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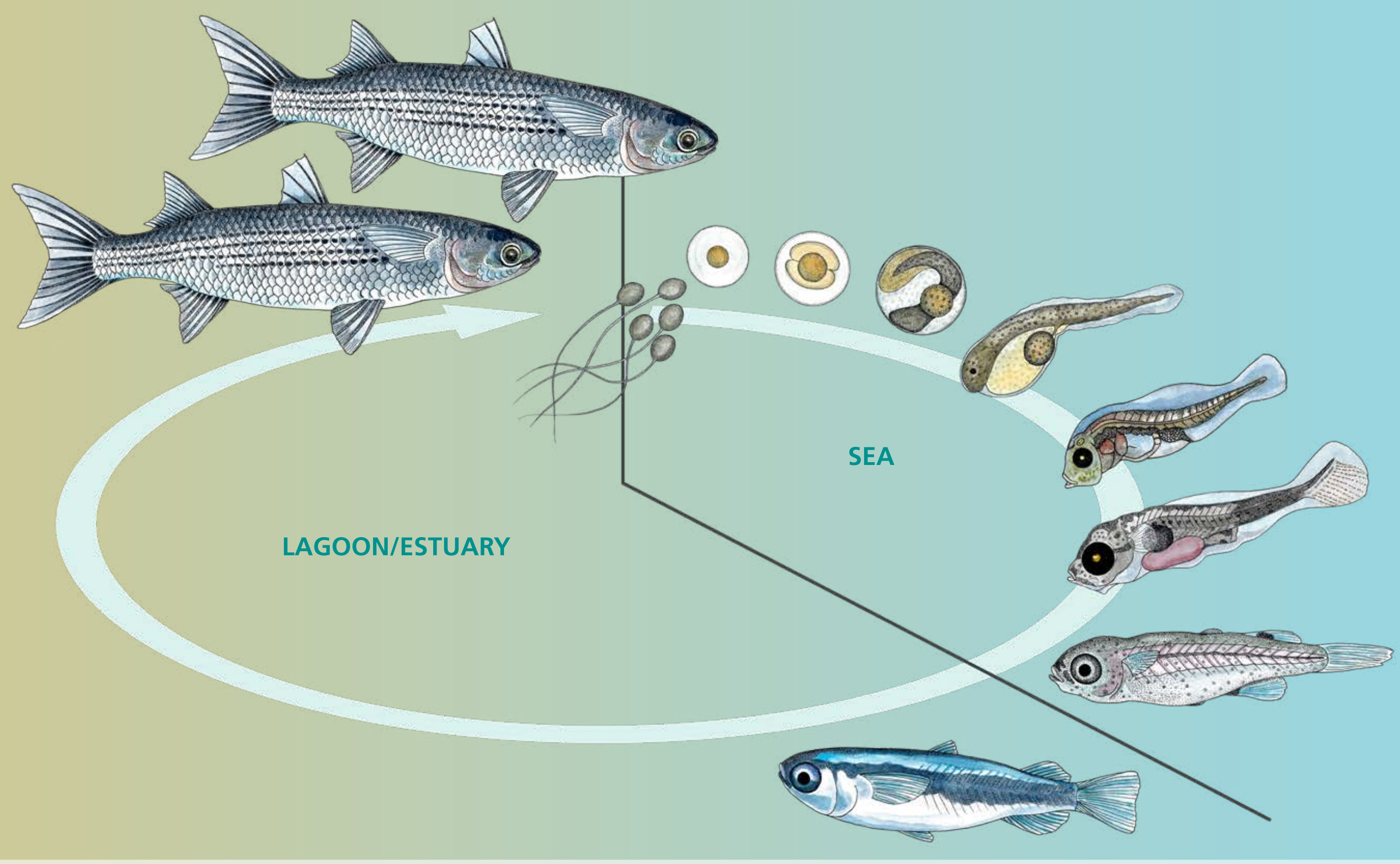

Cover photographs:
Life cycle of the flathead mullet (*Mugil cephalus*): spawning at sea, early growth, and juvenile migration to lagoons and estuaries (Illustration by Massimiliano Lipperi).

Hatchery, nursery and grow-out techniques for the flathead grey mullet (*Mugil cephalus*)

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Preparation of this document

This manual focuses on hatchery production of the flathead grey mullet, *Mugil cephalus*, and provides a practical compilation of techniques covering all stages of its culture. The species is a high-value, low-trophic marine fish with minimal environmental impact when farmed. Consistent and sustainable production is best achieved through hatchery-based seed supply.

The information presented on captive reproduction and larval rearing is based on scientifically grounded experimental studies on the culture of this species. The techniques described have been tested at laboratory and pilot scales and are considered suitable for implementation at commercial scale.

The manual also describes grow-out techniques with demonstrated potential for commercial production and presents hatchery methodologies applicable to both small- and large-scale operations.

The flathead grey mullet is considered a promising low-trophic species for the expansion of global aquaculture due to several desirable traits, including its adaptability to a wide range of rearing conditions, tolerance to challenging environments such as high temperatures and low dissolved oxygen levels, omnivorous feeding habits, and the high nutritional value of its products, including smoked fillets and *bottarga*.

