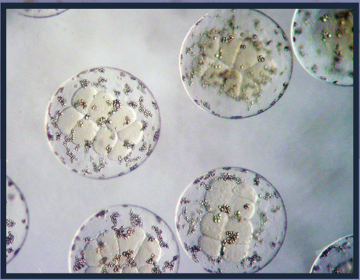

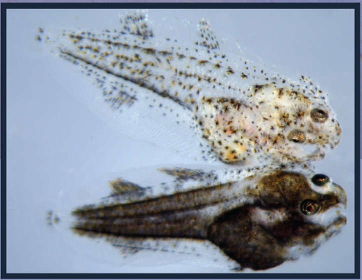

THE BIOLOGY OF SOLE

Editors

José A. Muñoz-Cueto
Evaristo L. Mañanós Sánchez
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A SCIENCE PUBLISHERS BOOK

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Preface

Senegalese sole, *Solea senegalensis*, is a flatfish highly appreciated in Spain and Portugal. Its name in Spanish (*"lenguado"*) and Portuguese (*"linguado"*) derives from the latin *"linguatus"*, meaning tongue-like shape. Actually, solea has a flat oval shape with numerous skin dots and shades that mimic the surrounding environment. This fish species is widely distributed from eastern Atlantic (southern Great Britain and Ireland) to western African (Angola-Senegal, herein its name), inhabiting coastal and estuarine sandy seabed. It is also found in western Mediterranean as far east as Tunisia, as a migration phenomenon through the Straits of Gibraltar.

Senegalese sole and common sole, *Solea solea*, are very similar in appearance, although the latter one has a compact black spot near the margin of the pectoral fin of the eyed-side. Historically, there is evidence for *S. solea* aquaculture back in the late XIX century in France, although its production never succeeded really despite research and production efforts made in France and the UK in the late XX century. *Solea senegalensis*, however, offered better prospects as this species proved stronger and better adapted to warm waters, assessing a great success when reproduction in captivity was achieved in the 1990s from wild-caught broodstocks. With sustained spawning and larvae supply, juvenile on-growing and mass production was finally possible, although major bottlenecks remained regarding (1) reproduction control and larval mortality, (2) optimum feeding protocols and diets, and (3) disease control and malformations.

Aquaculture of *S. senegalensis* started in Portugal and Spain in the late 1970s mid 1980s with very few tonnes per year. This species was introduced as a promising model for fish farming diversification in southern Europe. Sole farming has been expanding exponentially since then, reaching in 2017 over 1.600 tonnes, which were produced mainly in four countries: Spain (830 tonnes), Portugal, France and Iceland. Outside Europe, S. sole is being cultured in China, although there is no reliable production data (300–500 tonnes unofficial rough estimates).

In the last decade, devoted research programmes provided substantial advance in our scientific knowledge of Senegalese sole and helped solving problems and improving sole farming prospects. Actually, over seven hundred scientific papers have been published on *S. senegalensis*, involving researchers mainly from Spain (68%), Portugal (39%), France (7%) and UK (7%). This book brings together the work of the world's most relevant researchers working on different and complementary aspects of the general Biology, Physiology, Behaviour and Pathology of Senegalese sole.

The book is divided into two Sections: A (General Biological and Engineering overviews) and B (Specific Physiological functions). Section A begins with an introductory chapter (*A-1*) entitled *"An Overview of Soleid (Pleuronectiformes) Fisheries and Aquaculture"*, written by the most respected senior scientists working in sole: Bari Rhys Howell and Maria Teresa Dinis. They will stress the commercial interest of this flatfish species, discussing the current fisheries situation with declining catches and the increasing interest for fish farming to supply the market. The chapter introduces *S. senegalensis* as the best candidate because of its faster growth and greater tolerance to captivity conditions. The second chapter (*A-2*) deals with the "*Engineering of Sole Culture Facilities: Hydrodynamic Features*", providing technical advice for the design of aquaculture systems adapted to sole. This chapter highlights important particularities of flatfish and bottom feeding, and the need to optimize space introducing the concept of multilevel layer, flow-throw recirculating systems for intensive on growing facilities. Special attention is paid to tank geometry and water inlet configurations and velocities to safeguard the welfare of fish with bottom and sedentary habits.

Section B begins with 5 chapters devoted to Reproduction (B-1). The first one (*B-1.1*) is entitled "*Sensing the Environment: The Pineal Organ of the Senegalese Sole*", and focusses on phototransduction mechanisms and the key role of the pineal, which is a neural photosensory structure that encodes environmental information into rhythmic neural projections and neuroendocrine (melatonin) signals. This chapter put together current knowledge on the functional neuroanatomy of the pineal organ, the characterisation of its photoreceptor and melatonin-synthesizing cells, and the tract-tracing studies revealing the pinealofugal and pinealopetal projections. The second chapter (*B-1.2*) reviews the "*Neuroendocrine Systems in Senegalese Sole*", and reports as the brain, and particularly the forebrain, plays a central role in the neuroendocrine control of many physiological processes in fish, such as reproduction, feeding, metabolism, growth and stress. This chapter describes how external and internal signals are perceived by fish through specific sensory systems and are integrated into a cascade of neurohormones transported via neurosecretory fibres that enter through the pituitary stalk and are released from axon terminals directly into the surroundings area of the target adenohypophyseal cells. In the third chapter (*B-1.3*), the "*Reproductive Physiology and Broodstock Management of Soles*" are addressed. Different issues are discussed such as the anatomy, morphology and maturation of the gonads, the annual reproductive cycles, the endocrinology of reproduction, sex differentiation and the hormone-based therapies developed to stimulate sole spermiation and spawning. The fourth chapter (*B-1.4*) is devoted to "*Sperm Physiology and Artificial Fertilization*", as most breeders are still wild-caught and gamete management is required for *in vitro* fertilization. Issues regarding factors leading to poor sperm quality will be reviewed and the use of new techniques (apoptosis, DNA damage and transcript analysis) will be discussed. The last chapter (*B-1.5*) focus on the "*Mating Behaviour*" of sole as their reproduction failure in captivity is most likely linked to unpaired behavioural responses of the broodstocks. Courtship patterns and dysfunctions of sole raised in captivity are discussed based on behavioural analysis of different aspects such as dominance, mating systems, genetics, reproductive development, chemical communication and learning.

The next section of the book is related to Early Development (B-2) and comprises 4 chapters. The first one (*B-2.1*) introduces "*The Biological Clock of Sole: From Early Stages to Adults*", starting with the characterization of the molecular clock in Senegalese sole and its development during embryonic and larval stages. The synchronizing effect of light during these early stages is discussed, highlighting its role in the onset of the molecular clock mechanism and gene expression. The second chapter (*B-2.2*) is entitled "*Embryonic and Larval Ontogeny of the Senegalese sole: Normal Patterns and Pathological Alterations*" and considers the main events during embryogenesis, larval development and metamorphosis. The complex flatfish metamorphosis and the changes in the brain, neuroendocrine, bone, digestive, epidermal, cardio-respiratory, hematopoietic, excretory and immune cell-tissues, as well as pigmentary disorders and skeletal abnormalities, will be reviewed.

The third chapter (*B-2.3*) is focused on the "*Effects of Light and Temperature Cycles during Early Development*", as these two main environmental factors synchronizes the clock that triggers hatching rhythms. The underwater photo-environment and the effect of light wavelengths on larval performance are also discussed, as well as the long-lasting effects of daily thermocycles on reproduction rhythms and sex ratio. Finally, the last chapter (*B-2.4*) reviews the "*Larval Production Techniques*", introducing key issues of the production techniques used for Senegalese sole larvae and post-larvae, focusing on rearing systems, feeding protocols and early nutrition.

The following section deals with Nutrition (B-3) and contains one chapter (*B-3.1*) devoted to "*Macronutrient Nutrition and Diet Formulation*". Here, the nutritional requirements of Senegalese sole are discussed in order to design high quality, cost-effective feeds. This chapter reviews major achievements in research on macronutrient requirements and recommended levels to include in diets for juveniles. The feasibility of using new feedstuffs to replace the marine sources by more sustainable aqua feeds is also addressed, with particular focus on plant sources.

Welfare issues will be dealt in this section (B-4) along three chapters. The first one (*B-4.1*) is entitled "*Welfare, stress and immune system*" and looks at current research on the S. sole stress response and the crosstalk with the immune system. This chapter further explores circadian rhythms in the hypothalamus-pituitary-interenal (HPI) stress axis and its connection with the thyroid axis. The second chapter (*B-4.2*) is focused in "*Ecotoxicology*" and reviews the bioaccumulation of chemicals and their effects in S. sole as early-warning biomarkers to monitor water pollution and ultimately human health. The last chapter (*B-4.3*) is devoted to "*Pathology and Diseases Control*", which reviews the main diseases caused by bacteria, viruses and parasites in both farmed and wild sole. Special attention is paid to the particularities of sole and its susceptibility in rear conditions in captivity, suggesting guidelines for disease prevention management.

The osmoregulatory capabilities of S. sole to cope with changes in environmental salinity is discussed in the Section B-5 in one chapter (*B-5.1*) entitled "*Osmoregulation*". This chapter summarizes current knowledge on osmoregulatory tissues (gills, intestine and kidney), ion transport strategies and metabolic changes in sole inhabiting coastal waters and riverine estuaries. The endocrine control (cortisol, growth hormone, insulin/insulin-like growth factors, thyroid and renin/angiotensin system) is also discussed.

The last section is dedicated to Genetics (B-6), which is reviewed in the chapter *B-6.1*, "*Genetic and Genomic Characterization of Soles*". The book ends summarizing the advances made recently in genetic and genomic resources in *Solea*, including main transcriptome assemblies, genetic maps and existing molecular markers for genetic studies. This information is further used to research on epigenetics and the implementation of genetic breeding programs for the sole aquaculture industry.

This book represents a dissemination activity of the research projects CRONOSOLEA (AGL2010-22139-C03), SOLEMBRYO (AGL2013-49027-C3) and BLUESOLE (AGL2017-82582-C3), developed by the editors of this book and funded by the Spanish Ministry of Economy and Competitiveness (MINECO).

Contents

Section A. Fisheries, Aquaculture and Engineering

Section B. Reproduction, Chronobiology, Development, Nutrition, Welfare, Ecotoxicology, Pathology, Osmoregulation and Genetics

Section A

Fisheries, Aquaculture and Engineering

A-1.1

An Overview of Soleid (Pleuronectiformes) Fisheries and Aquaculture

Bari Rhys Howell[1,*] **and *Maria Teresa Dinis***[2]

1. Introduction

Flatfish occur throughout the world and numerous species are caught and highly valued as a source of flavorsome and nutritious food. A high demand, often combined with diminishing resources, results in high prices that make the fish attractive to fishermen and farmers alike. In some parts of the world species of the family Soleidae are among the most favoured. This chapter reviews the status of catch fisheries for these species and the progress made towards increasing supply through the development and application of aquaculture techniques.

Reviews of flatfish catch fisheries are included in a recent publication on the biology and exploitation of flatfish (Gibson 2005). Information on soleid fisheries has largely been sourced from three chapters of that book that comprehensively review Atlantic (Millner et al. 2005), Pacific (Wilderbuer et al. 2005) and tropical (Munroe 2005a) flatfish fisheries. Data on landings have been updated from the most recent FAO fisheries statistics (FAO 2014).

