Chapter 5

Early development and metabolism

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Chapter contents

Abstract: This chapter reviews the ontogeny and physiology of larval development in the Sparidae family and provides a comparative view of the larval biology of the species, highlighting those aspects that are relevant for their culture. Despite extensive similarities in larval biology, development and phenotype, species-specific differences exist, partly related to different temperature optima among species. Sparids typically release a large number of small eggs that hatch in an early stage of development. Larvae are very vulnerable during early development requiring relatively strict biotic and abiotic conditions to survive and grow. Sparids spawn buoyant pelagic eggs containing a single oil globule. Fertilized eggs take only a few days to hatch. Decreased hatching, increased larval mortality, and abnormalities occur outside optimal temperature ranges. Newly hatched larvae are small, have a large yolk sac, and typically start feeding within 3–5 days. The early ontogenetic events focus on the development of the organs and structures necessary for growth and survival, including the sensory organs, mouth, trunk and tail muscle mass, and the digestive system. The young larvae have poor visual acuity that improves as the eye diameter increases, and new photoreceptors and structures appear. Skeletal development and ossification have been well studied, in part because malformations are an issue in cultured fish. The pattern of anatomical changes and structural differentiation is related to functionality, behavior, and environmental preferences. The digestive system including gut, gall bladder, liver, pancreas, and gastric glands develops rapidly. The enzymes responsible for digestion of proteins, lipids, and carbohydrates are present at first feeding. The functional ontogeny of the digestive tract and pancreatic enzyme activity patterns are similar to those described in other fish groups. Growth in larvae is influenced by many exogenous factors with temperature and food being the most important. Other key abiotic factors in culture include oxygen, salinity, turbidity, and light. Optimal requirements are species-specific and

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change during larval ontogeny. The pattern of development is typically from a longitudinally elongated body shape to a longitudinally compressed form often characterized by a large skull and jaws. Sparids grow relatively quickly during early development. The critical stage of initial swim bladder inflation usually occurs soon after complete yolk absorption. The transient physostome larvae inflate their swim bladders by gulping air at the water surface. Factors influencing swim bladder inflation include temperature, salinity, turbulence, light, genetics, egg quality, water quality, and tank hydrodynamics. There is a considerable body of research on Sparid larval nutrition. Highly unsaturated fatty acids (*n*-3 HUFA) are essential for good growth and survival. Amino acids (AA) are a major energy source during the early life stages. The supply of dietary protein is paramount for optimal larval growth. The metabolism of larvae and juveniles is controlled by micronutrients, such as vitamins and minerals but there is little published literature. The vitamin A concentration of live feeds can be an important determinant for normal skeletal development. The generalized feeding regime for larvae starts with rotifers followed by *Artemia* and then formulated feeds. On a weight-specific basis, Sparid larvae tend to ingest more than 100% of their own weight in the first days of feeding. The specific ingestion rate decreases with age and more efficient digestion. Experimental microdiets have allowed investigation of early nutritional requirements, sustain survival comparable to those of live feeds, but growth is typically poor. Microdiet attractiveness improves with the inclusion of protein hydrolysates or free AA. Microbound, microencapsulated, and microcoated diets have been used with increasing effectiveness. Further improvements in culture will come from a better understanding of basic nutritional requirements of larval sparids, as well as of their interactions with abiotic factors, such as temperature, light, and hydrodynamics at key developmental stages.

Key words: gut functionality; larval growth; larval nutrition; metabolism; ontogeny; yolk sac absorption

5.1 Introduction

Sparids were one of the first groups of marine fish in which larval rearing techniques were developed. Japanese development of modern techniques began in the late 1800s and early 1900s. In 1887, the first rearing trial of red sea bream (*Pagrus major*) was carried out in the Okayama Prefecture, and successful larviculture was attained by Kajiyama and Nishioka in 1930 (Research Group of Central Pacific Region 1988; Fushimi 2001). Yamashita (1963) closed the life cycle of red sea bream in 1962. Large-scale production started in 1966 at the Hakatajima Station, Seto Inland Sea-Farming Association (SISFA) with 55,000 juveniles (8–15 mm in total length, TL) produced and 9000 cultured fish (25–40 mm TL) used for stock enhancement. This was the first time a marine finfish was successfully cultured in Japan (Fushimi 2001). Kasahara *et al*. (1960) then cultured blackhead sea bream (*Acanthopagrus schlegelii*, synonyms *Mylio macrocephalus* and *Sparus macrocephalus*) using similar techniques. The growing interest in Sparid culture during the late 1960s and 1970s was then driven by studies on red sea bream in Japan (Okanamoto 1969; Fukuhara 1976a, 1976b; Foscarini 1988; Fushimi 2001) and gilthead sea bream (*Sparus aurata*) in the Mediterranean Sea (Lumare & Villani 1971, 1973; Barnabé & René 1973; Alessio *et al*. 1975; Arias 1976; San Feliú *et al*. 1976; Pitt *et al*. 1979; Person-Le Ruyet & Verillaud 1980), and early studies on feeding physiology of Western Atlantic sea bream (*Archosargus rhomboidalis*) in North America (Houde 1974; Houde & Potthoff 1976; Stepien 1976). These studies increased significantly the knowledge of the early life history of sparids, as well as in marine fish in general. Since the 1980s, many other species of Sparid larvae have been studied, most of them as a prospecting strategy for diversification of new species for aquaculture (Fukuhara & Fushimi 1981; Hussain *et al*. 1981; Divanach *et al*. 1982; Tsukashima & Kitajima 1982; Oshima 1984; Faranda *et al*. 1985; Tucker 1987; Jug-Dujakovic & Glamuzina 1988; Pankhurst *et al*. 1991; Battaglene & Talbot 1992; Abellán *et al*. 1994; Fushimi 2001; Partridge *et al*. 2004; Mihelakakis *et al*. 2005). Now after more than four decades of continuous research, following the first published studies in Japan, there is a mass of scientific literature on larval biology and culture of different species belonging

to the Sparidae genera *Pagrus*, *Sparus*, *Diplodus*, *Archosargus*, *Acanthopagrus*, *Rhabdosargus*, *Dentex*, and *Pagellus*.

This chapter reviews the ontogeny and physiology of larval development in the Sparidae family and provides a comparative view of the larval biology of the various species, highlighting those aspects that are relevant for their culture. Reared species inhabit temperate and subtropical waters of the Atlantic, Indian, and Pacific Oceans having a rapid embryonic and larval development. Most sparids are carnivorous with benthopelagic habits (see Chapter 1 in this book). Pelagic larval periods end when juvenile characteristics and feeding habits are completely acquired, and the fish move to deeper habitats. Sparids have an altricial reproduction and developmental strategy, releasing a large number of small eggs that hatch in an early stage of development (Balon 1981). Sparid larvae are very vulnerable during early development stages requiring relatively strict biotic and abiotic conditions to survive and grow. Like other marine fish with altricial larvae, three different stages are observed during early development and feeding: the endotrophic stage from hatching to the opening of the mouth, lasting a few days during which the embryo develops from yolk reserves; the mixed endoexotrophic stage from the mouth opening up to the complete exhaustion of yolk reserves (very short or absent in sparids); and the exotrophic stage reliant on exogenous feeding. The exotrophic stage is the longest stage, during which larvae grow continuously at a relatively high rate, and undergo important morphological and physiological transformations. The first signs of juvenile features and habits can be detected a few weeks after hatching, and following notochord flexion. However, definitive shape, anatomical and physiological characteristics, and feeding habits are achieved some weeks or months later when the fish reach between 20 and 60 mm TL and an age of 6–24 weeks, depending on species and temperature (Tanaka 1971; Houde & Potthoff 1976; Hussain *et al*. 1981; Leu & Chou 1996; Tucker & Alshuth 1997; Koumoundouros *et al*. 1999a; Boglione *et al*. 2003; Yúfera *et al*. 2004; Russo *et al*. 2007). In spite of the great similarities in larval development within the group, there are some species-specific characteristics.

5.2 Anatomical development, general characteristics, and sensory organs

Knowledge of larval development and physiology has been achieved mainly from laboratory studies carried out between 12 and 30°C. Sparids spawn buoyant pelagic eggs with mean diameters ranging between 0.8 and 1.1 mm (Table 5.1), containing a single oil globule with 180–250 μm diameter. Fertilized eggs take from 1 to 2.5 days to hatch depending on species and temperature (Figure 5.1), and to a lesser extent on salinity (Mihelakakis & Kitajima 1994; Mihelakakis & Yoshimatsu 1998). Embryonic developmental time decreases with increasing temperature in the range of 12–30°C with some small differences among species. In temperate species, decreasing hatching rates, increasing larval mortality, and the presence of abnormalities are more evident below 15°C and above 22°C (Camus & Koutsikopoulos 1984; Polo *et al*. 1991; Mihelakakis & Yoshimatsu 1998). In subtropical species, such as the Western Atlantic sea bream, the incubation temperatures to obtain viable larvae are higher and best survival is obtained between 26 and 30°C (Houde 1974; Stepien 1976). Newly hatched larvae are from 1.6 to 3.1 mm TL, and have a large yolk sac that provides nutrition to the developing embryo until exogenous feeding starts after 3–5 days when larvae are 2.6–3.9 mm TL (Table 5.1).

As generally occurs in altricial larvae of marine teleosts, at hatching larvae have incomplete or undifferentiated organs (Govoni *et al*. 1986). Eyes are formed, but nonfunctional and the mouth is absent (Houde & Potthoff 1976; Hussain *et al*. 1981; Sarasquete *et al*. 1995; Tucker & Alshuth 1997). The early ontogenic events focus on the development of the organs and structures necessary for growth and survival (Osse *et al*. 1997; Russo *et al*. 2007; Yúfera & Darias 2007). These include those required to begin and improve exogenous feeding, as well as to develop predator avoidance (Blaxter 1986). The primary structures necessary to obtain food and to process it efficiently are the sensory organs, mouth, trunk and tail muscle mass, and the digestive system.

Table 5.1 Egg diameter (mm) and total length (TL mm) at hatching and first feeding of Sparid larvae