This publication aims to support the development of sustainable aquaculture and to improve its performance in producing nutritious and high-quality aquatic foods. It contributes to the strategic vision of the Food and Agriculture Organization of the United Nations (FAO) under the FAO Blue Transformation Roadmap, which promotes the sustainable intensification and responsible expansion of aquaculture to strengthen food security, livelihoods and environmental sustainability. The approaches and practices presented are consistent with the principles of the Guidelines for Sustainable Aquaculture (GSA), which provide a global framework to guide responsible aquaculture development that supports food security, equitable livelihoods, climate resilience and healthy ecosystems.

Abstract

The global production of flathead grey mullet, *Mugil cephalus*, has increased in recent years, reflecting the growing importance of this species in aquaculture. This manual provides a comprehensive overview of the biology, life history, hatchery design and operational management of *M. cephalus*, a species valued for its adaptability and economic importance.

Mugil cephalus exhibits biological and anatomical characteristics that contribute to its wide geographic distribution and successful life cycle. Key aspects, including sex differentiation, reproduction and larval ontogeny, are described, with particular attention to the development of the digestive and visual systems, fins, axial skeleton, pigmentation and skeletogenesis. Understanding these biological processes is essential for the effective culture of the species.

Hatchery site selection and design are critical factors influencing production success. This manual reviews different culture systems, including open, semi-closed and closed systems, and discusses the biological, environmental, technical, legal, social and economic considerations involved in site selection. Hatchery infrastructure is also described, including seawater supply systems, live feed production units for microalgae and zooplankton, and facilities for larval and juvenile rearing.

Operational aspects of hatchery management are addressed, including the culture of microalgae and rotifers and the production of brine shrimp, *Artemia*. Broodstock management practices are presented, covering the capture and maintenance of wild and captive broodstock, as well as handling, nutrition and spawning under controlled conditions.

The production cycle of *M. cephalus*, from egg incubation and early larval management to larval rearing and post-larval culture, is described with emphasis on environmental parameters, feeding strategies and growth patterns. The larval stage lasts approximately 20–25 days post-hatch (DPH) at 24–25 °C and 30–35 DPH at lower temperatures, after which larvae metamorphose into fry with scales, fully formed fins and an adult-like body form. During metamorphosis, the fish shift from pelagic to more benthic behaviour and can be weaned onto formulated feeds.

Common diseases and parasites affecting *M. cephalus* are also reviewed, and general guidelines for health management in aquaculture systems are provided.

This manual synthesizes current knowledge and practices for the culture of *M. cephalus*, providing guidance for researchers, aquaculture practitioners and industry stakeholders. The integration of biological knowledge with appropriate hatchery design and management practices is essential for the sustainable development and expansion of flathead grey mullet aquaculture.

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Abbreviations

BW	body weight
DHA	docosahexaenoic acid
DNA	deoxyribonucleic acid
DO	dissolved oxygen
DPH	days post-hatching
EIA	enzyme immunoassay
EPA	eicosapentaenoic acid
EU	European Union
FAO	Food and Agriculture Organization of the United Nations
FSHR	follicle-stimulating hormone receptor
GnRHa	gonadotropin-releasing hormone analog
hCG	human chorionic gonadotropin
HDPE	high density polyethylene
IMC	Fondazione IMC – International Marine Centre (Italy)
IRTA	Instituto de Investigación y Tecnología Agroalimentarias (Institute of Agrifood Research and Technology) (Spain)
IU	international unit
LED	light-emitting diode
PCR	polymerase chain reaction
PIT tags	passive integrated transponder tags
PUFA	polyunsaturated fatty acid
PVC	polyvinyl chloride
RAS	recirculation aquaculture system
rGth	recombinant gonadotropin
rLh	recombinant luteinizing hormone
SD	standard deviation
SL	standard length
SNP	single nucleotide polymorphism
SOP	standard operating procedure
TL	total length
UV	ultraviolet
VNN	Viral Nervous Necrosis

CO_2	carbon dioxide
H_2O_2	hydrogen peroxide
KOH	potassium hydroxide
NaOH	sodium hydroxide
NH_3	ammonia
NH_4^+	ammonium ion
O_2	oxygen

≥	equal or greater than
≤	equal or less than
>	greater than
<	less than
nm	nanometre
µm	micron
mm	millimetre
cm	centimetre
m	metre

m^2	square metre
ha	hectare
µg	microgram
mg	milligram
g	gram
kg	kilogram
t	tonne
µl	microlitre
ml	millilitre
L	litre
m^3	cubic metre
s	second
min	minute
h	hour
y	year
µmol	micromole
ppm	parts per million
%	percent
°	degree (angle)
°C	degree Celsius
Lux/lx	illumination unit
W	watt
mJ	millijoule