Research on the development of aquaculture techniques for soles has a long history dating back to the beginning of the 20th century. The somewhat spasmodic progress is presented in roughly chronological order together with an explanation of the changing context that motivated the prioritization of the research. This historic account provides the basis for recent advances that have enabled, and will undoubtedly continue to underpin,

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long-awaited commercial developments. These most recent scientific advances are the principal motivation for this book and are described in detail in the following chapters.

2. Fisheries

2.1 Commercial importance of flatfish

Important industrial fisheries for flatfish have become established in areas where larger species are sufficiently abundant. Invariably, national or international management measures have been designed to maintain a maximum sustainable yield. Despite their popularity as food, the contribution of flatfish to global commercial fisheries is relatively small. In 2012, for example, the global capture production of fish, crustaceans, molluscs and other invertebrates was about 92 million tonnes (FAO 2014). Apart from minor fluctuations, this amount has changed little over the past 10 years. Of this total, 12 million tonnes derived from inland waters with the remaining 80 million tonnes coming from the marine fishing areas defined by FAO (Fig. 1). Catches of marine fishes accounted for most (66 million tonnes) of this. The contribution of all flatfish was just under 1 million tonnes, i.e., about 1.5% of all marine fish landed. This was slightly less than the contribution of cod (*Gadus morhua* L.)!

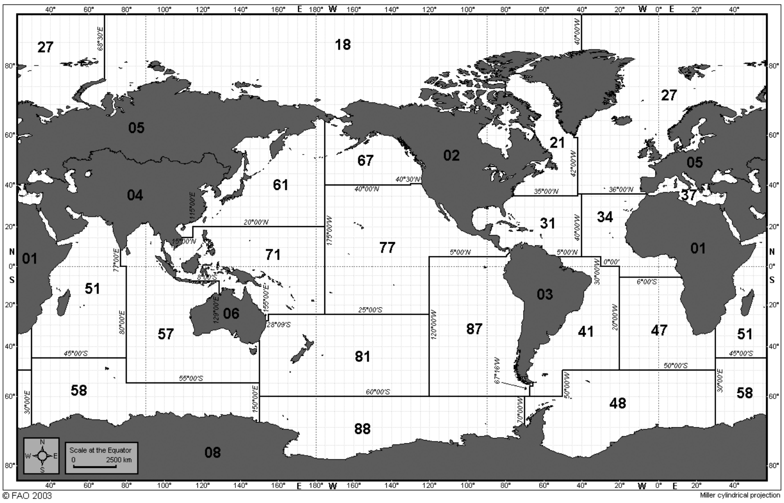

Fig. 1. FAO marine fishing areas. The areas referred to in the text are: 1 (Egypt, Inland Waters), 21 (Atlantic, Northwest), 27 (Atlantic, Northeast), 34 (Atlantic, Eastern Central), 37 (Mediterranean and Black Sea), 41 (Atlantic, Southwest), 47 (Atlantic, Southeast), 51 (Indian Ocean, Western), 57 (Indian Ocean, Eastern), 61 (Pacific, Northwest), 67 (Pacific, Northeast), 71 (Pacific, Western Central).

The significance of flatfish catches expressed in terms of weight somewhat understates their worth because in many instances the value of flatfish per unit weight is considerably greater than that of many other exploited species. Their economic contribution is consequently greater than would appear from weight data only.

2.1.1 Commercial importance of the Soleidae

A brief overview of dominant flatfish families by FAO fishing areas reveals the importance of the Pleuronectidae in world flatfish fisheries. This family is dominant in the north Atlantic and throughout the Pacific. Exceptions include the south-west Atlantic, where

Table 1. Recorded landings of soleid species in 2012 (data from FAO, 2014).

Common name	**Species**	**FAO Fishing Areas**	**Landings (t)**
Common sole	*Solea solea* (L.)	1, 27, 34, 37	32,746
Sand sole	*Solea lascaris* (Risso, 1810)	27, 34	239
Senegal sole	*S. senegalensis* (Kaup, 1858)	27	60
Wedge sole	*Dicologlossa cuneata* (Moreau, 1881)	27, 34, 37	1,198
Thick-back soles	*Microchirus* spp.	27	202
West coast sole	*Austroglossus microlepis* (Bleeker, 1863)	47	1,561
Agulhas or mud sole	*A. pectoralis* (Kaup, 1858)	47, 51	338

paralichthyids dominate, and the south-east Atlantic where soleids, bothids and cynoglossids are the most dominant. However, the most important soleid fisheries occur in the north-east Atlantic alongside pleuronectids and scophthalmids. This apparent restriction of the commercially important soleids to the eastern side of the Atlantic is illustrated in the 2012 catch data for soleid species shown in Table 1. The common sole, *Solea solea* (L.), is the only species for which there is a substantial targeted fishery, which in 2012 yielded about 11% of the total flatfish catch of the northeast Atlantic. The landings of other soleid species in this fishing area were relatively small and were largely by-catches of demersal fisheries targeting other species. This is also the case for the *Austroglossus* spp. fished in the south-east Atlantic which are mainly a by-catch of the productive hake (*Merluccius capensis*, Casteinan) fishery.

This overview of commercial soleid fisheries does not reflect the much wider global distribution of soleids. Although soleids are curiously absent from the west-Atlantic and only occur rarely in the eastern Pacific, the family is widely distributed throughout the Indo-Pacific with maximum diversity in the Indo-Malayan archipelago and north Australian waters (Munroe 2005b). Although the contribution of soleids in these and other areas is largely unquantifiable they undoubtedly have substantial local importance (see below).

2.1.2 Soleids in the northeast and east-central Atlantic, and Mediterranean

Common sole. The common sole is by far the most important commercial soleid in the world and the only one for which there is a targeted fishery. The species is widely distributed from the Trondheim Fjord in Norway southward, around the whole of the British Isles and most of the Mediterranean, to Senegal on the west coast of Africa. Annual landings from 2003 to 2012 ranged from 33 to 42 thousand tonnes (Fig. 2), showing a gradual decline that continued the trend of the 1990s that Millner et al. (2005) described and attributed to over-exploitation.

In 2012, the total landings were just less than 33 thousand tonnes (Fig. 2), the majority of which (73%) was caught in the northeast Atlantic. The remainder was caught in the Mediterranean (16%), the east-central Atlantic (7%) and in Egypt's inland waters (4%). The latter seemingly paradoxical contribution is presumed to have originated from Lake Qarun into which the local sole (named *S. vulgaris* at the time) was introduced in the 1930s and 1940s following salinization of the lake after its disconnection from the freshwater of the Nile (Ishak 1980). Within the productive northeast Atlantic area, the greatest landings were from the south and central North Sea but with significant landings from the eastern English Channel, the Bay of Biscay and the Irish Sea. Although significant, the size of the catch is a poor reflection of the value of the fishery. In 1998, Millner et al. (2005) calculated that, although the total catch of sole was only 10% of the total flatfish catch by weight, it represented 34% of its value.

Beam trawling is the usual form of capture, although there is an important gill net fishery for sole (as well as other species) along the Dutch coast. The seasonal spawning concentrations of sole close inshore is exploited by inshore fishing vessels that use trawls and fixed tangle nets, especially in the English Channel. However, it is the large fleets of trawlers from the Netherlands, Belgium and England that generate the greatest part of the catch through their exploitation of the stocks of the North Sea and the English Channel. The development of intensive targeted fisheries for sole began in the 1950s. Vessels from the Netherlands began using heavy chains to increase the effectiveness of the beam trawls in catching sole and this was accompanied by a rapid increase in the size and power of vessels. By the 1980s these developments had spread to all fleets around the British Isles (Millner et al. 2005).

The decline in stocks since the late 1980s emphasized the need for effective management. This is primarily achieved by regulations governing the gear used and the imposition of quotas that aim to maintain the spawning stock biomass (SSB) above the threshold at which recruitment is impaired, while maintaining fishing mortality below the level that would drive the SSB to that threshold. In 2013, nearly all stocks of sole in the northeast Atlantic were assessed as being fished inside safe biological limits. Only the Irish Sea stock was deemed to be overfished and operating outside safe biological limits. These assessments are made by the International Council for the Exploration of the Sea (ICES) that provides independent scientific advice to countries bordering the North Atlantic, including the European Union (Seafish 2013). In the Mediterranean the abundance of sole is too low to justify a targeted fishery and so its capture is part of a multi-species fishery in which vessels rely on the capture of a large number of species for their income.

Other Soleidae. French, Portuguese and Spanish boats, fishing in the northeast Atlantic, eastern-central Atlantic and Mediterranean land small quantities of four other soleid species which are caught as by-catches rather than targeted fisheries. Of these species the Senegal sole, *Solea senegalensis* Kaup, is of particular interest because it is almost indiscernible from the common sole and the two species may not always be differentiated in areas where their distribution overlaps. This includes the Bay of Biscay, northwest Africa and the western

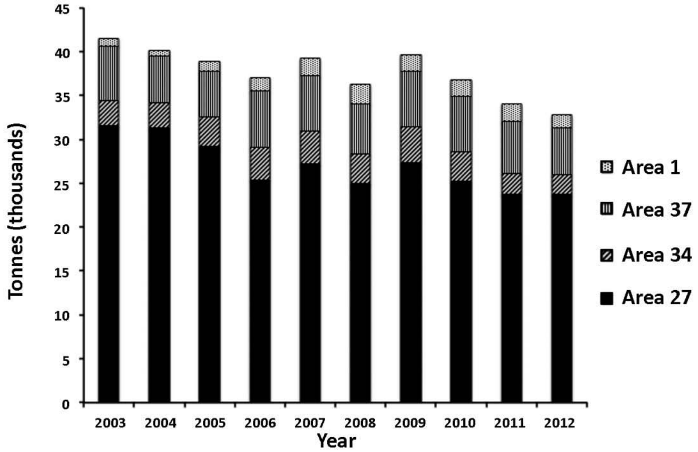

Fig. 2. Landings of common sole, Solea solea, by FAO fishing areas from 2003 to 2013.

Mediterranean. The catch data for these two species in these areas may not therefore be very reliable. In 2012, for example, the common sole catch in the eastern-central Atlantic was reported to be 2,223 tonnes with a further 8,274 tonnes of 'unidentified' soleids, about 70% of which was landed by the Nigerian fleet. Surprisingly, no catches of Senegal sole were reported from this area, the only catch of that species recorded (FAO 2014) was 60 tonnes from French vessels in the Bay of Biscay. It seems possible that the reported catch of *S. senegalensis* has been underestimated either by being grouped with the common sole or by being included in the unidentified Soleidae category.

2.1.3 Soleids in the southeast Atlantic

According to FAO statistics (FAO 2014), *Austroglossus* spp. are the only commercially significant soleids in the southeast Atlantic. The two commercially important species, the west coast sole (*A. microlepis* Bleeker) and the Agulhas or mud sole (*A. pectoralis* Kaup), represent only a small proportion of the total catch as a by-catch of the hake fishery. However, although hake accounts for up to 70% of the catch by weight the soles are by far the most important finfish species because of their higher value per unit weight. They are consequently the main target of small trawlers particularly off the south coast (Diaz de Astarloa 2002).