Common name	Species	Temperature (°C)	Egg diameter (mm)	TL at hatching (mm)	TL at first feeding (mm)	Reference
Yellowfin sea bream	*Acanthopagrus latus*	21.4	0.83	2.03	2.95	Leu & Chou 1996
Sheepshead	*Archosargus probatocephalus*	23	0.82	1.68–1.78	2.64	Tucker & Alshuth 1997
Western Atlantic sea bream	*Archosargus rhomboidalis*	24	0.80–0.94	2.00–2.20	—	Houde & Potthoff 1976
Sobaity sea bream	*Sparidentex hasta*	21	—	1.87	3.20	Hussain *et al*. 1981
Gilthead sea bream	*Sparus aurata*	17	1.07	—	—	Lahnsteiner & Patarnello 2003
Gilthead sea bream	*Sparus aurata*	19	—	2.26	3.93	Polo *et al*. 1991
Gilthead sea bream	*Sparus aurata*	19	1.00	—	—	Yúfera, personal observation
Snapper	*Pagrus auratus*	17–22	0.80–1.00	—	—	Pankhurst *et al*. 1991
Snapper	*Pagrus auratus*	21.5	0.90–1.00	—	3.10	Battaglene & Talbot 1992
Red sea bream	*Pagrus major*	18.5	1.08	—	—	Mihelakakis & Yoshimatsu 1998
Red sea bream	*Pagrus major*	18.7–21.5	—	2.10	3.40	Moteki *et al*. 2001
Red sea bream	*Pagrus major*	20	0.92	2.01	—	Fukuhara 1985
Red porgy	*Pagrus pagrus*	16–20	0.89–0.93	—	—	Machinandiarena *et al*. 2003
Red porgy	*Pagrus pagrus*	18	0.90	—	—	Arastizabal 2006
Red porgy	*Pagrus pagrus*	18	1.08	3.18	3.90	Mihelakakis *et al*. 2001
Red porgy	*Pagrus pagrus*	18	0.84	—	—	Kolios *et al*. 1997
Red porgy	*Pagrus pagrus*	18	0.99	—	—	Büke *et al*. 2005
Common dentex	*Dentex dentex*	17	0.96	2.17	3.55	Glamuzina *et al*. 1989
Common dentex	*Dentex dentex*	18	1.03	—	—	Saka *et al*. 2004
Common dentex	*Dentex dentex*	16–20	—	2.74	—	Crespo *et al*. 2001
Pink dentex	*Dentex gibbosus*	20	0.96	2.097	3.35	Fernández-Palacios *et al*. 1994
Sharpsnout sea bream	*Diplodus puntazzo*	22	0.85	1.69	—	Faranda *et al*. 1985
White sea bream	*Diplodus sargus*	16.8	0.98–1.02	—	—	Divanach *et al*. 1982
Common two-banded sea bream	*Diplodus vulgaris*	17	1.01	2.63	3.90	Jug-Dujakovic & Glamuzina 1988
Blackspot pandora	*Pagellus bogaraveo*	14	1.19	—	—	Peleteiro *et al*. 1997
Common pandora	*Pagellus erythinus*	19	0.78	2.03	—	Güner *et al*. 2004
Common pandora	*Pagellus erythinus*	19	—	2.32	—	Klaoudatos *et al*. 2004
Common pandora	*Pagellus erythinus*	15–21	0.77	1.71–1.81	—	Klimogianni *et al*. 2004
Common pandora	*Pagellus erythinus*	18.5–20	—	2.55	3.18	Micale *et al*. 2006
Goldlined sea bream	*Rhabdosargus sarba*	19.2–22.3	1.03	—	—	Leu 1994
Goldlined sea bream	*Rhabdosargus sarba*	15–23.5	—	2.06–2.52	—	Mihelakakis & Kitajima 1994
Goldlined sea bream	*Rhabdosargus sarba*	—	0.95–1.02	—	—	Mihelakakis & Kitajima 1995

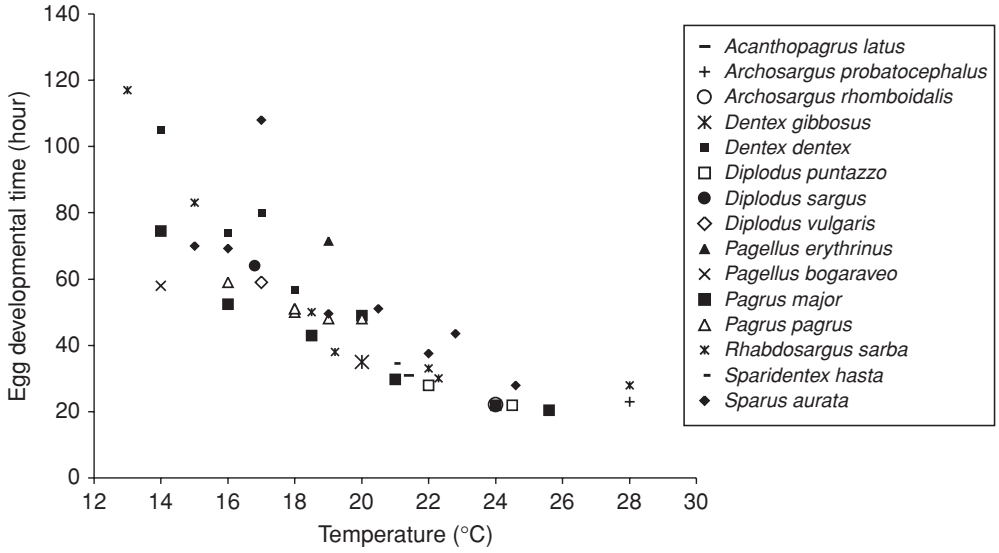

Figure 5.1 Egg developmental time in different Sparidae species in relation to incubation temperature. Sources: Yellowfin sea bream, *Acanthopagrus latus* (Leu & Chou 1996); sheepshead, *Archosargus probatocephalus* (Tucker & Alshuth 1997); Western Atlantic sea bream, *Archosargus rhomboidalis* (Houde & Potthoff 1976); common dentex, *Dentex dentex* (Glamuzina *et al*. 1989; Saka *et al*. 2004); pink dentex, *Dentex gibbosus* (Fernández-Palacios *et al*. 1994); sharpsnout sea bream, *Diplodus puntazzo* (Faranda *et al*. 1985); white sea bream, *Diplodus sargus* (Divanach *et al*. 1982); *Diplodus vulgaris* (Jug-Dujakovic & Glamuzina 1988); blackspot sea bream, *Pagellus bogaraveo* (Peleteiro *et al*. 1997); common pandora, *Pagellus erythinus* (Güner *et al*. 2004; Klaoudatos *et al*. 2004; Klimogianni *et al*. 2004); snapper, *Pagrus auratus* (Pankhurst *et al*. 1991; Fielder *et al*. 2002); red sea bream, *Pagrus major* (Fukuhara 1985; Mihelakakis & Yoshimatsu 1998); red porgy, *Pagrus pagrus* (Kolios *et al*. 1997; Mihelakakis *et al*. 2001; Machinandiarena *et al*. 2003; Büke *et al*. 2005; Arastizabal 2006; Morris *et al*. 2008); goldlined sea bream, *Rhabdosargus sarba* (Leu 1994; Mihelakakis & Kitajima 1994); sobaity sea bream, *Sparidentex hasta* synonym *Acanthopagrus cuvieri* (Hussain *et al*. 1981); gilthead sea bream, *Sparus aurata* (Camus & Koutsikopoulos 1984; Polo *et al*. 1991; Lahnsteiner & Patarnello 2003).

The sensory organs necessary for searching for food and detecting prey and predators have been studied in larvae of several species (see reviews by Evans & Browman 2004; Døving & Kasumyan 2008; Loew & Wahl 2008). Eye development has been studied in some sparids, such as the red sea bream (Kawamura *et al*. 1984), snapper (*Pagrus auratus*) (Pankhurst 1994; Pankhurst & Eagar 1996), red porgy (*Pagrus pagrus*) (Roo *et al*. 1999), Southern black bream (*Acanthopagrus butcheri*) (Shand *et al*. 1999, 2002), and goldlined sea bream (*Rhabdosargus sarba*) (Ibrahim *et al*. 2006). Sparid larvae are visual feeders as has been demonstrated in several fishes (Dowd & Houde 1980; Tandler & Helps 1985; Fielder *et al*. 2002) and, consequently, their eyes become functional at the time of first feeding. At mouth opening, the retina is equipped with a monospecific layer of single cones already pigmented and the optic nerve is connected to the optic tectum (Kawamura *et al*. 1984; Pankhurst & Eagar 1996; Roo *et al*. 1999). Nevertheless, as studies with snapper demonstrate, in early development the larvae have poor photopic acuity and have severe visual constrains (Pankhurst 1994). With progressive development, the eye diameter increases, new photoreceptors and structures appear, such as rods, double cones, and mosaic patterns, allowing better acuity and vision in deeper waters with low illumination intensity.

There are also several studies on the development of mechanical and chemical receptors in the family. Free neuromasts able to detect water movements, and an olfactory epithelium are present in the head and rostral area in early larvae of blackhead sea bream (Fukuhara 1977; Su & Wang 1990), red sea bream (Iwai 1980; Yamasita 1982), sharpsnout sea bream (*Diplodus puntazz*o) (Boglione *et al*. 2003), common pandora (*Pagellus erythrinus*) (Boglione *et al*. 2006), and gilthead sea bream (Orzali 2008). With development and larval growth, the free neuromasts increase in number and density. Canalized neuromasts within the cephalic and trunk lateral line confer a better perception of water acceleration (Hofer 1908). Completion of the nares appears in the last stages of transformation to juveniles (Boglione *et al*. 2006). Taste buds, chemosensory organs responsible of gustation, appear in the buccal cavity, usually a few days after first feeding, and their density increases throughout development being more evident and numerous after two to three weeks (Iwai 1980; Boglione *et al*. 2006; Sánchez-Amaya *et al*. 2007; Micale *et al*. 2008). In some cases, taste buds have been detected only after several weeks of feeding (Boglione *et al*. 2003; Micale *et al*. 2008).

Development of the mouth is a key issue for food uptake. A wide range of mouth gapes, between 70 and 300 μm, has been reported at first feeding for the different species. Mouth gapes of 70–80 μm (Klaoudatos *et al*. 2004) and 235–286 μm (Güner *et al*. 2004) have been measured in common pandora, 160–240 μm in the yellowfin sea bream (*Acanthopagrus latus*) (Leu & Chou 1996), 140–340 μm in red porgy (Machinandiarena *et al*. 2003), 250 μm in gilthead sea bream (Fernández-Díaz *et al*. 1994), and 155 μm in red sea bream (Moteki *et al*. 2001). After mouth opening, the gape widens with increasing larval length, but this escalation is noticeably quicker during the first few days (Fernández-Díaz *et al*. 1994; Moteki *et al*. 2001; Yúfera & Darias 2007). With further development and after the onset of feeding, the mouth is structurally fully formed with cartilage and bones (Kohno *et al*. 1983; Matsuoka 1987; Koumoundouros *et al*. 1999b; Moteki 2002), and finally with buccal and pharyngeal teeth whose organization becomes more complex in the later stages of larval development (Koumoundouros *et al*. 2000; Boglione *et al*. 2003). These changes improve greatly the catching and feeding capacity of older larvae.

Skeleton development and ossification have been the subject of many studies in sparids, due to the occurrence and importance of malformations observed in cultured fishes. Thus, different general and specific studies on skeletal structures have been investigated for red sea bream (Fukuhara 1976a, 1985; Kohno *et al*. 1983; Matsuoka 1987; Moteki 2002), blackhead sea bream (Fukuhara 1976a), Western Atlantic sea bream (Houde & Potthoff 1976), sheepshead (*Archosargus probatocephalus*) (Mook 1977), gilthead sea bream (Koumoundouros *et al*. 1997; Faustino & Powers 1998), common dentex (*Dentex dentex)* (Koumoundouros *et al*. 1999b, 2001b), white sea bream (*Diplodus sargus*) (Koumoundouros *et al*. 2001a) (Figure 5.2), common pandora (Sfakianakis *et al*. 2004), and sharpsnout sea bream (Favaloro & Mazzola 2006). The cartilaginous structures can be observed in larvae below 3 mm TL (Matsuoka 1987) and ossification starts when the notochord reaches 5–6 mm, following the completion of cartilaginous ontogeny and just after flexion (Fukuhara 1985; Faustino & Powers 1998). During these early stages the only fins present are the primordial finfold and pectoral bud cartilages (Fukuhara 1976a; Koumoundouros *et al*. 1997; Santamaría *et al*. 2004). The caudal fin is the next to develop. Jaws and branchial arches develop first and shortly after the hypural of the caudal fin complex. The finfold starts to be reabsorbed after notochord flexion and is replaced by the respective unpaired fins. Ray segmentation, first and

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Figure 5.2 Ontogenetic development of the vertebral column, dorsal and anal fins of white sea bream, *Diplodus sargus*. White areas stand for cartilage, shadowed areas for ossified structures (ossification state of rays is not drawn; intermediate rays in F are not drawn). *Drl*—dorsal ribs; *Ep*—epurals; *HS*—hemal-processes; *Hy*—hypurals; *Nc*—notochord; *NS*—neural processes; *Pp*—parapophyse; *Pr*—pleural rib; *Prd*—predorsal; *PrH*—parhypural; *Prx*—proximal pterygiophore; *R*—lepidotrichium; *Rd*—distal radial; *S*—hard spine; *U*—vertebral centra; *Ur*—urostyle. Scale bars = 0.5 mm. (Reproduced from Koumoundouros *et al*. 2001a, with kind permission from Springer Science+Business Media).