Introduction

Global production of mullets from 1950 to 2024 shows a long-term expansion driven initially almost entirely by capture fisheries, followed by a rapid and increasingly important rise of aquaculture from the late 20th century onward. From 2000 to 2024, production rose from 504 000 t to over 1.07 million tonnes, with growth increasingly driven by aquaculture. Aquaculture output grew rapidly from roughly 100 000 t in 2000 to more than 400 000 t by 2024, raising its share of total production from about 20 percent to nearly 37–38 percent. This expansion was particularly strong in the early and mid-2000s, when aquaculture's contribution peaked above one-third of total supply, followed by some fluctuations in the early 2010s before resuming steady growth in the late 2010s and early 2020s. In contrast, capture fisheries remained relatively stable in trend, increasing more gradually from around 400 000 t to about 650 000–670 000 t, and thus declining in relative importance despite continued growth in absolute terms. Overall, the period is characterized by a structural shift toward aquaculture as the main engine of production growth, leading to a more balanced contribution between farmed and captured mullets by 2024. In 2024, global production of mullets reached 1.07 million tonnes, including 400 606 t (37.4 percent) from aquaculture.

Among more than 20 genera and over 70 species in the family Mugilidae, the flathead grey mullet (*Mugil cephalus* Linnaeus, 1758) is the most widely cultured species. Its adaptability to different water salinities – ranging from freshwater to full strength seawater – makes it particularly suitable for aquaculture. Furthermore, owing to its benthic feeding behaviour, this species contributes to improving sediment quality in polyculture systems. The flathead grey mullet has a relatively fast growth rate (approx. 1 kg/y), efficiently converts feed into biomass, and is a highly marketable species valued for its high-quality protein content and desirable organoleptic properties. Its fatty acid profile indicates that mullet flesh provides excellent nutritional benefits for human consumption, as reflected in lipid quality indices such as the omega-6/omega-3 ratio. A 150 g serving of *M. cephalus* flesh can largely satisfy the weekly recommended intake of essential fatty acids. In addition, mullet flesh provides a rich source of amino acids and essential minerals, making it a valuable component of a balanced and healthy diet.

Their commercial significance varies among country, with the species being highly valued as food in Egypt, Tunisia and Taiwan Province of China. Mullet can be processed in various ways – filleted, salted or smoked – to produce appealing products with extended shelf life. In the Orbetello Lagoon (Tuscany, Italy), smoked mullet fillets are marketed as a premium product, commanding prices of up to EUR 36/kg.

The salted and dried roe from gravid females, known as *bottarga* in Italy or *boutarque* in France (derived from the Arab word *batarekh*, meaning dried fish eggs), is an expensive delicacy in southern Mediterranean and Asia. In specific regions, such as the western coast of Italy and northern and western Greece, it assumes significant economic importance, with prices exceeding EUR 200/kg. A prime example of this regional significance is the high-quality Greek mullet roe *Avgotaraxo Messolongiou*, a product with protected designation of origin (roe of the flathead grey mullet fish harvested from the Messolongi and Aitoliko lagoons).

Mullet roe is harvested from the gonads of 3–5-year-old females, typically captured at coastal lagoon fish barriers during their spawning migration back to the sea, a phenomenon that occurs in late summer and early autumn. Recognizing the species' remarkable attributes, Nash and Shehadeh (1980) predicted a promising future for the

Mugilidae family in aquaculture, stating that "the Mugilidae have the brightest future of all marine and brackish water finfish in the developing technology of aquaculture." However, despite its wide distribution and popularity, the cultivation of this species continues to face persistent challenges, particularly the ongoing dependence on wild fry collection to supply the aquaculture sector.

STATUS AND TREND OF GLOBAL FLATHEAD GREY MULLET PRODUCTION

The flathead grey mullet is a popular fishery and aquaculture species with a long production history. The harvest of mullets dates back approximately 4 300 years, as evidenced by ancient Egyptian hieroglyphics depicting local aquatic food procurement practices. The first flathead grey mullet production records were published in 1950 (1 040 t), with 90 percent of the production originating from Eastern Asia, where China alone contributed over half of the total output.

Efforts to close the grey mullet's life cycle in captivity began in the early 1970s and achieved initial success in 1977, mainly within experimental and semi-commercial settings. However, large-scale commercial breeding has yet to be fully realized. Despite advances in mullet culture during the 1970s and 1980s, it never reached its full potential. Mullet farming remains almost entirely dependent on wild-caught juveniles. In 2021, Egypt collected 49.5 million mullet fries from the wild to use as seed in aquaculture, including flathead grey mullet (LFRPDA, 2021).

In recent years, the contribution of key producing regions has shifted noticeably. Israel remains a dominant and consistent producer of flathead grey mullet, with an estimated output of 2 050 t annually in both 2023 and 2024, accounting for roughly 34 percent of global production. Taiwan Province of China recorded strong growth in 2024, reaching 2 838 t, up from 1 760 t in the previous year. In contrast, traditional producers such as Greece and Singapore have experienced declining trends. Greek production fell to 124 t in 2024, down from 386 t in 2021, while Singapore's output decreased to 255 t from a peak of 621 t in 2021. Italy has reported zero production of *Mugil cephalus* since 2018, likely reflecting aggregation with other mullet species or differences in national reporting practices, despite continued production of other mullet species.