The west coast sole is distributed from northern Namibia to Cape Town, South Africa while the Agulhas sole is mainly found off the south coast of south and east coast of South Africa. During the 1970s and 1980s annual catches of west coast sole off South Africa were variable but showed peaks of around 2000 tonnes. Landings diminished to virtually zero during the 1990s (Millner et al. 2005) and have not recovered since. This reduction in catches by South African fishermen coincides with Namibia becoming independent in 1990 with the consequence that South African boats were limited to the area south of the Orange river, which marks the border between South Africa and Namibia (Diaz de Astarloa 2002). Landings of this species since that period are now limited to Angola & Namibia but with a significant proportion being caught by Korean Republic fishermen (Fig. 3).

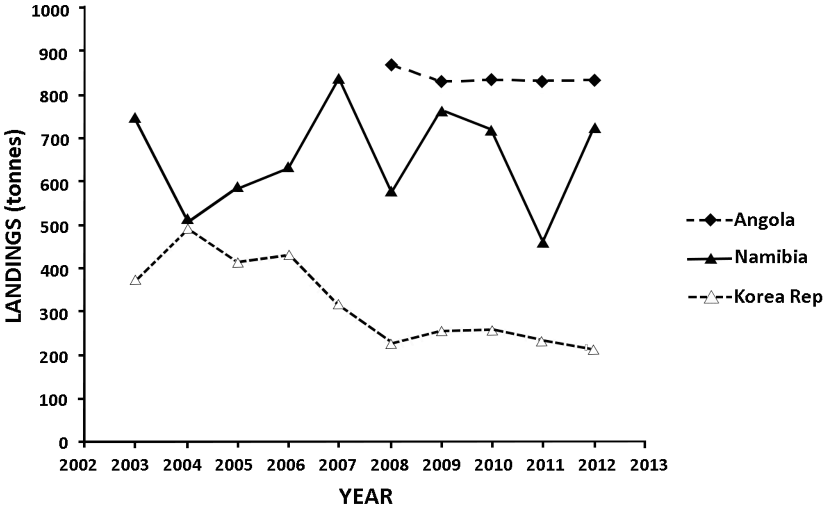

Fig. 3. Landings of the west coast sole from 2003 to 2012.

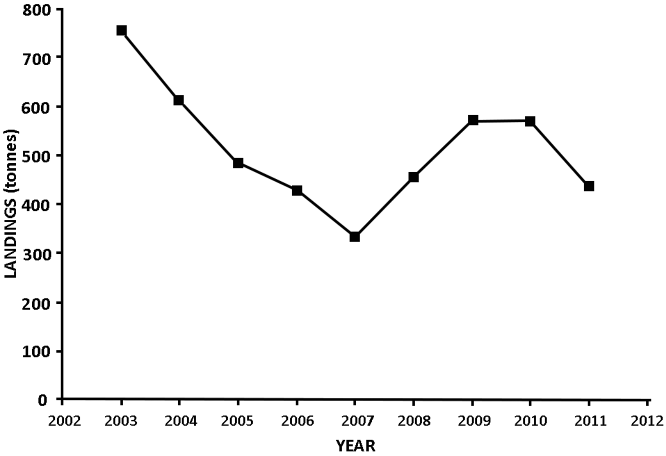

Fig. 4. Landings of the mud sole from 2003 to 2012.

The distribution of the Agulhas sole does not extend to the African west coast and is mainly caught off the south coast of South Africa. During the 1980s and 1990s the annual landings averaged about 850 tonnes (Millner et al. 2005) but from 2003–2012 landings had decreased to an average of about 500 tonnes (Fig. 4). They remain, however, one of South Africa's most valued fish.

2.1.4 Soleids in the tropics

Despite the absence of quantitative data on the commercial value of soleids in tropical waters, Munroe's (2005a) review of flatfish in these areas provides some quantitative evidence of their value.

Flatfish are common in suitable soft-bottom habitats but most are too small and too sparsely distributed to be directly targeted as a commercial fishery. Larger fish caught as a by-catch can be marketed for human consumption and provide an important source of nutrition in areas where they are abundant. In the coastal fisheries of Indo-west Pacific, several soleid genera are prominent including *Solea* spp., *Synaptura* spp., *Brachirus, Zebrias* spp. and *Pardachirus* spp. (Table 2). In the estuarine waters of the Indo-West Pacific, the ovate sole, *Solea ovata* Richardson, also has some prominence as does the black sole, *Achlyopa nigra* Macleay, whose distribution extends to the Northern Australian estuaries.

The type of fisheries ranges from traditional subsistence and artisanal fisheries to large-scale industrial fisheries. Subsistence and artisanal fisheries play a major role in providing food for local communities as well as an occupation for a high proportion of the population.

The fishing gear used in the tropics is similar to that used elsewhere for flatfish. Beam trawls and other benthic trawls are used in deep waters and mini-trawls, barrier or stake nets, seines, traps and many other gears are used in shallow water. In African waters, artisanal canoes are the most important part of the fishing fleet with the fishermen landing more fish (50–90% of the total landings) than the industrial fisheries in their region. The industrial trawl fisheries use large boats with modern equipment targeting specific or groupings of fish. Unlike their more traditional subsistence and artisanal counterparts, the majority of the by-catch is invariably discarded or processed into fishmeal or other products.

Table 2. Examples of soleid species marketed from by-catch in the Indo-west Pacific (compiled from Munroe 2005).

FAO fishery area	Species
Oriental sole	*Brachirus orientalis* (Bloch & Schneider 1801)
Elongate sole	*Solea elongate* (Day, 1877)
Convict zebra sole	*Zebrias captivus* (Randall, 1995)
Indian zebra sole	*Zebrias synapturoides* (Jenkins, 1910)
Finless sole	*Pardachirus marmoratus* (Lacepède, 1802)
Stanaland's sole	*Solea stanalandi* (Randall & McCarthy, 1989)
Commerson's sole	*Synaptura commersonii* (Lacepède, 1802)

In these tropical areas landings of soleids, as for most flatfish, cannot be estimated with any degree of accuracy mainly because only a small proportion of catches is identified to species level. Even the proportion identified to family level is relatively small. Landings of all flatfish, however, though variable, have increased during the last decade. In the Indo-Pacific region, for example, catches increased from about 45,000 tonnes in 2003 to 65,000 tonnes in 2012. The financial contribution of soleids is impossible to estimate, but in terms of the provision of nutritious food is likely to be significant in local areas.

3. Aquaculture

3.1 Early pioneers

The development of farming techniques for flatfish has had a long and sporadic history since its origins in the latter half of the 19th century. The 'early hatchery movement', as it was known and described in some detail by Shelbourne (1964), aimed to arrest the decline of natural stocks by the release into the sea of countless millions of eggs and yolk-sac larvae. By the 1930s this approach to fish stock management had fallen into disrepute having failed to demonstrate the efficacy of such a practice, but the demonstration that marine fish eggs could be fertilized successfully and hatched in extremely large numbers did, however, lay the foundations and inspiration for subsequent work on the rearing of marine fish larvae, including those of soles. The first reported success was that of Fabre-Domergue and Biétrix (1905), working at the Concarneau Laboratory in France. They reared common sole from eggs through to the completion of metamorphosis in 50-litre barrel-shaped glass containers. The larvae were fed first on a flagellate culture followed by plankton collected from local rock pools.

Further attempts to rear any marine fish beyond the yolk-sac stage generally foundered on an inability to provide the larvae with adequate nourishment. The discovery by the Norwegian biologist Rollefsen (1939) that the nauplii of the brine shrimp, *Artemia salina* (L.), were a suitable food for plaice larvae was therefore of paramount importance to the subsequent development of rearing methodologies. It was not until the 1960s, however, that the impact of this discovery was fully realized when Shelbourne (1964) demonstrated that larval plaice, *Pleuronectes platessa* L. could be mass-reared using brine shrimp nauplii as food. In addition to meeting the nutritional requirements of the larvae he attributed his success to the use of good quality eggs from stocks acclimated to captive conditions, the control of bacterial infestation of the eggs with antibiotics, the provision of high light levels and black tanks to promote a good feeding response, and the maintenance of high standards of hygiene.

The methodology developed for plaice was readily adapted to the common sole with appropriate adjustments to water temperatures (Shelbourne 1975). In small-scale experiments using 35 l tanks up to 80% of eggs survived to the completion of metamorphosis with up to 90% normal pigmentation. Encouraged by these results Shelbourne undertook larger scale trials in 6 m^{-3} tanks and achieved a survival rate of just over 40%, normal pigmentation of more than 95% and a final survivor density of four to five thousand juveniles per m^2 at an average length of just under 2 cm.

At that time these unequaled achievements were regarded with considerable global interest and were the stimulus for work on a wide range of species. There is certainly no case for detracting from Shelbourne's achievements but, in retrospect, he seems to have been somewhat fortunate in his choice of species, i.e., the plaice and common sole. Firstly, the larvae of these species are relatively large-mouthed and, in contrast to many other species of commercial interest, such as the turbot, *Scophthalmus maximus* (L.), and lemon sole, *Microstomus kitt* (Walbaum), are able to ingest brine shrimp nauplii as a first food. It was sometime later that the rotifer, *Brachionus plicatilis* Müller, was subsequently identified as a suitable first food for most smaller-mouthed species and was also shown to be a suitable alternative for *Artemia* nauplii for sole (Howell 1973). Secondly, and perhaps most importantly, neither of these two organisms form part of the natural diet of marine fish larvae and it was only after it was appreciated that these prey organisms were deficient in certain essential fatty acids (EFAs) that the rearing of some of the more valuable flatfish species, such as the turbot and the Japanese flounder, *Paralichthys olivaceous* Temminck & Schlegel, was accomplished. Success with these species depended on enriching the EFA content of the prey organisms offered by first feeding them on algae rich in these substances (Watanabe et al. 1978, Howell 1979). Fortunately for Shelbourne, the requirement of the sole (and of the plaice) for these EFAs appears to be less stringent than for many other species, high survival through the larval stages being achievable without any such enrichment of the prey organisms. However, enriching the EFA content of the live food fed to sole larvae has subsequently been shown to have other important beneficial consequences (see below).

The vast majority of subsequent studies on soleids remained focused on the common sole although in the last decade or two the emphasis has strongly shifted to its more southerly counterpart, the Senegal sole. Some studies have been undertaken in South Africa on the white margined sole, *Dagetichthys marginatus* (Boulenger), but as yet there is no record of any commercial developments (Ende and Hecht 2011).

3.1.1 Further development of rearing techniques for the common sole

The ability to mass-produce juvenile fish not only met the pre-requisite for stock enhancement trials as envisaged by Shelbourne (1975), but stimulated a considerable interest in the possibility of on-growing the juveniles to market size in land-based systems. In colder countries, such as the UK, the possibly of promoting faster growth rates by using a continuous supply of warm water generated by nuclear power stations was also considered. The common sole was one of many target species identified because of their high market value and depressed availability through over fishing.

