGR, mean ± SD, % day^{-1})		
Days 0 to 22	0.64 ± 0.9 [a]	1.06 ± 0.8 [b]
Days 23 to 42	1.23 ± 0.8	1.36 ± 0.7
Total (Days 0 to 42)	0.92 ± 0.8	1.20 ± 0.7
Survival % (number of fish) (running sum)		
Day 22	94.4 (68)	92.6 (25)
Day 42	94.4 (68)	92.6 (25)
Day 67	94.4 (68)	--
Day 87	94.4 (68)	--
Day 108	94.4 (68)	--
Tag retention % (number of live tagged fish)		
Day 22	98.5 (67)	--
Day 42	98.5 (67)	--
Day 67	97.1 (66)	--
Day 87	97.1 (66)	--
Day 108	95.6 (65)	--

[a,b] Values in the same row with different superscripts are significantly different at $P < 0.05$.

4. Discussion

The results of this study showed that tagged shortfin eels had a depressed growth rate compared with untagged eels at the first sampling event (Day 22), but these initial lower rates did not lead to significant differences at Day 42. This observation implies that the initial negative effect of PIT tagging on eel growth rate decreases with time. Moreover, the fact that at Day 22 it was possible to observe a differential growth rate between does not necessarily mean that the negative effect lasts for the full 22 days. It might have persisted for a shorter period.

There are no other studies on the effect of PIT tagging on the growth rate of eel species to compare with this experiment. However, studies in other fish species that were PIT-tagged in the abdominal cavity have also noted decreased growths rate immediately after tagging, with lower values lasting for 2 to 3 weeks and then presenting growth rates similar to untagged fish. Prentice *et al.* (1990) reported that the growth of tagged juvenile Chinook salmon (*Oncorhynchus tshawytscha*) was slightly depressed during the first 20 days, after which it was approximately equal for the control and tagged fish. Similar results have been found for juvenile steelhead trout (*Oncorhynchus mykiss)* and Atlantic salmon (*Salmo salar*) using 11.5 or 23 mm long transponders (Sigourney & Horton, 2005; Bateman & Gresswell, 2006). Navarro *et al*. (2006) studying fingerlings of gilthead seabream (*Sparus auratus*) found a lower growth rate in this species during the first two weeks after tagging, which did not lead to differences at the end of the 26-day experiment. Studies on juvenile Eurasian perch (*Perca fluvialitis)* indicated a depressed growth of PIT-tagged fish with high tag-body weight ratio during the first post-tagging days, but after 2 weeks their growth was similar to that of control fish (Baras *et al*., 2000).

The present study has shown no significant differences between the survival rate of the tagged fish and the control group. This innocuous effect of PIT-tagging on mortality has been observed also in studies with other eel species. In a 11-week experiment, Winter *et al.* (2005) found no significant difference in mortality rate between the control (15%) and the tagged (10%) European eel

(*Anguilla anguilla*) groups during their silver eel stage. The Baras & Jeandrain (1998) 70-day experiment with European eel showed no differences in mortality between the groups. Likewise, Zimmerman & Welsh (2008) had no tagging mortality in their trials with American eel (*A. rostrata*). These results on eels are also in agreement with numerous investigations on survival of other PIT-tagged fish species, which show no differences in mortality due to tags (Prentice *et al.,* 1990; Baras *et al*., 2000; Zydlewski *et al*., 2001; Mueller *et al.,* 2006, Navarro *et al*., 2006; Acolas *et al*., 2007).

Shortfin eels showed a high PIT-tag retention rate (95.6%) after 108 days, in concordance with the few other studies that have evaluated PIT performance of eels that were tagged in the body cavity. Zimmerman & Welsh (2008) working in culture with small (20 to 37 cm) American eel (*A. rostrata*) did not observe tag loss in their 60-day experiment. A field study on *A. rostrata*, found a final retention rate of 89% after 60 days (Morrison & Secor, 2003). Baras & Jeandrain (1998) tagged European eel (> 300 g) with 1.7 g transmitters and no tag expulsion was observed. Likewise, in the Winter *et al.* (2005) investigation on transponder implanting in big European eel (> 680 g), none of the fish lost its tag.

As a general rule, it is recommended to avoid tags bigger than 1 to 2% of fish weight (Marty & Summerfelt, 1986; Buckley & Blankenship, 1990; Winter, 1996; Baras *et al*., 2000; Jepsen *et al*., 2002; Winter *et al.,* 2005). In the present study, the tag/fish weigh ratio was always under 0.14%, well below the upper limit. In respect of unclosed incisions, regular practice is to leave the cut open (Prentice *et al.,* 1990) for small implants in fish. Baras & Jeandrain (1998), in their experiment on the tagging of European eel, indicated that there were no differences in tag retention rate due to open or closed incision.

There are three ways by which an implanted tag can be lost; through the incision, through an intact part of the body wall and through the intestine (Jepsen *et al.,* 2002). Most expulsions occur through the incision shortly after tagging when the wound is still open (Thomassen *et al*., 2000, Feldheim *et al*., 2002). Expulsion through the body wall has been observed for rainbow trout

(Lucas, 1989) and Atlantic salmon (Moore *et al.,* 1990), and trans-intestinal expulsion has also been observed in rainbow trout (Chisholm & Hubert, 1985) and catfishes (Marty & Summerfelt, 1986; Baras & Westerloppe, 1999).

In the present research a total of three PIT-tags were lost. One tag was expulsed when the wound was presumably still open, before sampling Day 22. The two remaining tags were lost after the wounds were completely closed. For one of these tags, the expulsion through a part of the body wall was confirmed for the damaged skin on the belly (*Figure 1c*), but for the other tag no mark, wound or cicatrisation was observed in the fish skin. These observations suggested a trans-intestinal expulsion for the third tag. Thus, is highly probable that the three ways by which an implanted tag can be lost occurred in the present study.

In conclusion, the present study provides evidence that the PIT tagging is a suitable method for marking yellow shortfin eels. Nevertheless, it is suggested to start the rearing experiment on eels two or three weeks after tagging, to avoid the initial lower growth rates of the tagged fish.

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