Egypt represents a unique case due to the sheer scale of its production. Although specific aquaculture figures for *Mugil cephalus* appear relatively low in some global datasets, total mullet production – largely reported under "other mullets" – has continued to expand. Output increased from 366 383 t in 2022 to 377 796 t in 2024. This sustained growth reflects more than four decades of expansion in Egypt's mullet aquaculture sector since the first official records in 1984.

Despite these advances, a significant gap persists between fry supply and demand. Most seed producers remain unable to meet the needs of local fish farmers, forcing continued reliance on wild-caught juveniles. Recent breakthroughs in captive breeding offer promising prospects for the development of reliable commercial fingerling production – an essential step toward reducing dependence on wild stocks and supporting the sector's long-term sustainability.

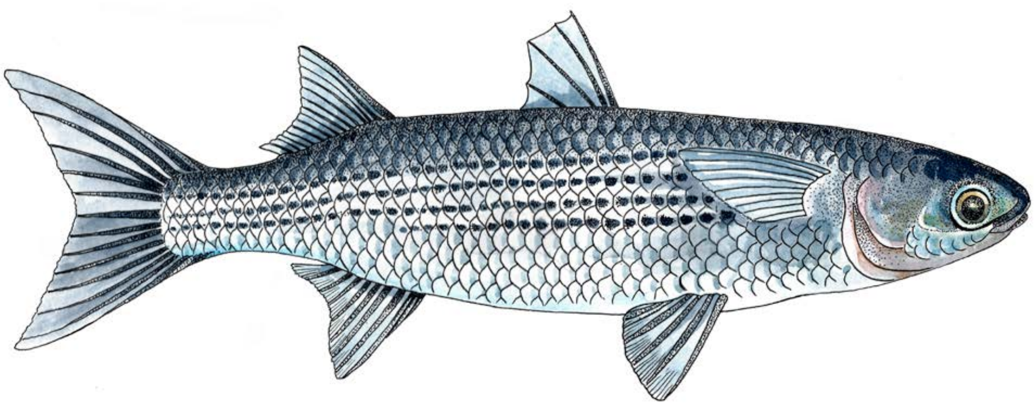
1. Biology and life history

1.1 HABITAT

The flathead grey mullet, *Mugil cephalus*, is an Actinopterygian teleost belonging to the Order Mugiliformes, Family Mugilidae (Figure 1.1). This migratory species can be found in various aquatic environments throughout its lifecycle including estuaries, the marine littoral zone (at depths of up to 20 m), and the open ocean. Notably, it shows euryhaline adaptation, with adult specimens thriving across a salinity gradient from 0 parts per thousand (ppt) to 75 ppt. The flathead grey mullet can also survive in waters with a diverse range of dissolved oxygen levels and can inhabit both clear and turbid waters, as well as sandy and muddy substrates.

Dietary habits of the flathead grey mullet vary with its life stages. *Mugil cephalus* larvae are planktivorous while in the open ocean and during the initial entry into estuaries. Juveniles, consume small invertebrates in the water column before transitioning to a benthic diet. Upon reaching the sub-adult and adult stages, the primary diet consists of detritus (including particulate organic matter) and benthic microalgae (especially diatoms) along with foraminifera, filamentous algae, protists, meiofauna and small invertebrates. This omnivorous diet places the flathead grey

FIGURE 1.1
Illustration of an adult flathead grey mullet, *Mugil cephalus*



mullet at a relatively low trophic level in the food chain. They exhibit both solitary and group feeding behaviour with their communal feeding habits reflecting strong schooling behaviour from late larval throughout adulthood.

1.2 ANATOMY

The flathead grey mullet has a subcylindrical body shape with an oval cross-section and a gently curving form (Figure 1.2a). Their dorsal coloration is typically greyish-green or blue, silvery sides and horizontal dark stripes, with no visible lateral line. The underside is lighter, often with a yellowish tone. As the largest mullet species, they can reach lengths of up to 120 cm. There are no discernible morphological distinctions between males and females.

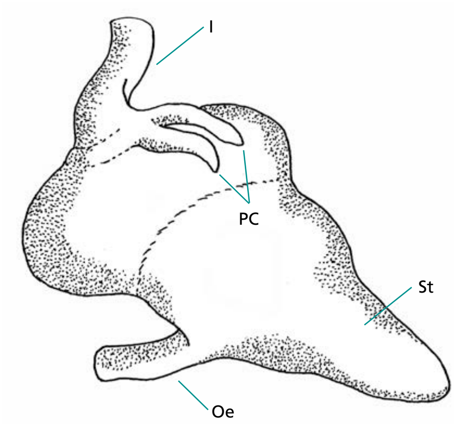
The development of scale types in this species begins with a transition from percomorph and cycloid scales in early juveniles to ctenoid scales in later stages. While spine and ray counts are not always reliable for distinguishing species within the Mugilidae family, they remain a convenient visual tool for identification. Typically, the dorsal fins are well-defined: the first dorsal fin contains four spines, whereas the second dorsal fin has one spine and eight branched rays. The pelvic fins are sub-abdominal, with one spine and five branched rays. The anal fin comprises three spines and eight branched rays.