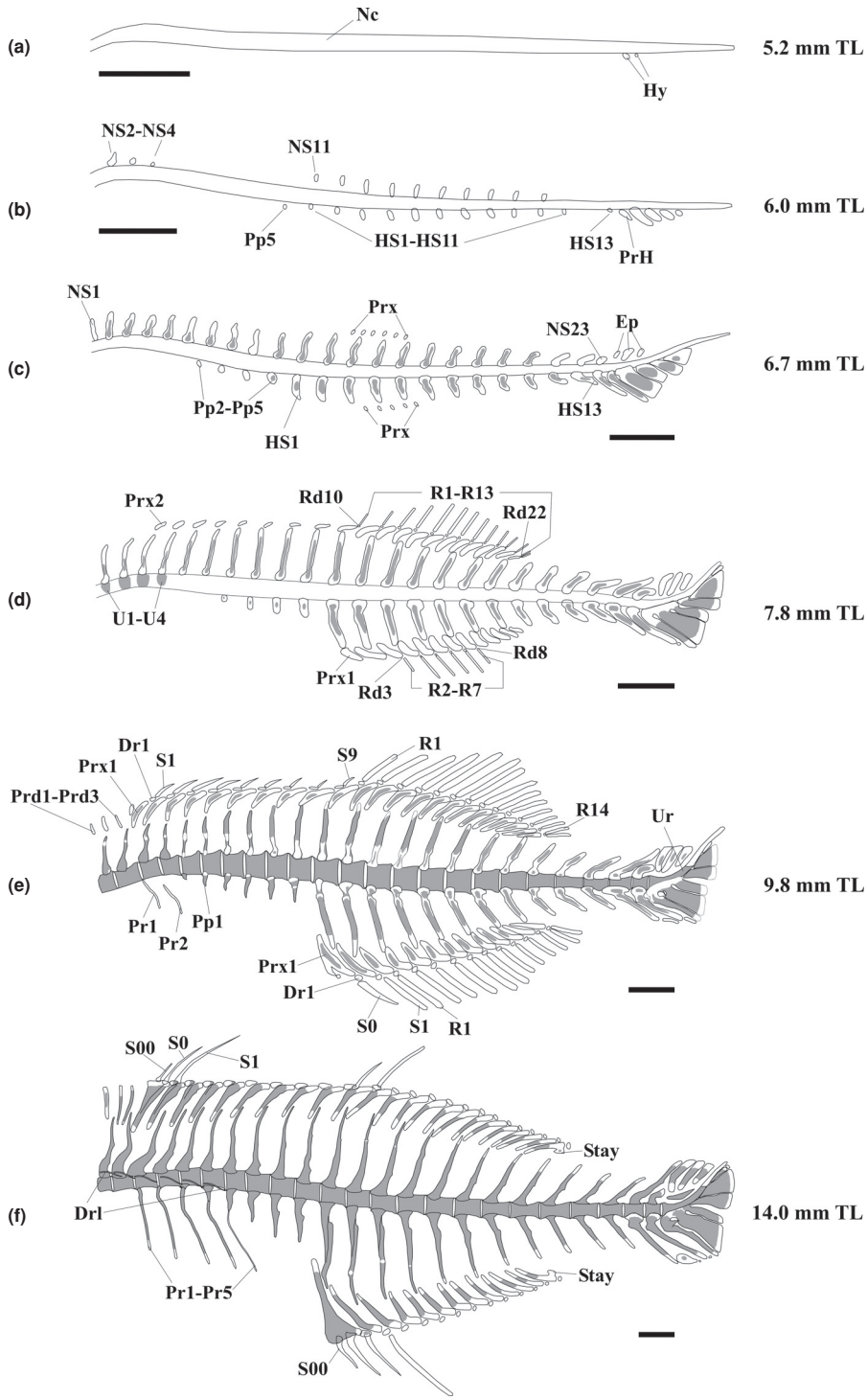

Figure 5.2 (*Continued*)

subsequent branching, occurs during the transitional phase from postlarva to juvenile, starting when the larvae, respectively, attain 5–6 mm and 8–10 mm TL (Fukuhara 1976a, 1985). The final structure and shape is reached after several weeks of development in juveniles of 20–40 mm TL (Fukuhara 1985; Koumoundouros *et al*. 1997).

The anatomical features and developmental pattern described above apply to most sparids and many other marine species. The pattern of anatomical changes and structural differentiation is related to functionality, behavior, and environmental preferences throughout larval development. At the onset of feeding, Sparid larvae have very limited movement ability and short distance detection and reactive capacity. Larvae detect potential prey by sight, aided by movement and chemical stimuli, mainly from amino acids (Kolkovski *et al*. 1997). The selection of prey and their ingestion by swallowing depends almost exclusively on their recognition and size, which is limited by the mouth gape. Locomotion and hunting ability improve notably within days as the larvae increase in TL, trunk, and tail muscles, and have better visual acuity and orientation capacity. An increase in taste buds allows for food palatability assessment and enables the larvae to better discriminate among prey and to decide whether to ingest (Boglione *et al*. 2003). Larger mouth gape allows ingestion of progressively larger prey. Progressive jaw development and ossification, together with the appearance of teeth, allows the shift from catching by simple sucking to catching by sucking and biting (Kohno *et al*. 1983; Koumoundouros *et al*. 2000). With the completion of squamation, ossification, the formation of the fins and teeth, the development of rod, and mosaic pattern of photoreceptors in the eye retina, and approaching of the final body shape, the juveniles are prepared better for deeper habitats and predation on epibenthonic prey.

5.3 Organogenesis and functionality of digestive system

5.3.1 Gut ontogeny

Organogenesis and ontogeny of digestive functionality in larval fish has been recently reviewed comprehensively by Zambonino-Infante *et al*. (2008). In this chapter, we summarize those aspects relevant for sparids. Development of the digestive system and associated organs has been well-described in several species of sparids (Tanaka 1969a, 1969b, 1971, 1972a, 1972b; Tsukashima & Kitajima 1982; Sarasquete *et al*. 1993a, 1995, 2001; Calzada *et al*. 1998; Domeneghini *et al*. 1998; Roo *et al*. 1999; Crespo *et al*. 2001; Boglione *et al*. 2003; Ortiz-Delgado *et al*. 2003; Elbal *et al*. 2004; Santamaría *et al*. 2004; Micale *et al*. 2006, 2008; Darias *et al*. 2007a; Sánchez-Amaya *et al*. 2007a). During the endotrophic stage, just after hatching, the digestive tract of Sparid larvae appears as a single undifferentiated straight tube, curved in the caudal portion, lying dorsally to the yolk sac with both ends closed. The epithelium of the digestive tract has a monostratified layer of columnar or cubical cells (Sarasquete *et al*. 1995; Calzada *et al*. 1998; Micale *et al*. 2008). Neither mouth nor anus is yet formed. The liver and pancreas are small, undifferentiated, rounded cell masses between the heart and intestine. During this stage, tissues and organs develop quickly. Throughout the yolksac period, three digestive tract sections can be observed: the foregut in the anterior part, midgut and hindgut posteriorly located with the first signs of pyloric sphincter, and ileo-rectal valve (Calzada *et al*. 1998; Roo *et al*. 1999; Sánchez-Amaya *et al*. 2007). The gall bladder lying between the liver and the pancreas is only distinguished at the end of yolk sac stage. As for most of the Acanthopterygii, the digestive tract in Sparid larvae is a loop. The looping of the growing intestine allows it to be accommodated in the visceral cavity and usually appears a few hours to days after hatching and can clearly be seen at or just after first feeding (Elbal *et al*. 2004; Micale *et al*. 2006). Gills and pseudobranches are not yet developed at this early stage and, consequently, osmoregulation and respiration functions are carried out by chloride cells of the buccopharyngeal epithelium and through the skin (Sarasquete *et al*. 2001; Santamaría *et al*.

2004; Sánchez-Amaya *et al*. 2007). By the end of the endotrophic stage, the pancreas, liver, and gall bladder become differentiated and connected with the gut (Guyot *et al*. 1995, 1998; Sarasquete *et al*. 1995; Micale *et al*. 2006, 2008). The pancreas exhibits acinar organization and sometimes the first endocrine cells form an islet of Langerhans, discernible within the exocrine tissue. The liver shows mature hepatocytes with accumulation of glycogen and protein granules in the cytoplasm. Finally, the mouth and anus open, at which point exogenous feeding begins and the vitelline reserves are almost exhausted.

The endoexotrophic stage has not always been detected in sparids, depending on the species, culture conditions, and the efficiency in which yolk is utilized at different temperatures (Polo *et al*. 1991). In any case, during the period immediately after the opening of the mouth it is already possible to distinguish the buccal cavity without teeth, the esophagus with a squamous stratified epithelium, and the intestine with a monostratified columnar epithelium with microvilli. The buccopharynx and esophagus still lack mucous secreting goblet cells, which appear a few days later (Sarasquete *et al*. 1995; Calzada *et al*. 1998; Ortiz-Delgado *et al*. 2003; Elbal *et al*. 2004; Darias *et al*. 2007a; Sánchez-Amaya *et al*. 2007). The esophagus then elongates, showing longitudinal folds. The future stomach appears as a little dilatation at the posterior section of the esophagus and is lined with a simple epithelium of cubic cells with some apical processes, but lacking any signal of secretion. The ileocecal valve separates the midintestine from the hindgut, and the rectum is short and formed by a simple cubic epithelium. Once feeding starts and gut contents are detected, the intestine becomes much wider, most notably in the anterior segment. The mucosa becomes thicker and the intestinal folding is already apparent in some species, such as white sea bream, redbanded sea bream (*Pagrus auriga*), and sharpsnout sea bream (Ortiz-Delgado *et al*. 2003; Sánchez-Amaya *et al*. 2007; Micale *et al*. 2008). The mouth size also shows a noticeable increase during the first few days. In general, the organs related with prey catching and digestion exhibit a positive allometric increase (Osse *et al*. 1997; Sala *et al*. 2005; Yúfera & Darias 2007). The absorption of nutrients is evidenced through the formation of lipidic vacuoles and inclusions in the enterocytes. Thus, the anterior-median intestinal enterocytes have supra- and infra-nuclear vacuoles related to lipid absorption (Diaz *et al*. 1997, 2002; Elbal *et al*. 2004; Yúfera & Darias 2007) and acidophilic supranuclear inclusions appear in the enterocytes of the posterior intestine, and contain abundant proteins (Tanaka 1971, 1972b; Sarasquete *et al*. 1995; Elbal *et al*. 2004). The vacuoles and inclusions are absent in unfed larvae (Yúfera *et al*. 1993b; Crespo *et al*. 2001). From first feeding, the exocrine pancreas shows acidophilic protein granules and the first islet of Langerhans may be observed (Tanaka 1969b; Sarasquete *et al*. 1993a, 1995; Guyot *et al*. 1998). The liver shows mature hepatocytes with accumulation of glycogen and protein granules in the cytoplasm (Tanaka 1969b; Guyot *et al*. 1995; Micale *et al*. 2006, 2008; Darias *et al*. 2007a). The hepatic sinusoids and vacuolization proliferate with increasing larval size, the glycogen being progressively stored in the liver.

During the first period of the exotrophic stage the organs exhibit an increase in size, but few morphological changes. At first, goblet cells and taste buds appear in the buccopharynx. Pharyngeal and buccal teeth also appear, although, in different order and time sequence in each species. For example, pharyngeal teeth have been observed during the second week of life in red porgy and redbanded sea bream (Darias *et al*. 2007a; Sánchez-Amaya *et al*. 2007) and only after a month in common pandora (Micale *et al*. 2006). The pyloric sphincter connecting the stomach with the anteromedian intestine becomes gradually more obvious. Likewise, the intestinal folds increase in length and complexity, and the intestinal mucous cells appear among the enterocytes proliferating from the anterior to posterior intestine (Ortiz-Delgado *et al*. 2003; Elbal *et al*. 2004).