Further research on the common sole was greatly facilitated by the ability to provide a regular supply of naturally spawned eggs from captive stocks of 'wild' fish fed natural feeds (Baynes et al. 1993). Larvae were reared using techniques largely based on those developed by Shelbourne (1975) with the exception that eggs were generally incubated without antibiotics but in containers gently agitated by aeration and continuously irrigated with a continuous flow of fresh seawater. Research focused on the principal requirements of an intensive farming approach, namely, formulated feeds for weaning and on-growing, good growth rates under crowded conditions and effective protocols for disease prevention and control.

Weaning and on-growing. Initially, juvenile plaice and sole of about 1 g in weight were successfully weaned from brine shrimp nauplii on to another live food, the oligochaete worm *Lumbricillus rivalis* (Levinsen) (Kirk and Howell 1972). These were abundant in rotting seaweed in the littoral zone but, although ample quantities could be gathered to support laboratory experiments, securing the quantities needed for commercial-scale production was not feasible. The use of formulated feeds was therefore the only realistic option but this proved to be much more challenging for sole than for other species such as turbot. Results of weaning trials were highly variable, usually yielding relatively low rates of survival and growth (Imsland 2003). This apparent dislike of fish-based feeds probably reflected the fact that the natural diet of sole is comprised of invertebrates and, as a nocturnal species, olfaction is a likely to be a dominant factor in prey selection. This was experimentally demonstrated by Mackie et al. (1980), who identified the precise chemical attractants (glycine betaine and certain L-amino acids) in mussel flesh that induced a strong feeding response. Subsequently, Cadena Roa et al. (1982) evaluated the use of these attractants in formulated feeds and found they elicited a greater response with corresponding higher survivals and growth rates than supplements of ground molluscs, polychaetes or brine shrimp. Significant progress had been made, but further advances were needed to secure the prospects of commercial farming (see below).

The effects of crowding on growth. Tolerance to crowding is an essential requirement for economic viability of intensive systems, the productivity per unit area being a function of both mean growth rate and the density at which the fish are stocked. High stocking densities can have adverse effects on growth through reduced water quality but in common sole it has been shown that even when such conditions are negated growth rate may still significantly diminish with increasing stocking density (Fig. 5). Such a decline in growth rate was not observed in a similar experiment with turbot (Howell 1998).

These results were interpreted as reflecting the different natural behaviour of the two species. Sole take food from the bottom, rather than the water column, and their intestine is adapted to eat small but frequent meals, rather than just one or two per day. These behavioural differences, coupled with the tendency of the oligochaete worms on which they were fed to form clumps, would create a situation in which a proportion of the stock could dominate the food supply either by active aggression or passive inhibition. This would suggest that any tendency for negative interactions within communal populations might be overcome by adopting appropriate feeding strategies. More recent studies (Schram et al. 2006, Lund et al. 2013) also found a negative correlation of stocking density with growth rate. However, their results differed in some important respects. Schram et al. (2006) obtained a positive correlation between size variation and stocking density implying that larger fish were growing faster than smaller fish. In contrast, Lund et al. (2013) found no such correlation, fish of all sizes growing at the same rate at each density. They concluded that at increased densities growth was depressed primarily due to a lower feed intake of all individuals, but also to less efficient use of the feed. The latter may have arisen from increased activity from social interactions and feed browsing, and possibly even from chronic stress.

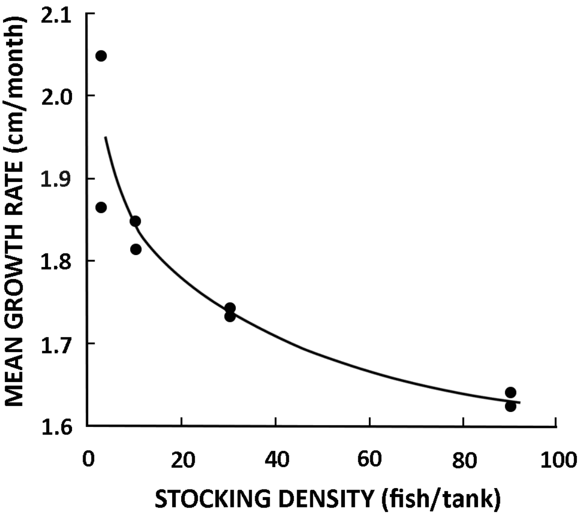

Fig. 5. The relationship between stocking density and growth rate of common sole on an ad libitum diet of the oligochaete worm Lumbricillus rivalis (from Howell 1998).

It seems clear that stocking density in common sole is an important determinant of growth rate although there is still some scope for conjecture regarding the behavioural mechanisms involved and hence the feasibility of minimizing these effects by adjusting rearing procedures.

Disease prevention and control. Disease rapidly becomes an issue with all species subjected to intensive rearing conditions. This proved to be the case in large-scale trials undertaken by the British White Fish Authority (now the Seafish Authority) at their site in Hunterston, Scotland. Juvenile sole were being weaned from brine shrimp nauplii on to either oligochaete worms or formulated feeds with some success until they experienced heavy mortalities caused by a disease known as Black Patch Necrosis (BPN). The condition was described by McVicar and White (1979) and caused by *Flexibacter maritimus* (Bernadet et al. 1990), now known as *Tenacibaculum maritimum.* Outbreaks devasted stocks and the only effective means of control appeared to be the provision of a sand substrate in which the fish could bury (McVicar and White 1982). This experience encouraged the view that sole was susceptible to disease unless cultured on a sand substrate, a requirement that would be a major impediment to the effective cleaning of tanks and the maintenance of hygienic conditions. Subsequent work (Baynes and Howell 1993) promoted a more optimistic view. In a small-scale experiment to assess the relative value of cooked and stored mussel with fresh mussel as food for juvenile sole, an outbreak of BPN appeared only among groups that received less than 2 feeds of fresh mussel per week (Fig. 6). The disease didn't spread to other groups despite the proximity of the tanks and the lack of stringent precautions to contain it. This experience suggested that a combination of a nutritious diet and hygienic conditions may be sufficient to avoid the occurrence of BPN without the provision a sand substrate. Common sole are, of course, susceptible to other diseases, for example vibriosis, but there is no evidence that the species is more vulnerable to disease than other species when subjected to appropriate rearing protocols.

New directions. Despite some positive outcomes, studies in these key areas did little to strengthen the belief that the intensive farming of sole was an economically viable option,

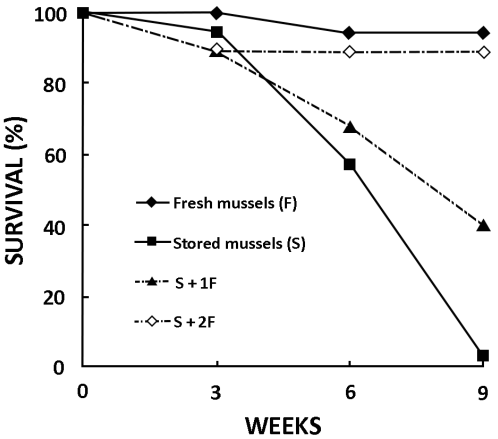

Fig. 6. The survival of groups of sole fed fresh mussel (F), cooked mussel (S) and cooked mussel with one (S + 1) or two (S + 2) feeds per week of fresh mussel (Redrawn from Baynes and Howell 1993).

other species, such as the turbot (*S. maximus*), appearing to be a more attractive candidate. By the onset of the 1980s the challenging problems of rearing turbot through its larval stages had largely been overcome and a dependable supply of juveniles could be assured. In contrast to sole, high juvenile growth rates of turbot could be achieved at high stocking densities on fish-based formulated feeds. The focus of commercial and research effort consequently shifted to turbot.

In the UK there was a further change of direction towards the end of the 1980s. Renewed concern about the continued decline of natural fish stocks rekindled interest in the possibility of arresting these trends by enhancing natural recruitment with hatchery-produced fish. This notion was encouraged by the considerable effort the Japanese had invested in this subject with an encouraging degree of success (see, for example, Howell and Yamashita 2005). In the UK, the sole was seen to be a suitable target because of the high value of the fishery and the relative ease of rearing the larvae beyond metamorphosis in large numbers.

Japanese and other studies suggested that, because of diminishing predatory pressures as fish grow, reared fish would need to be grown to a length of at least 5 cm before release in order to achieve a favourable return. It had also been established that the post-release survival of reared fish was significantly less than their wild counterparts (see reviews by Howell 1994, Olla et al. 1994). New research programmes were therefore focused on (1) developing methods for growing large numbers of metamorphosed juveniles to a length of at least 5 cm, and (2) assessing the suitability of reared fish for survival in the sea.

Further weaning and on-growing developments. Weaning sole onto formulated feeds under laboratory conditions had proved relatively successful but success on a commercial scale had not been demonstrated (Day et al. 1997). Apart from the importance of chemo-reception in stimulating a feeding response, the digestive system of the sole significantly differs from that of other candidates for farming. In particular, pepsin activity is not detected before day 200 and this is likely to impair the efficient digestion of certain feed components. For this reason, they assessed the effect of incorporating an enzymatically-hydrolysed fish meal into a weaning diet for 3 cm-long juvenile sole that had been reared on brine shrimp nauplii alone. The results were quite striking. The survival of the juveniles was directly dependent on the content of hydrolysed fish protein content (HFPC). Despite the inclusion of betaine-glycine to improve the attractiveness of the diets, increasing the HFPC content from 0 to 80% resulted in a progressive increase in survival from just over 40% to more than 75%, a survival rate approaching that of the control diet that consisted of high levels of invertebrate tissue.

Day et al. (1997) postulated that, although the hydrolysate may have increased the attractiveness of the diets over and above that of the added attractants, it was also possible that the more readily digested HFPC resulted in higher assimilation rates at a time when ingestion rates were relatively low. Such effects had been reported in European seabass, *Dicentrarchus labrax* (L.), by Cahu and Zambonino Infante (1995).

These trials were followed by weaning trials to evaluate a commercial larval feed produced by a process of agglomeration by the Norwegian Herring Oil and Meal Industry Research Institute (SSF), Fyllingsdalen, Norway (Day et al. 1999). The incorporation of water-soluble components into small-diameter feeds presented problems of particle stability and water pollution due to excessive leaching. SSF provided a technical solution that allowed high levels of soluble protein (30% of total protein) to be incorporated into small diameter feeds (100–2000 µm) while maintaining particle stability. This diet was customized for sole by the inclusion of a feeding attractant (betaine) at 6% of dry weight and by increasing the water-soluble protein to about 30% of the total protein. This allowed newly-metamorphosed sole weighing about 30mg to be weaned with a survival rate over 90% and with growth rates exceeding that on live foods. Weaned juveniles were successfully transferred from the agglomerated feed to a more conventional pellet with a 100% survival and without loss of growth rate. This positive result provided an effective means of growing juveniles to an appropriate size for stock enhancement purposes as well as greatly enhancing the prospect of developing viable intensive farming methods.

Fitness of hatchery-reared fish for survival in the sea. Morphological, such as abnormal pigmentation, and behavioural impairments, particularly those related to feeding and predator avoidance, had been shown to be among the major determinants of the post-release survival (Howell 1994, Olla et al. 1994) of hatchery-reared fish. Less predictable was the discovery in the common sole that temperature tolerance of juveniles was significantly influenced by nutritional factors during the larval stages. This was discovered when trials designed to determine the tolerance of reared juveniles to a winter temperature profile that simulated that in areas where they may be liberated produced widely differing results in consecutive years (Howell 1994). These conflicting results were subsequently experimentally demonstrated to be attributable to the poly-unsaturated fatty acid content of the live food fed to the larvae rather than that of the subsequent on-growing feed (Fig. 7) (Howell et al. 1996).