In addition to fin morphology, other distinguishing body features are useful for species identification. These include a broad, dorsally flattened head and a thick, soft, translucent adipose eyelid – the most developed among all mullet species. This eyelid covers most of the eye and features a vertical elliptical opening (Figure 1.2b and Figure 1.2c).

FIGURE 1.2
An adult flathead grey mullet, *Mugil cephalus*
Adult mullet specimen (a). The transparent adipose eyelid which covers part of the eye (b) has a vertical elliptical opening in the centre of the eye (c)

The internal anatomy, particularly the digestive system, offers additional morphological features, which aid with species identification. *Mugil cephalus* exhibits a conical stomach with two pyloric caeca of approximately equal length (Figure 1.3). The number of pyloric caeca can also be used to distinguish between species in the *Mugil* and other mullet genus.

FIGURE 1.3
Illustration of the stomach and pyloric caeca of *Mugil cephalus*
I: Intestine; PC: pyloric caeca; St: stomach; Oe: oesophaga



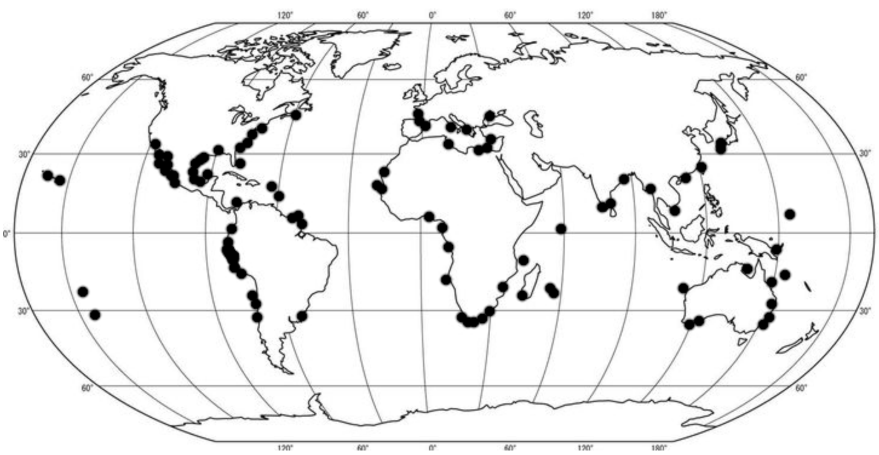
Mugil cephalus presents an oral and branchial filter-feeding system that includes gill rakers and a denticulate pharyngobranchial organ that filters ingested particles. The upper lip is characterized by its thin morphology, devoid of papilli. In addition, the labial setiform teeth of the upper jaw are tiny, straight and thick.

1.3 GEOGRAPHIC DISTRIBUTION

Flathead grey mullet is the most widely distributed species within the Mugilidae family, exhibiting a global presence across coastal regions characterized by temperate and tropical climates (Figure 1.4). This distribution spans between 42°N and 42°S, albeit with a discontinuous geographic range. It can be found in the Western Atlantic Ocean, from Canada to Brazil, including the Gulf of Mexico. However, it is notably absent in the Bahamas and the Caribbean Sea. In the Eastern Atlantic Ocean, the species extends from the Bay of Biscay in France to as far south as South Africa, including the Mediterranean and the Black Sea.

In the Eastern Pacific Ocean its presence ranges from southern California to Chile. It is present in the Pacific islands and in the Western Pacific from Japan to Australia. The species also inhabits the seas around the Philippines, Indonesia and the Indian Ocean from India to South Africa and Australia. It has a world-wide distribution in part because this species and its conspecifics have been able to disperse through both tropical and temperate waters, whereas strictly tropical or temperate mugilids have not dispersed into new habitats. The species wide global distribution suggests it could be a highly versatile candidate for aquaculture, with the ability to thrive in

FIGURE 1.4
Worldwide distribution of the flathead grey mullet (*Mugil cephalus*)



Note: Refer to the disclaimer on page ii for the names and boundaries used in this map.
Source: Modified from Whitfield, A.K., Panfili, J. & Durand, J.D. 2012. A global review of the cosmopolitan flathead mullet *Mugil cephalus* Linnaeus 1758 (Teleostei: Mugilidae), with emphasis on the biology, genetics, ecology and fisheries aspects of this apparent species complex. *Reviews in Fish Biology and Fisheries*, 22: 641–681.

both temperate and tropical environments, providing opportunities for aquaculture in diverse geographic regions.

1.4 LIFE HISTORY

Detailed information on the life-history of *M. cephalus* is limited. However, data from specific locations indicate a life cycle that involves marine and estuarine environments, and in some cases, freshwater habitats (Figure 1.5). Juveniles and sub-adults are primarily found in estuarine waters and coastal lagoons. Adults migrate to the sea once a year for spawning. In temperate regions, such as Europe, spawning typically occurs from summer to fall, whereas in subtropical regions, peak spawning activity occurs during winter. After fertilization, eggs and early larval stages drift with ocean currents. When larvae reach approximately 16–20 mm in length, they migrate to rivers and estuaries. Notably, some populations deviate from this pattern; in regions with intermittent river flows, the species may complete its entire life cycle in marine environments. For a chronological description of the early developmental stages of *M. cephalus* cultured at 23 °C see Table 7.1.