The primordial swim bladder is usually distinguished at the time of the mouth opening as a protrusion differentiated from the dorsal wall of the foregut (Santamaría *et al*. 2004; Sánchez-Amaya *et al*. 2007). After a few days, it is apparent as a small chamber connected to the gut by the pneumatic duct and the gas gland and *rete mirabile* are also apparent (Santamaría *et al*. 2004). The initial inflation usually occurs after the yolk resorption, generally in larvae with TL between 4 and 7 mm. In red sea bream inflation occurs at 10–12 days after hatching (DAH) at 3.61–4.29 mm TL (Kitajima *et al*. 1981), in gilthead sea bream at 7–10 DAH (Chatain 1986; Chatain &

Ounais-Guschemann 1990), at 7–9 DAH in snapper (Battaglene & Talbot 1992; Fielder *et al*. 2005), at 11 DAH in sobaity sea bream (*Sparidentex hasta,* synonym *Acanthopagrus cuvieri*) (Teng *et al*. 1999), at 11–12 DAH in common dentex (Santamaría *et al*. 2004), at 6–15 DAH in red porgy (Büke *et al*. 2005; Darias *et al*. 2007a) and between 8 and 20 DAH in common pandora (Güner *et al*. 2004).

Some weeks after first feeding, the stomach can be clearly discernible as an evagination, which expands as a blind sac dorsal to the midgut junction. Gastric glands appear progressively in the mucosa exhibiting characteristic patterns in each species (Ortiz-Delgado *et al*. 2003; Darias *et al*. 2007a). Pyloric caeca usually develop in parallel to gastric glands, although in sharpsnout sea bream they appear much earlier (Micale *et al*. 2008) and in red sea bream after the completion of gastric glands (Tanaka 1971). Caeca are formed through a projection of the intestine wall at the most anterior part, so that their epithelium is identical with that of the intestine. With larval growth, the liver becomes larger, more elongate, and bilobed with the esophagus resting in the dorsal groove. Toward the end of larval development, the anatomy of the digestive tract remains largely unchanged and is similar to that of adults. The main events of the larval development in sparids are summarized in Figure 5.3.

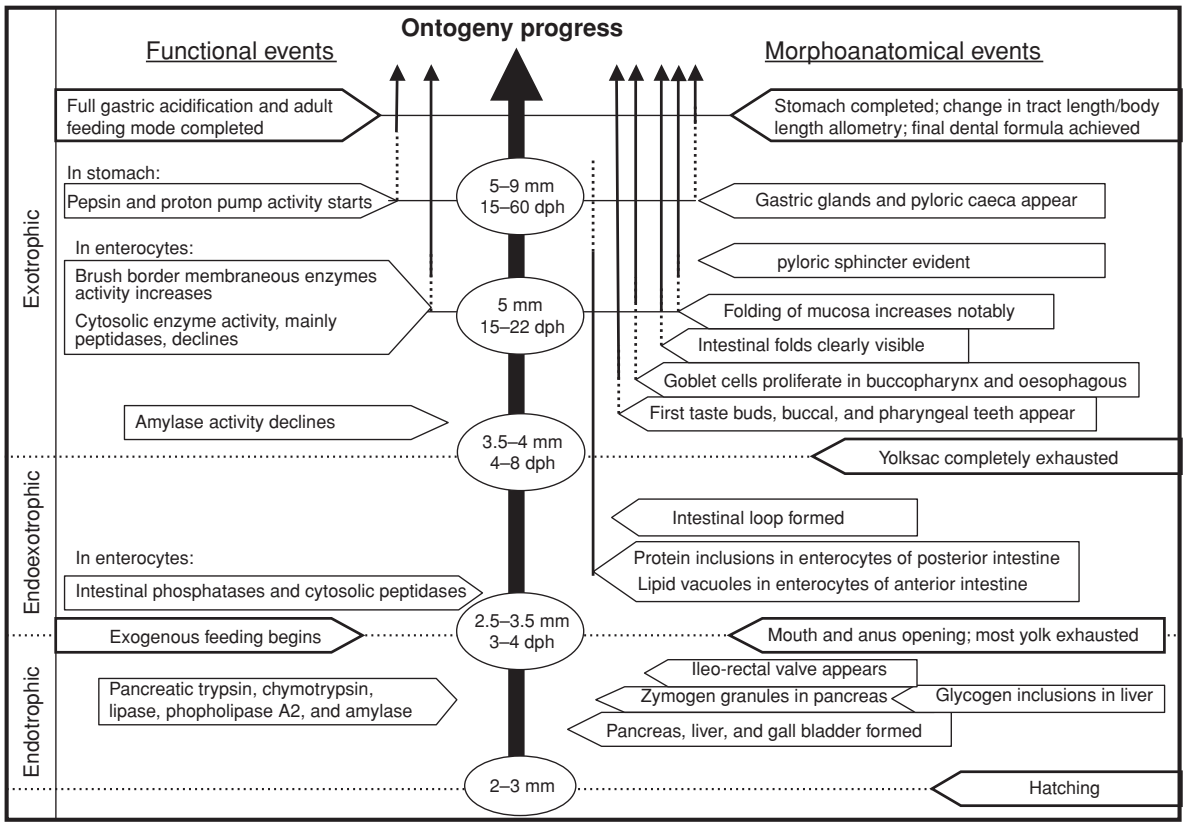

Figure 5.3 General pattern of the ontogeny sequence of morpho-anatomical and functional digestive events.

5.3.2 Functional development of the gut

The gut of larval fish is functional when feeding starts (Kawai & Ikeda 1971; Kawai & Ikeda 1973; Govoni *et al*. 1986; Yúfera & Darias 2007; Zambonino-Infante *et al*. 2008). In sparids, the enzymes responsible for digestion of proteins, lipids, and carbohydrates are already present at first feeding, and the larvae are able to absorb protein and lipids. Zymogen inclusions are present in the pancreas before or at the time of the opening of the mouth (Tanaka 1969b; Sarasquete *et al*. 1995; Diaz *et al*. 2002), and in the liver (Darias *et al*. 2007a). Food items are quickly transported backward and retained by the posterior constriction and excreted from the anus in a short time. The fat droplets present in the midgut enterocytes are indicative of lipidic nutrient absorption, while the vacuoles in the hindgut indicate the important role it is playing in absorbing intact protein macromolecules for their intracellular digestion. Pancreatic enzyme activity patterns during larval development and growth have been determined in several sparids, such as blackhead sea bream (Kawai & Ikeda 1973), gilthead sea bream (Moyano *et al*. 1996), white sea bream (Cara *et al*. 2003), redbanded sea bream (Moyano *et al*. 2005), common pandora (Caruso *et al*. 2001; Suzer *et al*. 2007), sharpsnout sea bream (Suzer *et al*. 2007), blackspot sea bream (*Pagellus bogaraveo*) (Ribeiro *et al*. 2008), and common dentex (Gisbert *et al*. 2009). In general, after the onset of feeding the alkaline protease specific activity tends to increase during early development, mainly due to the contribution of trypsin and chymotrypsin, decreasing in more advanced development stages when the gastric glands appear. Enzymes responsible for the digestion of lipids and carbohydrates are also present early in development. Lipase increases after first feeding and maintains its level of activity throughout larval development at a more or less constant level. Trypsinogen and bile-salt activation of lipase mRNA expression is also detected from hatching in the exocrine pancreas of red porgy and the expression level tends to remain relatively constant during the first month of life, decreasing thereafter (Darias *et al*. 2007b). In most Sparid studies, the amylase specific activity shows a pickup shortly after first feeding and then progressively decreases (Moyano *et al*. 1996; Cara *et al*. 2003; Suzer *et al*. 2006, 2007a; Ribeiro *et al*. 2008), although in common dentex a second increase can be observed during the fourth week of life (Gisbert *et al*. 2009). Gene expression of α-amylase in red porgy is detected from first feeding and is maintained at a high level during the first weeks of development, decreasing thereafter (Darias *et al*. 2006). Sarropoulou *et al*. (2005) found an upregulation of amylase mRNA just before first feeding in gilthead sea bream. This amylase activity pattern suggests the ability of larvae to use carbohydrates during the first days of feeding, a period of high energy demand.

Enzymes of the intestinal enterocytes (amino peptidases, acid and alkaline phosphatases, esterases) are also present at first feeding in sparids (Sarasquete *et al*. 1993b; Moyano *et al*. 1996; Cara *et al*. 2003; Gisbert *et al*. 2009), similar to other marine fishes (Zambonino-Infante & Cahu 2001; Zambonino-Infante *et al*. 2008). After a few weeks, but before the appearance of gastric glands, the digestion in the brush border membrane becomes more efficient, showing an increase in the aminopeptidase activity, while the cytosolic peptidase (leucine-alanine peptidase) activity tends to decline. These changes characterize the maturation of intestinal enterocytes during larval development in fish (Zambonino-Infante & Cahu 2001; Zambonino-Infante *et al*. 2008). With the appearance of the gastric glands, constituted by oxyntopeptic cells that secrete both pepsinogen and hydrochloric acid, there appears an increasing pepsin activity and a progressive decrease in gastric pH in fed larvae (Yúfera *et al*. 2004; Darias *et al*. 2005). Thus, with the development of the stomach, the proteolysis takes place mainly through the action of pepsin in an acid environment, followed by an alkaline proteolysis in the intestine (Yúfera *et al*. 2004). The detection and rising of the pepsin activity are in agreement with the appearance and proliferation of gastric glands in each species. The enzymatic activity is also in association with the detection level of mRNA codifying pepsinogen and proton pump (Darias *et al*. 2005, 2007c). At this transitional stage from larvae to juveniles, when the gastric gland becomes functional and pyloric caeca differentiate, acidophilic granules in the hindgut gradually disappear. These events indicate that the postlarval digestive system is approaching that of the adult, not only in its anatomical structure, but also in its functionality.

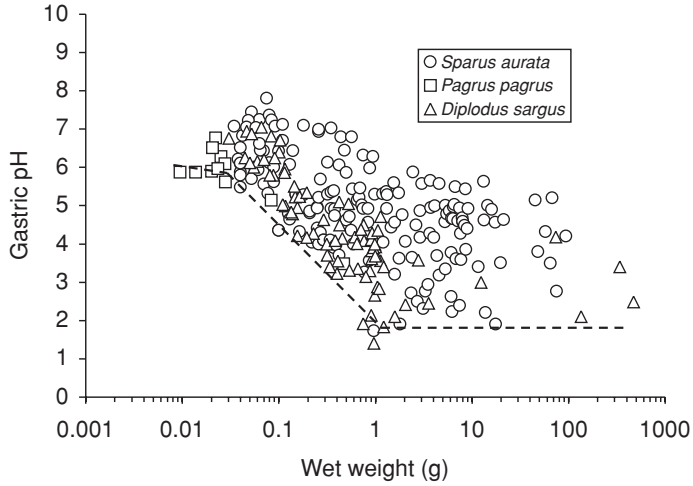

Figure 5.4 Changes in gastric pH in fed postlarvae and juveniles of three Sparidae species. Broken line indicates the minimum values determined throughout the transformation from larvae to juvenile. Sources: Yúfera *et al*. 2004; Darias *et al*. 2005; Yúfera & Moyano 2008.