Further work on other environmental factors, including salinity, high temperature and hypoxia, concluded that susceptibility to environmental stress was responsive to dietary n-3 manipulation, possibly due to altered tissue development or the overproduction of eicosanoids (Logue et al. 2000).

The practical implication of these studies is that survival during the larval stages is not necessarily a good indicator of subsequent 'hardiness'. This would have important implications if the fish were destined to be released into the sea but might also have a detrimental impact in more controlled intensive farming systems in which fish are exposed to other stressors such as handling and crowding.

Other studies generated further evidence of the importance of morphological and behavioural factors in determining the survival of reared fish released into a natural environment (Ellis et al. 1997). Post-release survival largely depends on evasion of

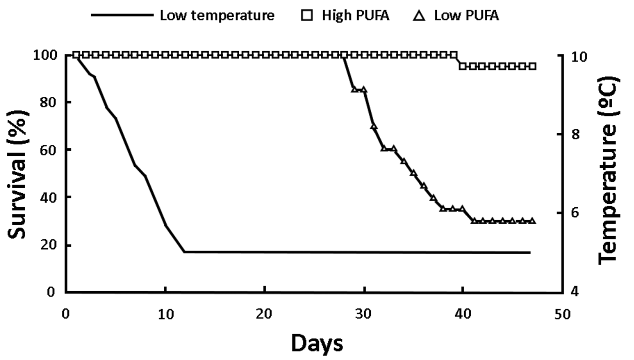

Fig. 7. The effect of low temperature (continuous line) on the survival of 8–10 cm long sole which had been reared to a length of 3 cm on Artemia nauplii of high (squares) and low (triangles) PUFA content (from Howell et al. 1996).

predators either by avoiding discovery or by escape. Sole, like many other flatfish, inhabit soft substrata largely devoid of vegetation. They adapt their skin colour to that of their surroundings and bury in the sediment to further reduce detection. The hatchery in which the fish are raised provides a very different environment with no sand substrate in which they can bury and tank colour and lighting that combine to induce a totally different skin colour to that of their wild counterparts. Their early life in hatcheries is also predator free and the fish will have become habituated to handling and presence of humans. Ellis et al. (1997) demonstrated that naïve (reared) sole are significantly disadvantaged with regard to factors such as burying ability and colour. The differences did, however, diminish when exposed to more natural conditions indicating that exposure to suitably designed pre-conditioning regimes for up to two weeks would enhance post-release survival.

3.1.2 Development of rearing methods for Senegal sole

Interest in the laboratory rearing of *S. senegalensis* began in the 1980s in Spain and Portugal where techniques for rearing other species of marine fish, particularly seabass, *Dicentrarchus labrax* (L.), and gilthead seabream, *Sparus aurata* L., were already quite advanced and showing considerable commercial promise. Work on the early life stages revealed considerable similarity to the common sole, although, as would be expected from their geographical distribution, the Senegal sole was adapted to higher temperatures regime. As with the common sole, a reliable supply of naturally-spawned fertilised eggs could be obtained from captive stocks fed on 'natural' food (squid and polychaetes in this case) and survival rates of larvae fed on brine shrimp nauplii and metanauplii to the completion of metamorphosis were similarly high (Dinis et al. 1999). Rotifers offered for the first few days of feeding were consumed but it was shown this did not improve survival or growth (Magalhães and Dinis 1996).

The reluctance of juveniles to accept formulated foods also reflected that of the common sole. The problem was to a large extent relieved by employing the same methods used for common sole but further advances have been made more recently by Engrola et al. (2009), who found that offering an inert diet at first-feeding together with brine shrimp nauplii promoted growth and better quality juveniles.

Progress was also made in the development of on-growing diets. For example, Coutteau (2001) (cited by Imsland et al. 2003) importantly demonstrated that formulations developed for other species were not necessarily optimal for sole. In terms of growth and feed conversion efficiency, sole performed better on a specially formulated feed with a crude protein to crude fat ration of 55/16 than on a standard commercial turbot feed with a protein/fat ratio of 52/20, though other factors, such as feed attractability, may have been involved.

Disease is a common feature of all cultured fish and the Senegal sole is no exception. Like the common sole, the Senegal sole is susceptible to tenacibaculosis (also know as fin-rot or Black Patch Necrosis) and vibriosis. Senegal sole is also susceptible to photobacteriosis, caused by a particularly virulent pathogen, *Photobacterium damsela* ssp. *piscicida*, which causes high losses in many cultured species, such as gilthead seabream (Morais et al. 2014). Several sole farms in the south of Spain have suffered high mortalities from this pathogen. Isolation from other fish species that are vulnerable to this disease is helpful as is the selection of sites or systems where temperatures do not rise above 22°C (Cañavate 2005).

Important differences between the species include that of their response to stocking density. Morais et al. (2014) observed that most studies reported that growth in Senegal sole is not affected by high stocking density and frequent handling stress, citing the work of Aragão et al. (2008), Costas et al. (2008, 2012, 2013) and Salas-Leiton et al. (2010). This would be a favourable trait in an intensive farming environment and is in contrast to the studies on the common sole cited above.

Growth rate is a key determinant of economic viability, and in this regard the Senegal sole would seem to have a significant advantage over common sole. Growth data to market size is sparse in the literature for both species. Howell (1997) used published experimental data to estimate the time required for common sole to reach the minimum market size of 24 cm (125 g) at a near optimum temperature of 19°C. The data indicated it would take 300 days, though different growth rates of the two sets of data used suggested that could have been an over-estimate. Nevertheless, that is a considerably slower growth rate than that of Senegal sole grown in an earthen pond at a naturally fluctuating temperature ranging from 15 to 24°C (Dinis et al. 1999). The fish were fed pellets but benthic invertebrates, notably polychaetes, were probably also exploited. After one year the fish had grown to a mean length of 35 cm (456 g). This evidence does suggest that Senegal sole may have a considerably faster growth rate than the common sole.

3.1.3 Further advances

By the end of the 20th century, the developments in feeding technology that facilitated ready transfer from live to formulated feeds with consistently good survival and growth rates encouraged industry to believe that the main obstacle to the farming of soles could be overcome. This coincided with saturated markets for established farmed marine species, such as turbot, seabass and gilthead seabream, and the desire of industry for further diversification. In an attempt to avoid further ‘false dawns’ in 2002 a workshop was organized at the CEFAS Laboratory, Weymouth, UK to provide an opportunity for researchers and commercial operators who had experience of working with soles to form a considered view of the potential for farming these species and to identify the obstacles that would still need to be overcome. The group reached a positive view and, because of the perceived usefulness of such a forum, four more workshops were held over the next 10 years, each one reassessing the status of the industry, its problems and the most recent research aimed at alleviating those problems (Howell et al. 2006, 2009, 2011). This period saw a significant shift in emphasis in research from the common sole to the Senegal sole as it became clear that the latter species offered the greater prospect of commercial viability. Comprehensive reviews of that research are presented elsewhere in this book.

3.1.4 Application of rearing technology

Stock enhancement. Shelbourne's (1964) main aim in developing mass-production techniques for flatfish was to enhance natural recruitment to depleted stocks with hatchery-reared juveniles. There had been no convincing evidence of the benefits of releasing eggs or yolk-sac larvae but the success of transplantation experiments using larger wild fish encouraged the belief that releasing older life stages when larger and less vulnerable to predation and starvation would be more productive (Shelbourne 1975). Shelbourne did not undertake any releases of sole but in 1964, large numbers of juvenile plaice were released in two sites off the west coast of Scotland. All the fish were lost, either through predation or other reasons (Blaxter 2000). Blaxter (2000) implied better results may have been obtained had there been some consideration of carrying capacity, predator abundance, optimal conditions for release and the fitness of the reared fish for release into the wild. There are no recorded releases of sole at that time or since, despite the vast amount of information now available to support the construction of appropriate protocols. Although catches of sole in the north-east Atlantic in 2012 were 44% less than in 1967, the preferred remedy remained the traditional management practice of limiting fishing mortality principally through quotas and gear restrictions. To attempt to make good such a deficit by the release of reared fish would certainly be a formidable challenge and highly expensive.

Extensive and semi-intensive cultivation. It has been recently estimated that there are over 92,000 ha of coastal wetlands in southern Europe that are already being used for extensive and semi-intensive production (Anras et al. 2010). Total fish production from these is far from trivial, Portugal, Spain and Italy collectively producing a total of about 13,000 tonnes per annum. About 77% of this production comes from semi-intensive systems. Detailed statistics for individual species are not available but sole is one of the main species listed for both Portugal and Spain (Anras et al. 2010).

Statistics for aquaculture production of soles (FEAP 2015) for the period 2005 to 2014 shows that production for Portugal and Spain was relatively constant from 2005 to 2008 (Table 3). Thereafter, Spanish and French production dramatically increased reflecting the onset and growth of intensive production. It may be deduced, therefore, that the 2005–2008 production is an approximation of production of soles from extensive and semi-intensive systems, i.e., 70–90 tonnes per annum.

Spain and Portugal have a long tradition of extensive cultivation in the now largely deserted salt ponds ('esteros' in Spain) of their southern coast. Yúfera and Arias (2010) describe the annual cycle that begins after the harvest at the end of autumn. The pond-monks are left open for several months so that the tidal flow of water will populate the pond with larval fish and other organisms. After this period of natural recruitment the pond-monks are kept closed except for periodic exchanges of water undertaken with netting installed in the monks to prevent escapes. The fish trapped in the pond feed entirely on natural food production within the pond until they are captured at the end of autumn. The catches mainly comprise mullets, seabass, gilthead seabream and sole with a small amount of eels and crustacean (crabs and shrimps). In a study in the Bay of Cádiz, the production after one year was 320 kg/ha of which only 5.1% was crustacea and 0.2% fish of no commercial value, a reflection of the high productivity of the area (Yúfera and Arias 2010).

Table 3. Annual aquaculture production of soles (tonnes) for the period 2005 to 2014 (FEAP 2015).

	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014
France	0	0	0	0	0	142	200	220	223	261
Portugal	11	9	8	13	14	14	50	100	35	60
Spain	60	80	60	55	180	204	110	194	313	786
Italy	0	0	0	19	14	14	10	0	0	0
TOTAL	**71**	**89**	**68**	**87**	**208**	**232**	**170**	**294**	**348**	**846**

Productivity is, of course, a key issue with farmers and the profitability of totally extensive aquaculture is highly marginal. Following successful trials in the mid-1990s many farmers converted to semi-intensive cultivation systems that deliver greater productivity and profitability (Anras et al. 2010). The main target species were seabass and gilthead seabream. Senegal sole occur in these ponds either having been carried in naturally or released after purchase from local hatcheries. They feed on the natural benthos and on any formulated feed left by the target species and may reach a size of about 400 g after 16 months (Morais et al. 2014). The use of reared sole is not, however, always successful. Failures have been attributed to stocking fish of too small a size (< 0.2 g) before they were weaned coupled with a failure to rid the pond of predators prior to release. Releasing the fish at a larger size and sun-drying the pond before use could avoid this problem (Imsland et al. 2003). As previously indicated, a degree of conditioning of the fish for release may also prove beneficial.