Regarding longevity, males have an average lifespan of seven years, while females typically live up to eight years, with an overall species average of about five years. The oldest recorded individual reached 13 years. The timing of wild fry seasons varies regionally, as spawning is strongly influenced by temperature and photoperiod. A general breakdown of life stages is as follows:

- *Tropical and subtropical regions* – Spawning often happens in late autumn to early winter. Fry appear inshore a few weeks later, typically between December–February.
- *Mediterranean and Red Sea* – Adults spawn offshore in July–December; fry migrate to estuaries, lagoons and coastal shallows around October–January.

FIGURE 1.5
Life cycle of *Mugil cephalus*
(a) Adults undergo a reproductive migration toward the sea; (b) spawning and fertilization of eggs occur in shallow coastal waters; (c–e) fertilized eggs hatch in 36–48 h; (f–h) the larvae metamorphose into juveniles in about 40 days; and (i) juveniles from 1 cm total length undergo a trophic migration from the sea to lagoons or estuaries

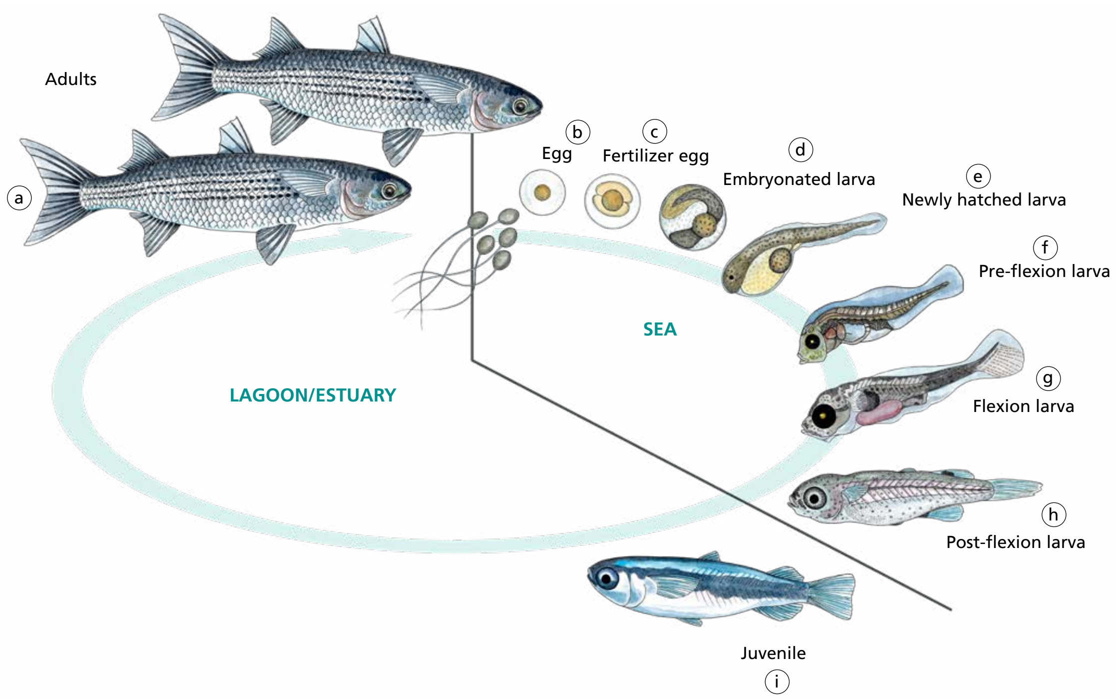


TABLE 1.1
Mean monthly coastal seawater temperatures (°C) during the spawning seasons of *Mugil cephalus* (highlighted in yellow) in various geographical areas

Areas	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr
Black Sea	20	23	24								
Turkey (Mediterranean)	24	26	28	26	20	18	17	16	16	18	20
Egypt (Mediterranean)	24	26	27	26	22	20	18	17	17	18	20
Morocco (Atlantic Ocean)	21	23	23	21	20	18	17	17	17	17	19
Caspian Sea	20	24	24	21	16	12	9	6	6	9	14
Adriatic Sea	22	25	26	22	19	16	14	12	11	13	18
Greece (Aegean Sea)	22	24	25	23	20	17	15	13	13	14	18
Greece (Mediterranean)	22	25	26	24	22	19	17	15	15	16	19
Tunisia (Mediterranean)	21	24	26	22	19	17	15	14	15	16	18
USA (Atlantic Ocean)	26	28	28	26	24	23	22	20	21	21	24
Mexico (Gulf of Mexico)	28	28	29	28	26	23	21	20	19	22	25
India (Indian Ocean)	29	28	28	29	28	27	27	29	29	30	30
Mauritania (Atlantic Ocean)	23	26	27	28	27	25	22	20	19	19	20
Sri Lanka (Indian Ocean)	29	28	28	28	28	28	27	26	29	30	30
USA (Atlantic Ocean)	26	28	28	27	25	23	22	20	20	20	24
USA (Atlantic Ocean)	26	28	29	27	25	23	23	21	20	23	26
Australia (Pacific Ocean)	22	21	21	22	22	24	25	26	26	25	24
South Africa (Indian Ocean)	22	22	21	21	22	23	24	26	26	25	24

Source: Modified from Whitfield, A.K., Panfili, J. & Durand, J.D. 2012. A global review of the cosmopolitan flathead mullet *Mugil cephalus* Linnaeus 1758 (Teleostei: Mugilidae), with emphasis on the biology, genetics, ecology and fisheries aspects of this apparent species complex. *Reviews in Fish Biology and Fisheries*, 22: 641–681.