The functional ontogeny of the digestive tract in sparids is similar to that described in other groups with altricial development. There are some variations among species in the time sequence for the appearance of some tissues and enzymatic activities. The biggest difference among species is perhaps the timing of stomach development, with the differentiation of first gastric glands at 16 DAH in redbanded sea bream (Sánchez-Amaya *et al*. 2007), 20 DAH in red sea bream (Tanaka 1971; Fukuhara 1985), 23 DAH in common dentex (Santamaría *et al*. 2004), 25 days in Western Atlantic sea bream (Tanaka 1971), 26 DAH in red porgy (Darias *et al*. 2007a), 30–39 DAH in sharpsnout sea bream (Micale *et al*. 2008), and >60 DAH in gilthead sea bream (Domeneghini *et al*. 1998; Elbal *et al*. 2004). After the appearance of gastric glands and pepsin activity, the completion of the stomach and full acidic digestion capacity takes some weeks or months depending on the species. Nevertheless, the acquisition of full acidification capacity seems to occur at a similar size irrespective of age (Figure 5.4). The formation of pyloric caeca ends with an allometric change in intestinal length and marks the transition to juvenile form in terms of digestive capacity.

5.4 Growth and energetics

5.4.1 Egg and yolk sac absorption

Sparids, as other teleost species, rely solely on endogenous reserves for only a few days. These reserves, in the form of a yolk sac, are the only nutrient source during the egg and yolk sac stages, that is, from fertilization until first feeding on exogenous prey/diet. Throughout the endogenous stage, fish embryos, or eleutheroembryos, use their yolk reserves for energy supply and growth. There is a high natural selection pressure for greater efficiencies in yolk utilization, resulting in bigger larvae at the onset of exogenous feeding. Bigger larvae are believed to be more competitive, more resistant to starvation, less susceptible to predation and are able to start feeding earlier (Blaxter 1988). Factors such as egg weight, yolk composition, temperature, oxygen, and salinity all affect the rate of yolk absorption and the efficiency of yolk utilization (Heming & Buddington 1988; Kamler 2008). The rate and efficiency of yolk absorption are clearly the main determinants of growth and survival during the early

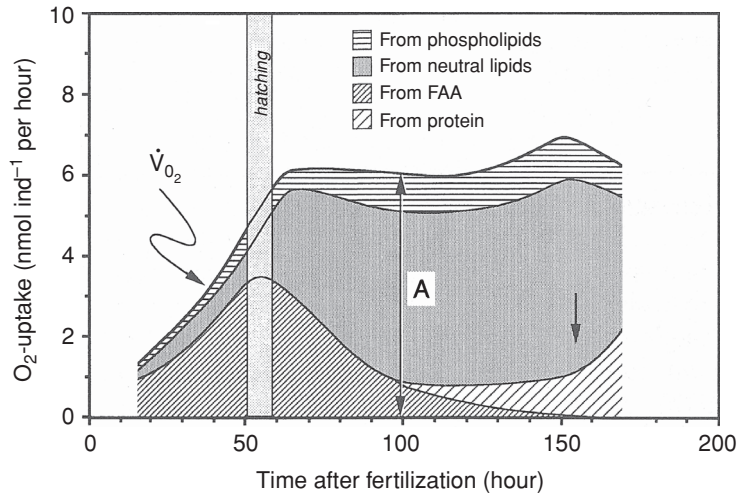

Figure 5.5 Proposed partition of aerobic energy metabolism in developing eggs and larvae of gilthead sea bream, *Sparus aurata*, at 18°C (Reproduced from Rønnestad *et al*. 1994, with kind permission from Springer Science+Business Media).

stages of larval development. For this reason, yolk sac larvae are often used as a biological model, representing a semiclosed system, where nutrient intake can easily be monitored, thus allowing detailed study of energy substrate preferences and dynamics (Rønnestad *et al*. 1999; Kamler 2008).

In teleosts, yolk is absorbed and digested in the syncytium (or periblast), and the resulting nutrients are released to the embryo (Heming & Buddington 1988). Observations using transmission electron microscopy in gilthead sea bream revealed a perivitelline circulation system closely related to the liver (Mani-Ponset *et al*. 1996). Normally, glycogen and other egg carbohydrates are used first as energy substrates in teleosts, followed by free amino acids (FAA) up until hatching (Rønnestad *et al*. 1999; Kamler 2008). This holds true for gilthead sea bream (Rønnestad *et al*. 1994; Mani-Ponset *et al*. 1996). Triacylglycerol mobilization normally follows after hatching in response to an increased energy demand. After depletion of the yolk sac, and unless sufficient energy substrates are provided by exogenous feeding, body protein-bound amino acids (AA) are mobilized (Rønnestad *et al*. 1999; Kamler 2008). For gilthead sea bream, and some other species, mobilization of protein AA occurs toward the end of the yolk sac stage and the absorption of the oil globule primarily after hatching, when the yolk sac becomes depleted (Rønnestad *et al*. 1994). Overall, FAA supply 60–70% of the energy during embryogenesis, while fatty acids (FA) from neutral lipids derived from the oil globule are the main metabolic fuel (80–90%) after hatching (see Figure 5.5), in gilthead sea bream (Rønnestad *et al*. 1994). Neutral lipids and FAA have also been described as the major energy substrates for red sea bream with an apparent greater importance of neutral lipids, in particular, after hatching (Seoka *et al*. 1997).

The sequence, and relative importance, of utilization of energy substrates seems to be species specific (Rønnestad *et al*. 1999). Species with oil globule(s), such as gilthead sea bream, red sea bream, and other sparids, rely less on AA oxidation, and more on neutral lipids, compared to species without oil globule(s) (Rønnestad *et al*. 1999). This may have important implications when formulating diets for first-feeding larvae. Furthermore, while temperature has a major effect on the rate of yolk absorption in both red sea bream (Fukuhara 1990) and gilthead sea bream (Yúfera *et al*. 1993a), no major differences seem to occur in metabolic substrate use during the yolk sac stage development of gilthead sea bream in the 16–24°C range (Yúfera *et al*. 1993a).

5.4.2 *Larval growth*

Growth in Sparid larvae is influenced by many exogenous factors with temperature and food (see Section 5.6) being the most important. Other important abiotic factors in culture include oxygen, salinity, turbidity, and light (intensity and photoperiod). Optimal requirements are species-specific and change during larval ontogeny (Barnabé 1990; Howell *et al*. 1998; Shields 2001). Using the ambient combinations of these abiotic factors or developmental features obtained from studies of larvae in the wild is not always optimal or relevant for intensively reared larvae (Tandler *et al*. 1989, 1995; Koumoundouros *et al*. 1999a; Fielder 2002). Sparid larval growth is affected by the duration of the transition period from endogenous to exogenous feeding (Yúfera *et al*. 1993a; Parra & Yúfera 2000). Development and growth is particularly affected during the early exogenous phase when larvae exhibit high developmental plasticity in allometric growth (Matsuoka 1987; Fukuhara 1991; Koumoundouros *et al*. 1999a; Russo *et al*. 2007). The pattern of development is typically from a longitudinally elongated body shape to a longitudinally compressed form often characterized by a large skull and jaws (Fukuhara 1985, 1987; Koumoundouros *et al*. 1999a; Loy *et al*. 2001). Sparid larvae tend to grow quickly, with considerable selective pressure for rapid growth to avoid predation, enhance organ development, and hence effective capture and digestion of prey, as discussed above (Howell *et al*. 1998; Koumoundouros *et al*. 1999a; Zambonino-Infante *et al*. 2008). Growth generally declines and becomes more variable during metamorphosis and weaning, associated with increased metabolic activity, then becomes more rapid following metamorphosis (Tsukashima & Kitajima 1982; Fukuhara 1991; Leu 1994; Ishibashi *et al*. 2005). The best information on the influences of abiotic factors on growth in sparids comes from studies within the genus *Sparus* (Barnabé 1990; Chatain 1997; Parra & Yúfera 2000; Shields 2001) and *Pagrus* (Foscarini 1988; Fukusho 1991; Fielder *et al*. 2002, 2005).

Sparids grow relatively quickly during early development reaching around 5 mm TL in 10 days, 5–10 mm in 20 days, and 10–40 mm in 40 days (Figure 5.6). Comparisons of growth among and within species are difficult

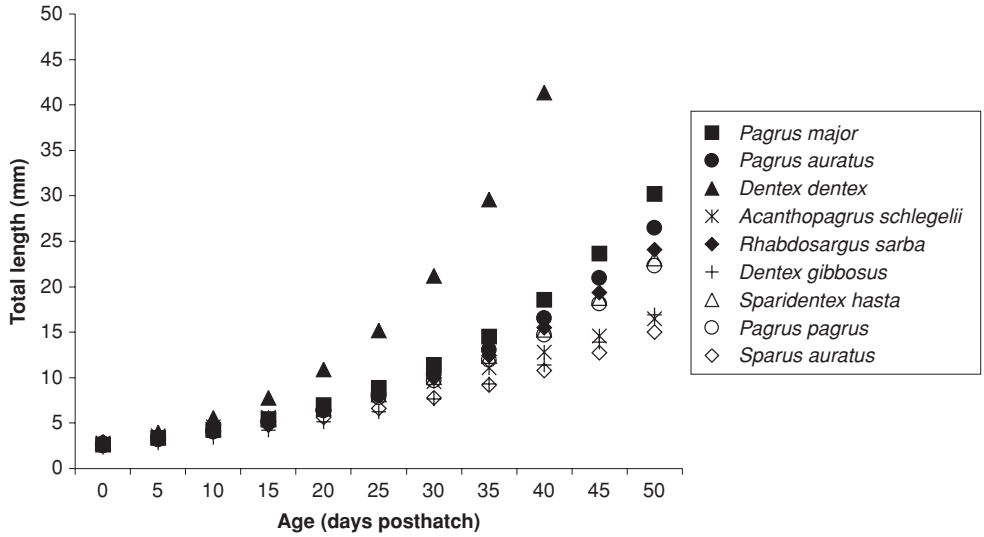

Figure 5.6 Larval growth in total length (mm) of different Sparid species with age (days). Sources: Sobaity bream, *Sparidentex hasta*, synonym *Acanthopagrus cuvieri* (Hussain *et al*. 1981); blackhead sea bream, *Acanthopagrus schlegelii* (Fukuhara 1987); gilthead sea bream, *Sparus aurata* (Russo *et al*. 2007); red sea bream, *Pagrus major* (Fukuhara 1985); red porgy, *Pagrus pagrus* (Roo *et al*. 2009); snapper, *Pagrus auratus* (Battaglene & Talbot 1992); common dentex, *Dentex dentex* (Koumoundouros *et al*. 1999a); pink dentex, *Dentex gibbosus* (Fernández-Palacios *et al*. 1994); goldlined sea bream, *Rhabdosargus sarba* (Leu 1994).

because larval data are reported in a range of metrics (e.g., total and standard length, wet and dry weight) over different rearing periods and for larvae reared under a wide range of densities, environmental, experimental, and culture conditions. Weight-specific data are available for common dentex, snapper, red sea bream, and gilthead sea bream under a range of conditions (Tandler & Helps 1985; Houde 1989; Koumoundouros *et al*. 1999a; Fielder *et al*. 2008). In general, temperature changes have a more profound effect during early life, and embryos and larvae tend to be more stenothermal than juveniles and adults (Rombough 1988). There is a positive relationship between increasing temperature, and growth and development for many sparids (Person-Le Ruyet & Verillaud 1980; Tandler *et al*. 1989; Fielder 2002). Tolerance to temperature and other abiotic factors temporarily declines around metamorphosis (Ishibashi *et al*. 2003). Postmetamorphosis sparids generally tolerate a wider temperature range, for example, snapper from 4 to 31.5°C (Battaglene & Talbot 1992; Fielder 2002). Similar results have been demonstrated for other early stage sparids, including gilthead sea bream (Person-Le Ruyet & Verillaud 1980; Tandler *et al*. 1989; Tandler 1993), red sea bream (Foscarini 1988; Mihelakakis & Yoshimatsu 1998), and goldlined sea bream (Mihelakakis & Kitajima 1994).