Intensive production. Extensive and semi-intensive systems have considerable value in terms of exploiting a natural resource in a sustainable way and providing a living for coastal communities. However, productivity is relatively low and outputs can be uncertain, being dependent on the vagaries of nature, so that the demands of the market cannot be fully met. To a large extent intensive cultivation may overcome these problems because the environment in which the fish are reared can be almost completely controlled. As the obstacles of rearing soles through the whole of their life history have been overcome, industry has progressively shown interest in adapting and developing systems that would permit the intensive production of the species.

Intensive farming is normally undertaken in fibre-glass, concrete tanks or shallow raceways, the latter of which one would naturally presume to be appropriate for flatfish, tank area having more importance than volume (Imsland et al. 2003). Producers have become attracted to these systems, often in conjunction with recirculation systems that, with the advantages of modern technology, enable a high degree of environmental control. There is no doubt that the use of these systems has brought about a dramatic improvement in disease control by both eliminating contact with other fish species and providing the required environmental control (Morais et al. 2014). Over the last 5 years the annual production of farmed soles has steadily increased and by 2014 had almost reached 1000 tonnes. Problems still have to be overcome but the sound scientific support that has been established and the experience and expertise of the industry will undoubtedly combine to secure the future of this relatively novel industry.

4. Markets

The demand for soles in Europe is widespread, especially in its coastal countries. The common sole is the most abundant species but, as pointed out above, other species, notably the Senegal sole, are also highly regarded and often not distinguished from the common sole in the market place.

The natural distribution of sole does not coincide with the pattern of its demand. In 2012, for example, the Netherlands and France caught about 50% of the total landings of the common sole with Belgium and the UK landing a further 15%. Spain and Italy, two countries with a high demand for soles, captured only 1 and 6% of the total catch respectively (FAO 2014) and consequently had to import much of their requirement from more northern countries. This follows a general trend for demersal fish as a whole in this region, many of which are competitors with sole in the market place (Bjørndal and Guillen 2014). In this case the principal 'producers' are Norway, Denmark and the Netherlands with the first two countries being the only countries that export significantly more fish than they import. The reverse is true for all other European coastal countries, especially Spain and Italy where the demand is high. In the case of Italy, 85% of their total supply of demersal fish is imported (data from FAO 2014). These are of course general trends and the situation for different species may vary. The UK, for example, is a net importer of demersal fish as a whole but a net exporter of the common sole (UKFS 2014), although the quantities are relatively small. In general, there is a significant shortfall in the supply of demersal fish, including soles, particularly in southern European countries and so there is almost certainly a ready market for any aquaculture production given the constraints of price and quality.

The role of aquaculture as a source of supply has become an attractive proposition in Spain as it has a favorable climate for the preferred species (Senegal sole), a consumer demand that greatly exceeds the country's natural resources, and a substantial body of research and industrial expertise that has become established through the development of its existing aquaculture enterprises. The over 40% reduction in sole catches from 1995 to the present has added to the pressure for, and commercial viability of, such innovations. An important consequence of the decline in catches is the accompanying decrease in average size of the fish. The restaurant sector favours large fish (400–600 g) and this provides a good market opportunity for the aquaculture industry if it can economically produce fish of that size, or larger (Bjørndal and Guillen 2014).

MercaMadrid, one of the largest fish markets in Europe, categorizes sole as small (< 0.5 kg), medium or large (> 1 kg). Over the period 2002–2013 the price of these size categories have ranged from 7–12, 13–18 and 18–25 €/kg respectively showing that large fish attract a price up to double that of small fish. The value of frozen fish can be up to 5 €/kg less than that of small fish. Farmed fish have been on the market for only a short time but the price was closer to that of small fish than that of medium fish, possibly because the fish were less than 0.5 kg in weight (Bjørndal and Guillen 2014).

Farmed fish bring the benefits that they are fresh, devoid of damage due to the rigours of capture, and their availability and size can, to a large extent, be reliably tailored to meet the demands of the market. Despite these advantages there is a tendency for the consumer to prefer 'wild' fish, being less comfortable with a 'novel' fish that may differ in appearance, such as its shape or colour, to its wild counterpart. Experience with other farmed species suggests that such prejudices do diminish with time, providing the product being presented has the necessary eating and safety qualities and is produced by ethical methods.

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A-1.2

Engineering of Sole Culture Facilities

Hydrodynamic Features

Joan Oca* **and** ***Ingrid Masaló***

1. Specific constraints in the design of sole culture facilities

In intensive land-based aquaculture, tank design should be adapted to the way species behave and swim while also reducing stress levels and improving fish welfare, which in turn contributes to enhancing its growth (Palstra and Planas 2011).

Sole has been described as a fish with low growth rates (Mas-Muñoz et al. 2011), presenting a high size dispersal when cultured. The low growth observed with sole and the high cost of the land surface makes it necessary to culture them in intensive systems, where growing technologies have to be adapted to their specific needs and behavior. Being one of the most sedentary flatfish species, sole rests on the bottom for long periods and shows a natural tendency to grouping when cultivated in a tank. Moreover, it feeds strictly from the bottom (De Groot 1971) and is adapted to eating small but frequent meals. Sole activity is limited to moving slowly over short distances across the bottom when they detect food; thus there is a time lag between when they detect and eat the food. The low swimming activity of sole has allowed development of specific techniques for determining sole biomass in tanks with laser scanning (Almansa et al. 2015) and for compiling a sole activity index with image analysis techniques (Duarte et al. 2009).

This chapter presents an analysis of growing technologies, tank geometry and hydrodynamic conditions in order to provide some specific guidelines for the design and management of sole facilities.

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2. Growing technologies

Solea senegalensis used to be cultivated in salt marshes that were associated with salt works in the south of Spain and Portugal. They were kept in polyculture with mugilids, sea bass and sea bream. With the decline of the salt industry over the 20th century, many ponds were partially adapted to extensive fish farming systems (Yúfera and Arias 2010).

Recent advances have been made in the knowledge of the reproductive biology and behavior of Senegalese sole in captivity as well as in their specific nutritional requirements. This has allowed the regular production of fry and the formulation and commercialization of improved species-specific diets.

These improvements have led to production systems undergoing major changes as they shifted from predominantly earth ponds or salt marshes with water renovation provided by tidal energy to intensive flow-through systems in fiberglass or concrete tanks that use a pumping system to supply a continuous water flow passing a single time through the facility before being discharged to the sea. Flow-through systems have enabled improvements with disease issues on sole farms by eliminating contact with other fish species, increasing the stocking densities and enabling greater control over environmental parameters.

The high cost of the farm's surface along the coast areas has led to adopting strategies that tend to optimize the use of land area and increase the potential area for locating the farm around the source of the water supply. This is done by reducing the required water flow rate.

Multilevel tank configurations have allowed taking advantage of the sole's morphology by breeding it in shallow tanks (around 20 cm depth), which are vertically stacked in order to reduce the farm's surface as well as vulnerability to disease, predators and natural disasters (Øiestad 1999, Labatut and Olivares 2004).

Kamstra et al. (2012) analyzed the economic rationale behind a multilevel shallow raceway system by comparing the capital costs for the rearing space as a function of productivity and the number of rearing levels. Their calculations were based on representative costs for building and land in The Netherlands. The analysis was based on a system with 7 levels that were 50 m long and in 4 rows, which was built at a price of 50 €/m^2 of rearing area. In this way, a 600 m^2 floor area supported 2000 m^2 of rearing area. The results showed that, assuming a productivity of around 20 kg/m^2/year, the capital costs per kg produced for a 7-level system was around 1.0 €/kg. Reducing the number of layers to 4, 2 and 1, the capital cost increased to about 1.5, 2.5 and 5.0 €/kg, respectively.

2.1 Flow-through versus recirculating systems

Introducing recirculating aquaculture technologies in single or multilevel facilities has been the key to reduce make-up water needs. This allows promoting versatility in terms of farm location and therefore reduces the costs associated to water transport. It has also made it feasible to control temperatures, which promotes consistent growth rates to market size throughout the production cycle and also contributes to a decline in many disease outbreaks that intensify when temperatures rise above 20–22°C (Morais et al. 2014). Furthermore, environmental and regulatory constraints are pushing fish farmers to move towards Recirculation Aquaculture Systems (RAS). In this type of system, fish are reared at high stocking densities and the make-up water needs are divided by 10 to 100 in comparison to flow-through systems (Martins et al. 2010). Consequently, wastewater flow rates decrease proportionally and waste concentration increases. Intensive or "fully-recirculating" RAS are typically defined as systems with water replacement ratios of less than 10% per day.

The use of water recirculating technologies has been increasingly adopted by fish hatcheries. Flow-through hatcheries that are supplied by marine water are subject to large fluctuations in water quality that are difficult to control. However, RAS technologies provide a rearing medium that is constant and adjustable, showing only slight and slow variations. Moreover, there is minimal heat loss in recirculated water systems, which normally operate above ambient water temperature. Despite the higher initial investment relative to flow-through systems, RAS technologies reduce production costs mainly because much less energy is required for heating, and the survival rate of the fingerlings is much higher (Blancheton 2000).

In comparing recirculating vs. flow-through systems for grow-out stages, the economic factors are usually determinant,in addition to the above mentioned aspects regarding the farm's surface, the water availability and the ability to control environmental parameters. The higher investment required by RAS technologies will condition the design and operation of the growing system, which will require continuous and intensive production, large production units and adequate farm size to minimize production costs. Also, when choosing flow-through or RAS technologies, it is necessary to consider not only the costs associated with moving water from the culture tanks to the different unit processes that restore used water quality, but also the costs linked to oxygen consumption by biological filters as well as those related to preventing catastrophic failures of the system (Timmons and Ebeling 2010).

Kamstra et al. (2001) (in Imsland et al. 2003) analyzed the prospects for growing *Solea solea* using a recirculation system based on a bio-economic model. The authors collected data from the literature and their own experimental research and projected these data into the infrastructure of a Dutch recirculation system. A case study was performed for a farm that produced 50 tons/year. The relative importance of the most important items in the total cost per kg of final product were estimated to be: fingerlings (5 g) 18.7%; feed 17.9%; electricity 9.2%; oxygen, gas and water 5.9%. Interest and depreciation were estimated to be 11.1% and 9.1%, respectively. Labor costs were estimated at around 26.4%.

2.2 Tank geometries and flow patterns

A proper tank design must combine hydrodynamics and the biological requirements of the species. It has to promote uniformity of rearing conditions, fast elimination of biosolids (non-ingested feed and faeces) and uniform distribution of fish throughout the tank (Tvinnereim 1988, Cripps and Poxton 1992, Timmons et al. 1998). Moreover, tanks should facilitate daily labors like feeding, removing dead fish and other routines like fish harvesting and grading.

Tank geometry, inlet/outlet characteristics and water flow rate determine the flow pattern and water velocities (Oca et al. 2004). Also the presence of fish and their stocking density have an effect on tank hydrodynamics by increasing the turbulence (Masaló et al. 2008) and modifying the tank bottom shape in flat fish facilities.