- *Temperate zones* – Spawning occurs between November-February, and fry may appear only in spring (March–May).

For further details on reproductive seasons across different geographical areas, see Table 1.1.

1.5 SEX DETERMINATION

The flathead grey mullet is gonochoristic, meaning individuals develop as either male or female. Initially, individuals may exhibit non-functional intersex or hermaphroditic gonads, which later differentiate into functional testes (males) or functional ovaries (females).

Unlike species with distinct heteromorphic sex chromosomes (e.g. XY or ZW systems), *M. cephalus* possesses homomorphic sex chromosomes, and its sex determination appears to rely on specific genetic loci. The LG9 chromosome region has been identified as significant in this process, with the follicle-stimulating hormone receptor (FSHR) gene identified as a primary candidate marker.

In Northern Hemisphere populations, a variant of the *fshr* gene (c.1759T>G) has been linked to male sex differentiation, but this relationship is not consistent across all populations. For instance, mullets in Queensland, Australia, are associated with a different single nucleotide polymorphism (SNP) at position 1834 G>T. These observations indicate that genetic mechanisms vary among populations, suggesting that other genes on the LG9 locus may act as "master" regulators. Furthermore, environmental factors, primarily water temperature, act as critical external modulators capable of overriding genetic commands through epigenetic regulation. This process, which can include the hypermethylation of the aromatase promoter (cyp19a1a), allows environmental stimuli to influence the sexual differentiation cascade, potentially leading to the development of "non-conforming" phenotypic males that possess a female genotype.

1.6 REPRODUCTION

The development of gonads in the flathead grey mullet is closely correlated with total length and age, while body weight shows a weaker relationship. Gonadal development progresses through three distinct stages:

1) **undifferentiated stage**, occurring in individuals younger than six months;
2) **differentiating stage**, in fish aged 7–14 months; and
3) **differentiated stage**, in individuals older than 15 months.

Oogenesis (formation of female gametes) begins in fish exceeding 21 cm in length, whereas spermatogenesis (production of male gametes) is first observed in individuals over 25 cm. Females typically attain sexual maturity during their fourth year at lengths of 40–42 cm, while males mature in their third year at 33–38 cm.

Female reproductive development

Flathead grey mullet exhibit group synchronous oocyte development, whereby a single batch of oocytes matures annually, resulting in one spawning event per year. Throughout the reproductive cycle, females display various stages of oocyte development (Figure 1.6).

Immature females possess previtellogenic oocytes (smaller than 150 µm), including primary growth and cortical alveoli oocytes (Figure 1.6a–b). At this stage, the ovaries are small and pinkish (Figure 1.7a). As vitellogenesis initiates, oocytes enter the secondary growth stage (Figure 1.6c–e), during which yolk globules accumulate in the oocyte, causing the oocytes to increase in diameter from 150 to 600 µm and ovaries to change colour from pink to yellow or orange (Figure 1.7b–c).

FIGURE 1.6
Flathead grey mullet oogenesis
(a) Previtellogenic early immature oocytes (primary oocytes) (70–100 µm); (b) previtellogenic late perinucleolar oocytes or cortical alveoli stage (100–120 µm); (c) early vitellogenic oocytes (250–300 µm); (d) final vitellogenesis (550–600 µm); (e) germinal vesicle break down, migratory nucleus (600–650 µm); and (f) ovulated oocytes, ova