Some species of sparids are euryhaline (Woo & Fung 1981; Tucker 1987; Foscarini 1988; Mancera *et al*. 1993; Kelly & Woo 1999; Kelly *et al*. 1999), an attribute that has enhanced their suitability for aquaculture (Wu & Woo 1983; Tucker 1987; Fielder *et al.* 2001; Fielder 2002). The ability to osmoregulate over a range of environmental osmolalities has been investigated for sheepshead (Tucker 1987), red sea bream (Woo & Fung 1981), goldlined sea bream (Kelly & Woo 1999), blackhead sea bream (Kelly *et al*. 1999), gilthead sea bream (Mancera *et al*. 1993), and snapper (Fielder *et al*. 2005). The interactive effects of temperature and salinity on embryonic and larval survival have been investigated for goldlined sea bream (Mihelakakis & Kitajima 1994), red sea bream (Mihelakakis & Yoshimatsu 1998), and snapper (Fielder *et al*. 2002). In most cases, the effect of temperature far outweighed that of salinity. Salinity in combination with light can affect buoyancy of sparid larvae, especially at night, which in turn can influence the ability of larvae to swim to the water surface to gulp air and inflate their swim bladder (Kitajima *et al*. 1993; Battaglene 1995). Similarly, osmotic stress associated with high salinity (~40–45‰) can also reduce swim bladder inflation in gilthead sea bream (Tandler *et al*. 1995). Successful swim bladder inflation is also reduced at the upper or lower extremities of the tolerable range for temperature or salinity in snapper (Fielder 2002) and gilthead sea bream (Tandler *et al*. 1995), impacting on growth.

Similar to most marine fish larvae, sparids are visual feeders and require light to feed and their growth can be increased by longer photoperiods and, hence, feeding duration (Tandler & Helps 1985; Boeuf & Le Bail 1999; Fielder *et al*. 2002). A minimum threshold of 100–150 lux is required to develop hunting activity in gilthead sea bream, and first feeding may take up to 2–3 days to learn (Ounais-Guschemann 1989; Parra & Yúfera 2000). Optimal growth occurs over a range of light intensities from 600 to 1300 lux and is also influenced by shading (Tandler & Mason 1983). The optimal photoperiod for survival is not necessarily the same as that for optimal growth and may change during ontogeny (Chatain 1997; Fielder *et al*. 2002). Tandler and Helps (1985) demonstrated that continuous light had a positive effect on growth rate of gilthead sea bream larvae compared to those grown under 12L:12D. While growth of larvae was greatest in continuous light (1000–3500 lux), survival was best at an intermediate photoperiod of 15L:9D (Peguin 1984, cited in Tandler & Helps 1985; Tandler 1993). Increasing the dark phase tends to promote swim bladder inflation in sparids (Kitajima *et al.* 1993; Battaglene 1995; Fielder *et al*. 2002). For example, swim bladder inflation was greatest at 12L:12D in snapper, but once larvae inflated their swim bladders, growth of larvae was best at a 18L:6D. Optimal light conditions are directly linked to feeding and larval densities, which in turn are influenced by addition of moderate amount of microalgae (greenwater technique) (Foscarini 1988; Partridge *et al*. 2004). Higher levels of prey can promote feeding and better larval growth under ambient light for Western Atlantic sea bream, but at low levels of prey larval growth increased with longer photoperiods (Dowd & Houde 1980).

Other factors influencing swim bladder inflation in sparids vary among species and include genetics, egg quality, water quality, and tank hydrodynamics (Chatain & Ounais-Gushemann 1990; Tandler *et al*. 1995). The

gilthead sea bream (Chatain & Ounais-Gushemann 1990; Sarasquete *et al*. 1995), red sea bream (Yamasita 1982; Kitajima *et al*. 1994), and snapper (Ling 1990; Battaglene 1995; Fielder *et al*. 2002) are three sparids species that have been studied in detail. The transient physostome larvae inflate their swim bladders by gulping air at the water surface (Kitajima *et al*. 1981; Chatain & Ounais-Guschemann 1990). The importance of low water turbulence and removal of surface films to optimize swim bladder inflation of these species has also been demonstrated (Foscarini 1988; Chatain & Ounais-Gushemann 1990; Battaglene & Talbot 1992). Swim bladder inflation usually starts when endogenous yolk sac reserves are depleted and exogenous feeding begins, and typically continues for several days until the pneumatic duct closes (Kitajima *et al*. 1981; Chatain & Ounais-Guschemann 1990; Battaglene 1995). Sparid larvae without functional swim bladders grow poorly, display high degrees of spinal deformities and experience significant stress-related mortality (Paperna 1978; Chatain & Dewavrin 1989; Battaglene & Talbot 1992; Kitajima *et al*. 1994).

Behavioral responses also influence growth, and growth variation in sparids with antagonistic behavior and cannibalism is common in various species, especially around metamorphosis and weaning (Hussain *et al*. 1981; Fukuhara 1985; Tawada 1986; Foscarini 1988; Tandler *et al*. 1989; Hecht & Pienaar 1993; Hecht *et al*. 1996; Leu 1997; Koumoundouros *et al*. 1999a; Teng *et al*. 1999). Faster growth in gilthead sea bream has been shown to promote size variation, which promotes aggressive behavior, and optimal temperatures for survival are lower than for growth (Tandler *et al*. 1989). Growth in larvae around metamorphosis is controlled by social interactions, variations in feeding activity, and the availability of food. Agonistic behavior is more rare in omnivores, such as sheepshead sea bream and goldlined sea bream (Tucker 1987; Leu 1994).

Although the optimal conditions for abiotic factors are clearly species-specific, there are close similarities in optimal rearing regimes between some sparids (Tandler 1993; Chatain 1997; Shields 2001; Fielder 2002). Stocking density is another important consideration in comparing growth rates among studies, which may vary from 6 to 15 larvae L^{-1} for snapper (Fielder 2002), 12–72 larvae L^{-1} for red sea bream (Fukusho 1991), and 50–100 larvae L^{-1} for gilthead sea bream (Tandler *et al*. 1989; Chatain & Ounais-Guschemann 1990; Parra & Yúfera 2000).

Microbial management of culture water and live feeds through water treatment, disinfectants, probiotics, and stimulation of the immune system can also have important influences on growth of sparid larvae (Grisez *et al*. 1997; Balebona *et al*. 1998; Nakase *et al*. 2007). A range of other water quality parameters have been shown to influence growth in sparids and these include ammonia, pH, and oxygen, often in combination with temperature and salinity (Parra & Yúfera 1999, 2002; Fielder 2002; Hassell *et al*. 2008). Ammonia can depress growth at low levels (0.05–0.15 mg L^{-1} NH_3–N) in red sea bream (Guillen *et al*. 1993), and gilthead sea bream are reportedly highly sensitive at >0.024 ppm NH_3–N (Parra & Yúfera 1999).

5.5 Larval nutrition

5.5.1 Macronutrients

There is little knowledge on macronutrient requirements of sparids, as in marine fish larvae in general. Watanabe *et al*. (1978b) evaluated the nutritional quality of live feeds as protein sources by determining their amino acid composition, digestibility, protein efficiency ratio (PER), and net protein utilization (NPU). There were no marked differences in amino acid composition of various kinds of live feeds, except a low threonine content in *Artemia* spp. The digestibility of protein in rotifers (*Brachionus* spp.) was as high as 89–94%, regardless of whether they were cultured in baker's yeast, freshwater, or marine *Chlorella*. Some more detailed studies exist for early juveniles of red sea bream (e.g., Takeuchi *et al*. 1991) suggesting high protein and *n*-3 HUFA requirements. This is also likely to be true for red sea bream larvae. Sugita *et al*. (2007) reported that red sea bream fingerlings cannot utilize effectively a high carbohydrate diet, which is also most probably valid for larvae as well.

5.5.2 Essential fatty acids

After the development of mass culture technology for rotifers in Japan, using marine microalgae, mainly *Nannochrolopsis oculata* (Maruyama *et al*. 1986) and baker's yeast in the 1960s, the production of cultured red sea bream juveniles developed remarkably quickly. However, serious mortality and malformation still occurred in the 1970s (Takashima 1978; Kitajima *et al*. 1981), which prompted a series of seminal research studies into the nutrition of live feeds (Watanabe *et al*. 1978a, 1978b, 1978c, 1979). In summary, Watanabe *et al*. (1979) pointed out that *n*-3 highly unsaturated fatty acids (*n*-3 HUFA) were essential for red sea bream and that high mortality was due to an essential fatty acid (EFA) deficiency. To overcome the problem, a new kind of yeast (ω-yeast) was developed as a food for rotifers (Kitajima *et al*. 1980a). The ω-yeast was produced by supplementing Pollock liver oil or cuttlefish liver oil at levels of 8–15% into the culture medium of baker's yeast. The highest dietary value was obtained in the rotifers cultured with ω-yeast produced by supplementing cuttlefish liver oil at a 15% level. It was demonstrated that the content of *n*-3 HUFA in the rotifers was the principal factor in the nutritional quality of rotifers as a live feed for finfish larviculture (Kitajima *et al*. 1980a, 1980b). The minimum requirement for *n*-3 HUFA in larval red sea bream was determined to be 0.4% WW rotifer^{-1} (Izquierdo *et al*. 1989). Research then focused on determining which *n*-3 HUFA, for example, eicosapentaenoic acid (20:5*n*-3, EPA) and docosahexaenoic acid (22:6*n*-3, DHA) were the most important EFA for normal growth. Watanabe *et al*. (1989) and Takeuchi *et al*. (1990) determined the efficacy of EPA and DHA as the EFA for larval red sea bream by feeding rotifers enriched with methyl oleate, EFA, DHA, and a *n*-3 HUFA mixture. The growth and survival rates were most effectively improved by incorporation of EPA and DHA or an *n*-3 HUFA mixture into the rotifers. The level of assimilation of DHA was much higher than EPA in larvae, although EPA may have partly been converted to 22:5*n*-3, and there was no retroconversion of DHA. The DHA was found to be superior to EPA as an EFA for larval red sea bream (Watanabe *et al*. 1989). Takeuchi *et al*. (1990) demonstrated that the requirement of EPA and DHA was, respectively, around 1% and 0.5% dry matter in the diet of juvenile red sea bream, and the EFA efficiency of DHA was about twice as high as that of EPA. There was no additive effect of EPA and DHA on growth and feed efficiency (Takeuchi *et al*. 1990).

The growth rate of red sea bream juveniles was greatly influenced by dietary *n*-3 HUFA levels, a suitable level being approximately 20% in dietary lipid (Takeuchi *et al*. 1992). There exists a functional difference between EPA and DHA (Watanabe 1993). Both DHA and EPA are important structural components of biological membranes, while EPA is also a precursor of essential metabolites, such as eicosanoids (Sargent *et al*. 1989). Another important precursor of eicosanoids is arachidonic acid (20:4*n*-6, ARA) also an EFA (Bell *et al*. 2003). In addition, it has subsequently been shown that not only the absolute amounts, but also the ratios between these three EFA are crucial for optimal marine fish larval performance and quality (Sargent *et al*. 1999; Bell *et al*. 2003).

The DHA, EPA, and to a lesser extent ARA have a major role in growth performance and survival of gilthead sea bream larvae as well (Izquierdo *et al*. 2000). A low dietary intake of ARA has been proposed to improve stress resistance of gilthead sea bream (Koven *et al*. 2001a). Furthermore, high dietary ARA may also have negative effects when gilthead sea bream larvae are under chronic stress (Koven *et al*. 2003). Dietary DHA and EPA deficiency has also been shown to induce a reduced visual response in larvae, probably through a delay in the functional development of the brain and vision (Benítez-Santana *et al*. 2007). A low DHA content in rotifers has also been demonstrated to lead to a high incidence of skeletal deformities in red porgy larvae (Roo *et al*. 2009).