2.2.1 Raceway versus circular tank

Raceways and circular tanks are the most commonly used geometries in aquaculture. The advantages and disadvantages of raceways and circular tanks must be analyzed by considering: (a) the flow patterns and their repercussion on the environmental conditions into the tank, (b) the efficient use of the available land area and ease of fish handling, and (c) self-cleaning capacity. In sole growing facilities, raceways are the most commonly used tanks nowadays. This is due to their advantages in terms of point (b), despite the fact that circular tanks are more efficient in terms of points (a) and (c), as will be shown below.

Flow pattern and environmental conditions: A raceway is a rectangular tank with a length/width ratio of about 10 and a depth of less than 1.0 m (Summerfelt et al. 2000b). Water flows through the raceway in a plug-flow manner with minimal back mixing, generating gradients of environmental conditions which often promote a heterogeneous fish distribution. Sole's tendency to remain in groups will lead to weak individuals being displaced to zones with low water quality, enhancing hierarchies that are the major cause for growth heterogeneity (Salas-Leiton et al. 2010). On the other hand, the average water velocity in a raceway cross section can only be adjusted by modifying the flow rate per unit width or modifying the water depth. Both parameters are not easy to modify in commercial scale facilities, and the velocities achieved are frequently insufficient for effectively removing settled solids from the rearing area.

In contrast, water in circular tanks is usually injected tangentially to the wall, and the outlet is located at the bottom center of the tank, which creates a rotating flow that provides highly uniform water quality conditions (Westers and Pratt 1977, Ross et al. 1995), due to the effective mixing achieved (Ross and Watten 1998, Timmons et al. 1998). The rotating velocity in these kinds of tanks can be increased not only by increasing the flow rate of inlet water (Q), but also by reducing the size of the water inlet orifices, in order to increase the impulse produced by the water inlet jets. The capability of modifying the impulse force in rotating flow tanks without changing the flow rate of the incoming water makes it much easier to control average velocities, as will be explained in more detail below when we analyze the hydrodynamic conditions in flatfish tanks.

Besides the average velocity, the distribution of velocities was also analyzed by Oca and Masaló (2013), who proposed a model to determine the distribution of velocities in circular tanks. This was later improved upon by Masaló and Oca (2016) in order to introduce the influence of fish swimming in the water column.

Use of land area and fish handling: Circular tanks show some advantages from a hydraulic point of view. Nevertheless, the choice of tank geometry is also determined by the cost of floor space (Timmons et al. 1998). Furthermore, it is necessary to consider their ease of handling, for example, the ability to sort fish and perform routine tasks like cleaning, removing dead fish, etc. In sole facilities, fish have to be on the farm for long periods in order to reach a commercial size, due to their low growth rates. Therefore, two key points to consider are optimization of the culturing area and the ease of tank and fish handling.

The tank size used in the facilities depends on the species, growing stage, and economic considerations. Large tanks are used in grow-out stages, as they provide capital and labor cost savings because tank maintenance is rather independent of tank size (Timmons et al. 1998). Nevertheless, larger tanks are more difficult to handle, especially circular tanks. Summerfelt et al. (2009) proposed different technologies to improve harvesting and grading in large circular tanks; but their handling disadvantages increase with large diameters, while the length can be increased in rectangular tanks without increasing the difficulty in fish handling.

Also, the rectangular geometry allows better use of the available area, since the percentage of land not occupied by tanks is reduced when compared with circular tanks. Moreover, low water depths can be used in the culture of some flatfish species (especially sole), and this facilitates the stacking of shallow raceways on various floors (multilevel tanks), which in turn increases the ratio between rearing area and land area by a factor equivalent to the floors used.

Finally, another feature to be considered in analyzing the land area use is the stocking strategy adopted. Using a continuous stocking strategy rather than a batch strategy, the total system production increases (Watten 1992). Batch stocking will lead to poor space profitability, because maximum biomass will only be reached when fish are near the end of the production stage or close to commercial size. Nevertheless, continuous stocking of fish in large tanks is difficult, because regular grading and harvesting are required. Raceways allow farming different sized fish in one tank by delimiting different areas in the tank with movable partition nets, which is much more difficult to do in circular tanks.

Self-cleaning capacity: Sole faeces are semi-liquid, dissolve very fast in water, and are difficult to collect (Dias et al. 2010). Consequently, it is desirable to concentrate and eliminate biosolids from the rearing area as fast as possible by transporting them the shortest distance from their point of origin to the water outlet.

In raceways, solids removal from the tank is time- and labor-intensive and generally inefficient. To achieve self-cleaning properties in raceways, high velocities are needed for directing biosolids to the outlet faster, which means high flow rates. That, in turn, represents greater power requirements. It is common, and recommended (IDEQ 1998), to leave the end area of the raceway free of fish in order to concentrate biosolids and allow them to settle undisturbed. Such areas are known as quiescent zones.

Larger raceways that are used in intensive production contain more fish, which means more waste particles. Waste particle production is nearly homogeneous in all tank areas (when fish are evenly distributed), but their concentration increases with the distance from the inlet (Brinker and Rösch 2005). Thus, it may be necessary to place quiescent zones along the lengths of raceways in longer tanks.

Fish swimming can aid in resuspending particles by directing them to the outlet. Nevertheless, flatfish species rest on the bottom, and sedimentation is therefore enhanced. Merino et al. (2007b) studied the settling characteristics of solids in California halibut (*Paralichthys californicus*) tanks, and they showed that, in raceways that were stocked over 150% PCA (Percentage of Covered Area), the settled solids were resuspended by fish activity and swept out of the culture area of the tank.

In contrast to raceways, circular tanks show good self-cleaning properties. In circular tanks, the water injected tangentially to the wall creates a primary rotating flow parallel to the tank wall. The primary flow generates a secondary rotation in a thin layer next to the tank bottom and that flows radially inward, carrying settleable solids towards the bottom-center drain (this phenomenon is called the “tea-cup” effect) (Paul et al. 1991). Therefore, the distance travelled by solids is shorter in circular tanks, thus avoiding high concentrations on the tank floor as well as leaching.

Another advantage of circular tanks is the possibility of concentrating a significant percentage of solids in a small volume of water. The use of two drains in the tank center allows having a stream with high solids concentrations (secondary or concentrated flow) and a stream with low concentration (primary or clarified flow). These types of systems have been applied in aquaculture tanks and are known as Dual-drain systems (Fig. 1).

In dual-drain systems, only 5–20% of recirculating water is drained from the bottom centre of the tank (secondary or concentrated flow), but 80–90% of suspended solids are removed (Lunde et al. 1997, Van Toever 1997, Schei and Skybakmoen 1998, Summerfelt et al. 2000a, Davidson and Summerfelt 2004). More recently, triple-drains have been

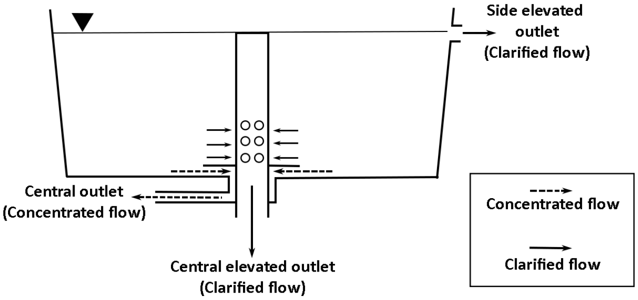

Fig. 1. Scheme of dual- and triple-drain systems.

designed (e.g., Wright et al. 2012), where three flows are differentiated (two clarified and one concentrated flow).

Dual-drains are in-tank systems that concentrate and separate biosolids in a small fraction of the water flow, allowing an increase in treatment efficiencies (Cripps and Bergheim 2000).

2.2.2 Raceway tanks with rotating flow cells

The ease of fish management and better land-use efficiency when employing raceways is in contrast to the advantages of circular tanks, specifically their self-cleaning and velocity control. Some authors have proposed tank designs that combine the hydrodynamic advantages of circular tanks with the handling and land-use advantages of rectangular tanks, creating rotating flow cells in rectangular tanks (Watten et al. 2000, Ebeling et al. 2005, Oca and Masaló 2007, Labatut et al. 2007a, 2007b). In these types of tanks, inlets are placed tangential to the cells and outlets in the center of each rotating flow cell.

Watten et al. (2000) designed the Mixed-Cell Raceway (MCR; Fig. 2), converting linear raceways (14.5 m long) into a series of hydraulically separated cells (each 2.4 m wide by 2.4 m long), with three inlets per cell.

Another similar but simpler design is the multivortex tank (Masaló and Oca 2014), where 4 rotating flow cells of 1 m diameter are created in a rectangular tank (4 × 1 m with 18 cm water depth) by injecting the water tangentially to the cells with only one inlet per cell (Fig. 3A). Baffles can be added between two consecutive water inlets to reduce the dissipation of energy due to the frontal collision of two entering plumes of water. The use of baffles increases the average velocities obtained without baffles by about 30%.

In tanks with rotating flow cells, the flow patterns obtained are very similar to those observed in circular tanks (Masaló and Oca 2014) (Fig. 3B), and no dead volumes or short-circuiting are observed (Watten et al. 2000), which indicates an adequate degree of mixing (Labatut et al. 2007a). In addition, average velocities are ten times higher than those which could be obtained in the same tank working as a linear raceway (Oca et al. 2004). Furthermore, they are proportional to impulse force (Masaló and Oca 2014), which is what happens in circular tanks (Oca and Masaló 2007) and thus allows for controlling velocities, as will be shown in the next section.

As in circular tanks, the distance travelled in these types of tanks by biosolids from tank bottom to outlet is shorter than in classic raceways, which allows for faster elimination from the tank bottom and a reduction in leaching.

The effect of flatfish on flow pattern in tanks with rotating flow cells was studied by Masaló and Oca (2013). These authors compared velocities obtained in the multivortex tank (18 cm water depth with 32% and 53% PCA), and they found an average velocity

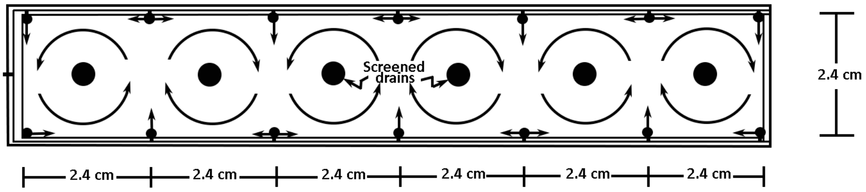

Fig. 2. The mixed-cell raceway (Watten et al. 2000).

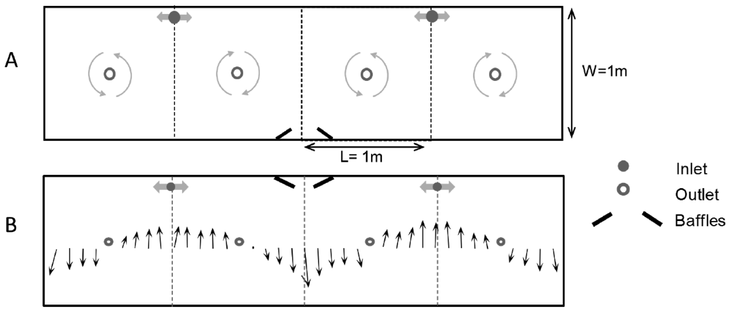

Fig. 3. (A) The multivortex tank proposed by Masaló and Oca (2014); (B) Velocity maps obtained in the longitudinal axis of the multivortex tank.

diminution of about 36% and 50% in respect to the tank without fish. Flatfish at the tank bottom increased the resistance to water flow and, consequently, the water velocities were lower.