5.5.3 Amino acids

Growth is essentially protein deposition and over 50% of dry matter (DM) of fish larval tissues is protein. In addition, amino acids (AA) are a major energy source during the early life stages of sparids and other marine

teleost species (Rønnestad & Fyhn 1993; Sivaloganathan *et al*. 1998; Parra *et al*. 1999; Rønnestad *et al*. 1999; Finn *et al*. 2002; Saavedra *et al*. 2008a). Together, this means that fish larvae have very high AA requirements in order to realize their tremendous growth potential (Kamler 1992; Conceição 1997; Otterlei *et al*. 1999). Therefore, the supply of dietary protein of suitable quality and quantity is paramount for optimal larval growth. Nonetheless, assessing protein quality for larval fish is a difficult task as the larval digestive system is immature at first feeding (Govoni *et al*. 1986; Rønnestad & Conceição 2005), and the capacity to digest and/or absorb complex proteins is limited (e.g., Rust 2002; Rønnestad & Conceição 2005; Rønnestad *et al*. 2007). Consequently, our knowledge of AA requirements of sparids and other marine fish larvae remains limited and is mainly qualitative rather than quantitative.

Larvae have higher total AA requirement than older fish (Dabrowski 1986; Fiogbé & Kestemont 1995). However, no precise requirements are available for sparids or other marine teleost larvae. This is related to the small size (∼2–3 mm) of most marine fish larvae at first feeding (Table 5.1), and difficulties in acceptance of inert microdiets by larvae. The use of live food in AA studies with fish larvae is also very limited by the difficulties in the manipulation of the AA composition of live prey (Kolkovski 2001; Conceição *et al*. 2003b; Aragão *et al*. 2004a), in particular the protein-bound fraction. Nevertheless, liposomes and lipid spray beads have been shown to be an effective tool to enrich live feed with free AA (Langdon & Buchal 1998; Tonheim *et al*. 2000; Önal & Langdon 2004, 2005; Barr & Helland 2007). Free AA contents can be boosted up to threefold using liposomes (Barr & Helland 2007). However, success in enriching rotifers with individual AA is variable, ranging from 1.2-fold for tyrosine to 4.7-fold for phenylalanine (Saavedra *et al*. 2008a, 2009a). Enrichment also depends on each AA's natural abundance in the rotifer free AA (FAA) pool and on the AA solubility in water. However, since FAAs comprise less than 6% of the total AAs of both rotifers and *Artemia* (Conceição 1997; Øie *et al*. 1997), the capacity to manipulate live prey's total AA profile remains limited. In addition, studies trying to manipulate AA composition of microdiets have also faced difficulties in controlling dietary AA levels, as high leaching losses often occur when crystalline AA are added to microdiets (López-Alvarado *et al*. 1994; Yúfera *et al*. 2002; Aragão *et al*. 2007). Losses in microdiets are not restricted to FAA, as soluble protein is also prone to leaching (Nordgreen *et al*. 2008).

The indispensable (or essential) AA (IAA) profile of larval fish carcass has been proposed as a good index of the IAA requirements of larval fish (Watanabe & Kiron 1994) as they are for juvenile and adult fish (Wilson 1994; Mambrini & Kaushik 1995). Beneficial effects of microdiets with balanced IAA profiles, based on the larval fish carcass AA composition, have been demonstrated in sparids. For example, survival of gilthead sea bream larvae was higher (Aragão *et al*. 2007), and the proportion of deformed larvae and ammonia excretion lower in white sea bream fed IAA balanced microdiets compared to an unbalanced IAA profile microdiets (Saavedra *et al*. 2009b). Available studies on changes in larval AA profiles of sparids suggest that AA profiles are relatively constant during ontogeny (Aragão *et al*. 2004b; Saavedra *et al*. 2006, 2007). Mean values are presented in Table 5.2. Still, a small but significant increase in arginine and a decrease in methionine contribution to the larval AA profile of gilthead sea bream were detected between early and late larval stages (Aragão *et al*. 2004b).

Larval AA profiles can be compared to dietary AA profiles to identify potential imbalances (Tulli & Tibaldi 1997; Conceição *et al*. 2003b). Rotifers in particular seem to have an unbalanced AA profile for Sparid fish larvae. Aragão *et al*. (2004b) suggested that rotifers are deficient in histidine, arginine, and lysine for gilthead sea bream larvae. Rotifers seem also unbalanced in histidine, arginine, lysine, threonine, and cysteine for both white sea bream (Saavedra *et al*. 2006) and sharpsnout sea bream (Saavedra *et al*. 2007). Histidine is probably the first-limiting AA when rotifers are fed to any of these three Sparid species. Also *Artemia* AA imbalances are apparent for sparids. Threonine, arginine, cysteine, and tyrosine are potentially in deficiency when *Artemia* metanauplii are fed to both white sea bream (Saavedra *et al*. 2006) and sharpsnout sea bream (Saavedra *et al*. 2007). However, AA imbalances when *Artemia* metanauplii are fed to gilthead sea bream seem mild (Aragão *et al*. 2004b).

Table 5.2 Whole-larvae amino acid profiles (g/100 g AA) for Sparid species

AA	*Sparus aurata*	*Diplodus sargus*	*Diplodus puntazzo*	*Pagrus major*
His	2.6	2.8	2.7	2.8
Thr	3.7	5.4	5.3	4.4
Arg	7.1	8.6	8.4	6.8
Val	5.8	5.3	5.6	4.8
Met	2.4	2.2	1.7	2.1
Phe	4.7	4.6	4.8	4.3
Ile	4.7	4.1	4.4	4.3
Leu	9.2	8.6	8.9	9.5
Lys	8.5	9.1	8.1	7.2
Tyr	4.4	4.4	4.5	3.9
Cys	0.3	1.6	0.8	—
Asp	10.7	8.6	9.7	9.6
Glx	13.2	13.5	13.1	17.2
Ser	5.5	5.7	6.0	4.5
Gly	6.5	5.4	5.5	6.2
Ala	7.2	5.9	6.0	6.5
Pro	3.6	4.8	4.9	5.8

Source: Aragão *et al*. 2004b, Saavedra *et al*. 2006, Saavedra *et al*. 2007, López-Alvarado & Kanazawa 1994.

Dietary AA imbalances may have a major impact in AA utilization by fish larvae. They tend to cause increased AA oxidation and lead to decreased food conversion efficiencies (Fauconneau *et al*. 1992). Aragão *et al*. (2004b) estimated that more than 60% of the AA intake may be unavoidably lost because of the imbalance between the rotifer and gilthead sea bream larval AA profiles. In fact, body proteins are the only storage of AAs in larvae, and AAs that cannot be used for protein synthesis will be either used for energy production, or irreversibly deaminated and directed to lipid synthesis (Conceição *et al*. 2003b). Such AA losses will also be reflected in higher AA requirements and may have particular importance in fish larvae, which have a growth potential of up to 50% per day (Houde 1989; Conceição *et al*. 1998a). However, some AAs are always used for energy production, even if there is a perfect match between dietary and larval AA profiles. Amino acids are a major energy substrate for fish larvae (Dabrowski 1986; Rønnestad & Naas 1993; Conceição *et al*. 1998b). However, dispensable (or nonessential) AAs are known to be preferentially used by fish larvae for energy production (Rønnestad *et al*. 2001; Conceição *et al*. 2002), and at least in fast growing (and high feed intake) fish larvae these obligatory AA losses are probably much smaller (in% of absorbed AA) than in slower growing larger fish (Conceição *et al*. 2003b).

In fact, the larval AA profile is only a rough indicator of larval AA requirements. Differential absorption and selective catabolism of individual AAs may reduce (or amplify) the impact of the dietary imbalances on AA losses. Methionine and arginine were shown to be better absorbed than tryptophan, tyrosine, and specially lysine by white sea bream larvae (Saavedra *et al*. 2008a, 2008b) using ^{14}C-AA and a tube-feeding methodology. Catabolism of individual IAA has also been shown to vary in white sea bream larvae (Saavedra *et al*. 2008a, 2008b), being higher for tyrosine, intermediate for methionine, tryptophan, and lysine, and lower for arginine. A method combining high resolution ^{13}C-NMR spectroscopy and the use of ^{13}C-labeled live food has been used to show that the relative bioavailability of individual AA is variable in gilthead sea bream larvae (Conceição *et al*. 2003a). A similar study using ^{15}N-enriched rotifers in combination with GC-IRMS in sharpsnout sea bream (Saavedra *et al*. 2007) showed several differences in relative bioavailabilities between AAs.

So far, AA requirements have been discussed in terms of growth. However, some AAs are precursors of molecules other than structural proteins, such as purines, hormones, and neurotransmitters. So, additional AA requirements to the ones for growth may exist. Phenylalanine supplementation in the diet decreased the incidence of skeletal deformities, while tyrosine supplementation increased resistance to a temperature stress in white sea bream (Saavedra *et al*. 2010), perhaps reflecting the role of these AAs as precursors in the synthesis of thyroid hormones and catecholamines.

5.5.4 Micronutrients

While the metabolism of Sparid larvae and juveniles is controlled by micronutrients, such as vitamins and minerals (Kolkovski *et al*. 1997), there is little published literature on the subject. Snapper larvae require dietary vitamin C (AsA) for normal development and can be cultured from early development stages by feeding only microbound diets containing relatively high contents of AsA of >768 mg kg^{-1} diet (Ren *et al*. 2008). Ascorbyl-2-monophosphate Na/Ca (AMP-Na/Ca) is a bioavailable AsA source for red sea bream juveniles, and supplements of >107 mg AsA kg^{-1} in diets improve blood chemistry, such as total cholesterol and triglyceride in plasma, and nonspecific immune function (Ren *et al*. 2008).

Tocher *et al*. (2002) investigated the interaction of the dietary antioxidant micronutrient, vitamin E (α-tocopherol), with antioxidant defense systems. Juvenile gilthead sea breams were fed with diets of identical unsaturation index supplemented with graded amounts of α-tocopherol. The relationships between dietary and subsequent tissue α-tocopherol levels were determined, as well as the effects of α-tocopherol supplementation on lipid and fatty acid compositions of both liver and whole fish, on the activities of the liver antioxidant defense enzymes, and on the levels of liver and whole-body lipid peroxidation products, malondialdehyde (thiobarbituric acid reactive substances, TBARS) and isoprostanes. Feeding the diet with the lowest α-tocopherol resulted in significantly decreased survival and growth. The indicators of lipid peroxidation were highest in fish fed with the unsupplemented diet and lowest in fish fed with the diet with the highest α-tocopherol.

Excess and deficiency of vitamin A in live food affect the health of cultured red sea bream. The vitamin A concentration of rotifers is an important issue in the occurrence of skeletal deformity. Rotifers with vitamin A concentrations of 212 IU g^{-1} are recommended for improved health of cultured juveniles (Fushimi *et al*. 2005). The vitamin A concentration of *Artemia* nauplii (50–2831 IU g^{-1}) does not cause skeletal deformities in red sea bream larvae previously fed on rotifers with an appropriate vitamin A concentration (Fushimi *et al*. 2005). Excess vitamin A in rotifers has also been shown to increase the incidence of skeletal deformities in gilthead sea bream larvae (Fernández *et al*. 2008).

White sea bream larvae fed vitamin B_2 (riboflavin) enriched rotifers had a better growth performance and survival (Souto *et al*. 2008). However, in the same study, vitamin B_2 levels in rotifers had no effect on gilthead sea bream performance. Therefore, different Sparid species may have different vitamin B_2 requirements during the larval phase.

Calcium (Ca) and phosphorus (P) are predominant minerals in fish and are mostly located in the skeletal tissues. Terrestrial animals absorb calcium and phosphorus mainly from their diet, but fish can uptake these elements from the water, in addition to the diet. The dietary calcium and phosphorus content and the Ca/P ratio affect noticeably the growth rate and feed efficiency of cultured red sea bream. The dietary Ca/P ratio, optimal at a 340 mg% Ca level for the growth of red sea bream, is about 1:2. The Ca/P ratio of blood serum reflects that the best growth of fish is achieved when fed with a diet that has a Ca/P ratio of 1:2 (Sakamoto & Yone 1973).