Rectangular tanks with rotating flow cells combine the superior hydrodynamic characteristics of circular tanks (high velocities and self-cleaning properties) with the easier fish management and better land use of the raceways, which could turn them into a good alternative for sole culture. This would be especially true if they could be vertically stacked in a multilevel system to achieve a much higher rearing area per land area unit, which is a critical factor in a slow-growth species like sole. Moreover, they can include dual-drain systems (Ebeling et al. 2005) to concentrate biosolids and reduce the effluent treatment cost.

3. Hydrodynamic conditions in flatfish growing tanks

3.1 Adjustment of water velocities in flatfish tanks

Water velocities in flatfish tanks must be adjusted to reach self-cleaning conditions and to optimize fish growth and welfare. Before analyzing the influence of water velocity in flatfish growth, it is important to identify the kind of strategies that can be used for fitting the required velocity in flatfish tanks. These strategies must always consider the flow rate required for maintaining water quality levels, but they will be very different in raceways than in circular or rotating flow tanks.

The minimal flow rate required in any fish tank will be related mainly to the amount of dissolved oxygen (DO) that must be transported by water to supply the needs of fish. This amount will be roughly proportional to the amount of feed consumed by fish. The water flow required per unit of biomass (q_0) can be calculated from Eq. 1, where M is the oxygen consumption per unit of biomass, and DO_{in} and DO_{out} are the oxygen concentrations in the inlet water and at the discharge, respectively.

$$q_0 = \frac{M}{DO_{in} - DO_{out}} \quad \text{Eq. 1}$$

For instance, if we estimate a feed consumption of around 0.01 kg/day per kg of biomass, an oxygen consumption of around 250 g DO per kg of feed and a decrease in DO concentration ($DO_{in} - DO_{out}$) of 3.5 gm^{-3}, the oxygen consumption per unit of biomass (M) would be 2.5 g/day per kg of biomass, and the specific water flow requirement (q_0) would be 0.714 m^3/day per kg of biomass.

In a raceway, the average water velocity in the cross section (V) will be determined by the flow rate (Q), water depth (h) and tank width (w) (Eq. 2). This means that, when the tank width is fixed, water velocity can only be modified by adjusting the flow rate per unit width (Q/w) or the water depth (h).

$$V = \frac{Q}{w \cdot h} \quad \text{Eq. 2}$$

With flatfish, the maximal fish biomass is not constrained by the water tank volume, but by the surface available ($L \cdot w$). A maximal stocking fish density per unit surface (SDS) is set. For this reason, water depths used in flatfish raceways are much smaller than in those for round fish. In grow-out stages, the depths of sole tanks range from 14 to 20 cm, giving a relatively low capacity for velocity control.

For a specific flatfish raceway, with known L, w and h, the required flow rate for oxygen supply (Q_0) and the corresponding water velocity (V_0) can be calculated using Eqs. 3 and 4.

$$Q_0 = q_0 \, SDS \, L \, w \quad \text{Eq. 3}$$

$$V_0 = \frac{q_0 \, SDS \, L}{h} \quad \text{Eq. 4}$$

Therefore, supposing a raceway with $q_0 = 0.714$ m^3/day per kg of biomass, and a maximal stocking density per unit surface (SDS) of 30 kg/m^2, the water velocity needed to deliver the required flow rate for oxygen supply (V_0) can be calculated using Eq. 4 and will be proportional to the ratio length/depth (L/h); giving $V_0 = 21.43 \cdot L/h$ (m/day) or $V_0 = 0.025 \cdot L/h$ (cm/s).

In the design of a flatfish raceway, we can optimize the cost of pumping by choosing the suitable tank length so that the flow rate imposed by oxygen needs matches the optimal water velocity required to reach the optimal growth and self-cleaning conditions in the tank. In the above mentioned case, with a water depth of 0.2 m and a tank width of 1 m, if the water velocity required to reach the optimal growth were 10 cm/s, the water flow rate required in each tank should be 20 L/s (Eq. 2) and the optimal length of the raceway, calculated from Eq. 4, would be 80 m. With this length, the required flow rate calculated from Eq. 2 for achieving a velocity of 10 cm/s coincides with those calculated from Eq. 3, based on fish oxygen needs. If we used a shorter tank, for example 40 m, we would need to double the raceways in order to maintain the same amount of biomass, and the flow rate in each raceway should also be 20 L/s if the optimal growing velocity of 10 cm/s is to be achieved (Eq. 2). Therefore, the total flow rate required for the same amount of biomass will also be twofold, with the corresponding increase in pumping costs.

The difference must be highlighted between the design criteria in raceways for flatfish and for round fish. For the latter, the maximal stocking biomass is not determined by the available surface, but by the water volume, as the tank design is constrained by the maximal stocking density per unit volume (*SDV*). Therefore, Eq. 4 should be replaced by Eq. 5, in which the velocity, calculated for oxygen requirements, is independent from the water depth. In practice, raceways for round fish are managed much closer to their design requirement for oxygen supply than for cleaning requirements or recommended velocities for fish conditioning, which are usually much higher (Timmons and Ebeling 2010).

$$V_0 = L\ SDV\ q_0 \qquad \text{Eq. 5}$$

The control of water velocity in rotating flow tanks, including circular and raceways with rotating flow cells, is totally different. The average circulating velocity (V_{avg}) is controlled by the water inlet impulse force, *Fi*, which can by calculated by Eq. 6.

$$Fi = \rho Q\ (V_{in} - V_{avg}) \qquad \text{Eq. 6}$$

where ρ is the water density, and V_{in} is the water inlet velocity.

Considering that the water inlet velocity (V_{in}) is commonly much higher than V_{avg}, Eq. 6 can usually be simplified as Eq. 7a, or as Eq. 7b if we substitute V_{in} with the flow rate (Q) divided by the total area of the water inlet orifices (A_O).

$$Fi = \rho Q V_{in} \qquad \text{Eq. 7a}$$

$$Fi = \frac{\rho Q^2}{A_O} \qquad \text{Eq. 7b}$$

Equation 7b shows that, with the same flow rate, *Fi* can be increased by reducing A_O.

Oca and Masaló (2007) defined a non-dimensional tank resistance coefficient (C_t) (Eq. 8), which is suitable for characterizing the resistance to water circulation offered by a specific rotating flow tank (circular or rectangular with rotating flow cells).

$$C_t = \frac{2QV_{in}}{A_w\ V_{avg}^2} \qquad \text{Eq. 8}$$

where A_w is the tank wet area of the circular tank or rotating flow cell.

Once C_t is experimentally determined, the desired average velocity can be obtained by using Eq. 9.

$$V_{avg} = \left(\frac{2QV_{in}}{C_t A_w}\right)^{1/2} = \left(\frac{2}{A_w \rho C_t}\right)^{1/2} Fi^{(1/2)} \qquad \text{Eq. 9}$$

Assuming A_w, ρ and *Ct* to be constant, it can be observed that, in a rotating flow tank, the average velocity is proportional to the square root of the impulse force *Fi,* and the constant of proportionality can be experimentally determined (Oca and Masaló 2007). Therefore, the control of velocities will be much easier than in raceways, because independently from the required flow rate for oxygen supply to fish, we have the capacity to modify the size of the water inlet orifices in order to achieve optimal water velocities. For a constant flow rate, reducing the size of water inlet area leads to an increase in *Fi* and therefore in the average velocity, as shown in Eqs. 7b and 9.

3.2 Optimal water velocities in flatfish growing tanks

Water flow velocities can be related to fish size through the estimation of a relative swimming velocity (*RSV*) that is expressed as body lengths per second (bl/s) (Hammer 1995).

Among flatfish species, *RSV* effects on growth have been quantified under farm-like conditions only for Japanese flounder (*Paralichthys olivaceous*) (Ogata and Oku 2000), Summer flounder (*Paralichthys dentatus*) (Bengtson et al. 2004) and California halibut (*Paralichthys californicus*) (Merino et al. 2007a).

Ogata and Oku (2000) analyzed the growth performance of juvenile Japanese flounder (initial mean body weight and length 5.7 g and 9.1 cm), reared at *RSV* 0.3, 0.9 and 2.1 bl/s. Weight gain and final length of the group reared at 2.1 bl/s were significantly lower than those of the group reared at 0.9 bl/s, and they suggested that the optimum water velocity in Japanese flounder occurred at about 1.0 bl/s. The feed efficiency at 2.1 bl/s was significantly lower than those at 0.3 and 0.9 bl/s. These findings are similar to those obtained by Merino et al. (2007a) for California halibut juveniles (initial mean body weight and length 1.53 g and 5.4 cm), which proved to grow faster and made more efficient use of the feed provided at *RSV* of 0.5 and 1.0 bl/s than at 1.5 bl/s.

Working with bigger sizes, Bengtson et al. (2004) found that summer flounder (124 g, 257 g and 387 g) grew best when reared at 0.5 bl/s, in comparison to those grown at an *RSV* of less than 0.3 bl/s or greater than 1.3 bl/s.

Almansa et al. (2011) evaluated the fish distribution in turbot raceways (*Scophthalmus maximus*) with higher sizes (mean weight 234.02 g, mean length 22.4 cm) and different water velocities. They observed that velocities between 0.33 and 0.46 bl/s promoted a highly homogenous distribution of turbot. Nevertheless, when they reduced the width in the middle of the unit and gave the fish the opportunity to choose between the central narrow area with higher velocities (trial 1: 0.58 bl/s and trial 2: 0.98 bl/s) or the upstream and downstream areas (maintained at 0.29 bl/s in both trials), the turbot avoided swimming against the 0.58 bl/s stream and, therefore, less fish biomass was observed in the area upstream than downstream from the narrowing. When the water velocity in the narrow area increased to almost 1 bl/s, the turbot were not able to maintain their position and were distressed, resulting in reduced feed ingestion.

3.3 Vertical oxygen stratification in flatfish tanks

A reduction in specific growth rates was reported by Ogata and Oku (2000), Bengtson et al. (2004) and Merino et al. (2007a) for flatfish species grown in still water. One possible explanation for this reduction has been proposed by Reig et al. (2007), who found that dissolved oxygen is depressed significantly in the immediate vicinity of California halibut at very low water velocities.

Almansa et al. (2014) analyzed the hydrodynamic conditions that determine the oxygen gradient that occurs in the layer of water adjacent to flatfish grown in tanks.

Experiments were conducted with *Solea senegalensis* tanks with two geometries and different water flow rates. Results showed that a vertical oxygen gradient is created in certain hydrodynamic conditions, as a consequence of oxygen consumption by fish resting on the tank bottom.

The vertical profile of oxygen in flatfish tanks is influenced by the fish oxygen consumption and by the presence of the boundary layer. Flatfish extract oxygen from the water layer immediately above them. This water layer can be a semi-stagnant zone with a depressed oxygen concentration due to fish consumption, causing an oxygen gradient