Manipulation of live food nutrition is essential for good hatchery protocols today. However, much less attention is paid to some nutritional components, such as trace elements. Recently, a new methodology has been developed to enrich rotifers and *Artemia* nauplii in zinc and manganese, to the level of these minerals in natural wild zooplanktons. Since minerals are important for skeletal development of fish, mineral supplementation to the level of that in natural zooplankton may be a benefit for normal growth and skeletal development of

cultured fish. Enrichment of rotifers and *Artemia* with zinc and manganese was performed successfully using microalgae as the uptake material. However, direct inclusion of zinc in the culture medium failed to enrich rotifers in zinc (Matsumoto *et al*. 2009). The effect of *Artemia* enriched with zinc and manganese on early growth, survival, proximate composition, and the occurrence of skeletal deformity in red sea bream has also received some attention. Fish fed *Artemia* enriched with zinc and manganese showed significantly improved growth performance and reduced occurrence of skeletal deformity (Matsumoto *et al*. 2009; Nguyen *et al*. 2009).

5.6 Feeding

5.6.1 Live prey

Sparid larvae generally start to feed the day after their mouth opens. The onset of feeding is not as synchronous as in other more voracious species. The percentage of larvae feeding increases during the first few days and in some species a high feeding incidence is attained only when those larvae unable to feed have died (Polo *et al*. 1992; Parra & Yúfera 2000). Similarly, the number of prey within the gut increases quickly during the first few days of feeding (Moteki *et al*. 2001). Only the more active larvae capable of ingesting suitable food are able to obtain energy and nutrients for further prey searching, and consequently to survive and grow. Larvae need to obtain high-quality food above a certain threshold to pass through this "critical period" (Tanaka 1972a). Those larvae that fail to feed, survive on endogenous reserves, and become feeble. The moment for irreversible starvation after the opening of the mouth depends primarily on temperature (Houde 1974; Yúfera & Darias 2007). In Western Atlantic sea bream, the point of no return occurs at 5 DAH at 22°C and 3 DAH 26°C (Houde 1974). In gilthead sea bream, it occurs between 7 and 8 DAH at 20°C (Yúfera *et al*. 1993b), but, as in other species, some effects of food restriction are detected early. Assuming these two examples are representative of the family, Sparid larvae have to start feeding within two days of mouth opening to prevent deleterious consequences.

Currently, the generalized feeding regime for Sparid larvae starts with rotifers as first feed followed by *Artemia* and then formulated feeds. Sparids have small larvae at first feeding and consequently benefit if small-sized *Brachionus* species are fed during the first few days (Yúfera *et al*. 1991; Polo *et al*. 1992). Before the establishment of rotifer mass culture technology, other planktonic prey were tested. The larvae of blackhead sea bream have been fed on dinoflagellates (*Oxyrrhis* spp.), followed by Spirotricha (*Stylonchia* spp.) when they reached 3–3.5 mm TL, then copepod or barnacle nauplii at 4–4.5 mm TL (9–12 DAH), *Artemia* spp. nauplii at 5 mm in TL (15 DAH) and young mysid shrimp at about 7 mm TL, 3–4 weeks after hatching (Kasahara *et al*. 1960). Finally, the larvae were able to feed on chopped fish at 15 mm TL, 6–7 weeks after hatching. Following this method, many trials for blackhead sea bream and red sea bream larviculture were conducted in Japan. In the 1970s, the usual feeding regime for these species was rotifers (or trochophore larvae of bivalve) as first feed, followed by copepoda and then minced fish (Foscarini 1988). Today the most common feeding protocol remains rotifers (small and large), followed by *Artemia* nauplii and metanauplii and commercial feeds. This feeding regime was developed during the 1980s, supported by the establishment of mass culture technology for rotifers, as described earlier (Person-Le Ruyet & Verillaud 1980; Yúfera *et al*. 1991; Fushimi 2001).

Okauchi *et al*. (1980) estimated that larval blackhead sea bream consume 18,500 rotifers from first feeding to 10 mm TL. Daily rotifer consumption increases during the first month from 299 rotifer ind^{-1} at 10 DAH (4.9 mm TL) to 4700 rotifer ind^{-1} at 29 DAH (11.4 mm TL). Similarly, for red sea bream, the larvae are estimated to feed on 60 rotifer ind^{-1} when 3.9 mm TL to 2150 rotifer ind^{-1} at 10.4 mm TL (Fujita 1977 cited in Kitajima *et al*. 1976 cited in Okauchi *et al*. 1980; Foscarini 1988). A similar estimate has been made for gilthead sea bream where ingestion increases during the first month from an average of 15–1000 μg dry matter larvae^{-1} per day (Parra & Yúfera 2001). On a weight specific basis, Sparid larvae tend to ingest more than 100% of their own weight in the first days of feeding. Then, the specific ingestion rate decreases with more

efficient digestion (Yúfera & Darias 2007). However, in gilthead sea bream the specific ingestion rate increases continuously during the first weeks of feeding (Tandler & Mason 1984; Parra & Yúfera 2000). In general, the ingestion rate of larval fish is affected by environmental condition and prey density. Ingestion has a saturation response with increasing prey density (Lika & Papandroulakis 2005). During early larval development, above a feeding rate of 1–2 rotifer mL^{-1} the amount consumed is similar (Okauchi *et al*. 1980; Parra & Yúfera 2000; Lika & Papandroulakis 2005), but relatively low prey densities tend to result in the selection of more vigorous larvae with higher specific ingestion rates (Parra & Yúfera 2000).

As commented above, sparids feed and grow better under appropriate illumination conditions (Tandler & Helps 1985; Huang & Hu 1990, Boeuf & Le Bail 1999; Fielder *et al*. 2002). A circadian rhythm has been observed in red sea bream during the light period. Feeding activity in larvae above 5 DAH is crepuscular with two peaks, at dawn and dusk. The dusk feeding is more vigorous than that at dawn (Kotani & Fushimi 2009). However, Huang and Hu (1990) did not detect significant changes in the feeding activity of goldlined sea bream under continuous illumination during early development. The high feeding activity after the onset of feeding induces a quick decrease in available prey in intensive larval rearing. Consequently, the feeding protocols for marine fish attempt to maintain prey density above a minimum threshold. By doing this, the daily prey demand is satisfied, relatively high predator/prey encounter occurs and punctual or persistent food restrictions are overcome. Rotifer densities between 5 and 20 rotifer mL^{-1} are usual in the larviculture of sparids during the first 2–4 weeks of feeding (e.g., Okauchi *et al*. 1980; Tandler & Helps 1985; Foscarini 1988; Leu & Chou 1996; Sánchez-Amaya *et al*. 2007; Giménez & Estévez 2008). *Artemia* nauplii (and then metanauplii) are generally supplied from 1 to 3 weeks after first feeding at an initial density of 0.5–5.0 nauplii mL^{-1}, increasing thereafter (Battaglene & Talbot 1992; Teng *et al*. 1999; Mihelakakis et *al.* 2001; Güner *et al*. 2004).

5.6.2 Inert diets

Despite considerable progress in microdiet technology in recent years, the first weeks of feeding in larval sparids, as in most other marine larval fish species, still relies mainly on live feeds. Nevertheless, several microdiet types have been used at the experimental level with some degree of success for red sea bream (López-Alvarado & Kanazawa 1994; López-Alvarado *et al*. 1994; Takeuchi 2001), gilthead sea bream (Yúfera *et al*. 1999; Yúfera *et al*. 2000; Koven *et al*. 2001b; Yúfera *et al*. 2005; Seiliez *et al*. 2006), and white sea bream (Saavedra *et al*. 2009a, 2010). In general, these studies showed that microdiets allow investigation of the nutritional requirements of sparids, sustain survival rates comparable to those of live feeds, but growth rates are typically much lower. In contrast with the difficulties in early life stages, weaning onto inert microdiets is already a reality for late-stage larvae of most Sparid species at the commercial scale. Three main problems hinder further progress in the use of microdiets in early larval stages of sparids, and other species: (1) microdiets tend to be less attractive to fish larvae compared to live prey, leading to lower ingestion rates; (2) digestibility of microdiets is usually lower, specially in relation to complex proteins; (3) high leaching losses of soluble molecules (e.g., free AA, peptides, vitamins, and minerals) upon contact with the rearing water. In addition, the lack of knowledge on the precise requirements for several nutrients makes it difficult to formulate complete and well-balanced diets.

The problem of microdiet attractiveness can be overcome partly by the inclusion of protein hydrolysates or free AAs, which have been shown to have a role as attractants (Kolkovski *et al*. 1997; Cahu & Infante 2001; Koven *et al*. 2001b). The free AAs alanine, glycine and arginine, as well as betaine, have been identified to stimulate feed intake in gilthead sea bream (Kolkovski *et al*. 1997). Koven *et al*. (2001b) have also demonstrated that a microdiet supplemented with phospholipids, in particular phosphatidylcholine, increases feed intake. Preparing a highly digestible microdiet while controlling leaching losses is a particularly difficult challenge (Yúfera *et al*. 2002; Kvåle *et al*. 2006). For instance, encapsulation techniques that prevent leaching tend to make digestion very difficult (Langdon 2003). In order to tackle these challenges, several microdiet types have been developed and tested (see review by Langdon 2003), but none seems to solve all problems. Microbound and cross-linked protein-walled capsules may be effective in delivering lipids and high-molecular-weight,

water-soluble nutrients to larvae, such as proteins and carbohydrates, while lipid-walled capsules, lipid spray beads and liposomes are potentially more useful in delivering low-molecular weight, water-soluble nutrients, such as AAs and water-soluble vitamins (Langdon 2003). Complex particles have also been proposed to take advantage of both lipid-walled capsules and microbound diets (Önal & Langdon 2005).

Different diet types have been used for nutritional studies with Sparid larvae with some success. Microbound and microcoated diets have been used to determine the dietary arginine requirements of red sea bream to be at least 2.5% of diet dry matter (López-Alvarado & Kanazawa 1994). Yúfera *et al*. (1999, 2000, 2005) developed two types of microencapsulated diets, the first using cross-linking of casein and other dietary proteins, the second an internal calcium gelation method. Both these diets have been used successfully for gilthead sea bream (Yúfera *et al*. 1999, 2000, 2005) and white sea bream (Saavedra *et al*. 2009a, 2009b, 2010), during the first weeks of feeding, specially when small quantities of rotifers were cofed, resulting in moderate growth rates. Similar results have been obtained in studies on lipid requirements in gilthead sea bream using microbound diets (Salhi *et al*. 1994, 1999; Bessonart *et al*. 1999; Seiliez *et al*. 2006, Ganuza *et al*. 2008).

5.7 Conclusion

Sparids are a commercially important group of fish being already cultured or evaluated for industrial production in many parts of the world. Many biological methods used to develop current commercial marine fish larval rearing techniques were originally developed for sparids. This is particularly true for methods developed first for red sea bream in Japan and gilthead sea bream in the Mediterranean region, whose mass culture was later adapted successfully to other species. Despite extensive similarities in larval biology, development and phenotype, species-specific differences do exist among sparids, partly related to different temperature optima among species. Skeletogenesis and swim bladder inflation are critical aspects in the rearing of most Sparid species. Great progress has been made in both areas, but persistent malformations in cultured fish remain and support an active area of research. Commercial pressure dictates ever-increasing growth performance and survival of juvenile fish, and production at reduced costs. Reducing or eliminating the use of live feeds remains a long-term goal. Overcoming other critical aspects and optimization of performance are likely to come from a better understanding of basic nutritional requirements of larval sparids, as well as of their interactions with abiotic factors such as temperature, light and hydrodynamics at key developmental stages. In addition, the importance of biotic factors, such as microbial control, is also an area of active research.

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