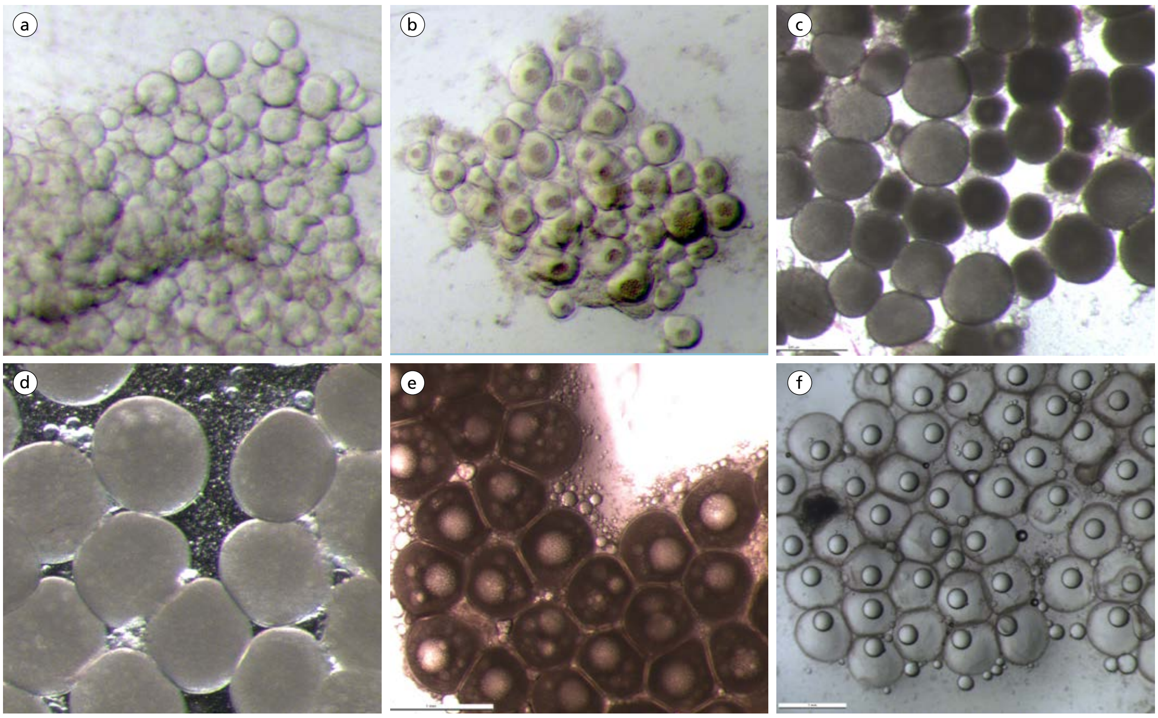


FIGURE 1.7
Flathead grey mullet female gonads at different stages of development
(a) Immature; (b) early-vitellogenesis; (c) advanced vitellogenesis; and (d) after spawning
Scale bar: 5 cm

Source: Reprinted under the editor permission from Ramos-Júdez, S., Estévez, A., González-López, W.A. and Duncan, N. 2023. Lipid and fatty acid composition of muscle, liver, ovary, and peritoneal fat in wild flathead grey mullet (*Mugil cephalus*) according to ovarian development. *Theriogenology*, 198: 317–326

Oocyte maturation follows, characterized by migration of the nucleus to the periphery, hydration and ovulation (Figure 1.6f). The resulting eggs are spherical (880–980 µm in diameter), transparent, and contain a single large oil globule that provides buoyancy. After spawning, the ovaries appear opaque pink to reddish due to increased vascularization (Figure 1.7d) and contain both atretic follicles and previtellogenic oocytes.

Male reproductive development

Male *M. cephalus* possess an unrestricted spermatogonial testis with a tubular structure. Spermatogenesis is of the cystic type, occurring within cysts formed by Sertoli cells. The testicular development cycle consists of several distinct stages reflecting the gradual maturation of the gonads.

In the immature stage, the testes are small and thread-like (Figure 1.8a), with narrow lobules showing active spermatogonial proliferation and interstitial spaces filled with connective tissue and blood vessels.

During the early developing stage, the testes contain primarily spermatogonia and primary spermatocytes, indicating the onset of spermatogenic activity. In the mid-developing stage, the seminiferous tubules enlarge and contain germ cells at various stages of maturation, showing active proliferation within the tubular lumen.

The late developing or initial ejaculation stage marks the first appearance of spermatozoa within the tubules, signifying the transition toward full functionality. In the fully mature stage, the seminiferous tubules are densely packed with mature spermatozoa and only a few residual early-stage germ cells. At this stage, the testes are fully developed, exhibiting a firm texture and a distinct white coloration (Figure 1.8b).

Following spawning, the testes enter the spent stage, characterized by reduced tubule size, thickened walls, and a few remaining spermatozoa. Degenerative changes in the germinal epithelium become evident as the gonads regress. Material from an immature *M. cephalus* is shown in Figure 1.9.

FIGURE 1.8
Flathead grey mullet male gonads at different stages of development
(a) Immature; and (b) fully developed testes
Scale bar: 5 cm

FIGURE 1.9
Fresh material from the cannulation of a *Mugil cephalus* male

Representative histological sections of adult female gonads are illustrated in Figure 1.10, depicting the progression of oocyte development from the early previtellogenic phase to the post-spawning condition. These micrographs highlight the distinct cellular characteristics and tissue organization associated with each reproductive stage. In contrast, Figure 1.11 presents histological preparations of male gonads at different developmental stages, showing the structural transformation of the seminiferous tubules during spermatogenesis.

Mugil cephalus is an oviparous species that releases pelagic eggs and sperm into the water column where external fertilization occurs. The timing of the spawning season varies depending on the geographical location and is influenced by water temperature.

Spawning typically avoids extreme temperatures; specifically, water colder than 17 °C or warmer than 28 °C. For instance, in the Eastern Mediterranean, spawning occurs during the warmest months, from June to October, when water temperatures range between 20–28 °C. In contrast, along the Atlantic coast of South Carolina (United States of America), spawning takes place during the cooler months, from October to April, with water temperatures ranging from 20–25 °C.

Female mullets exhibit high fecundity, producing approximately 650 to 850 eggs/g of body weight.

FIGURE 1.10
Histological sections of female *Mugil cephalus* gonads across developmental stages

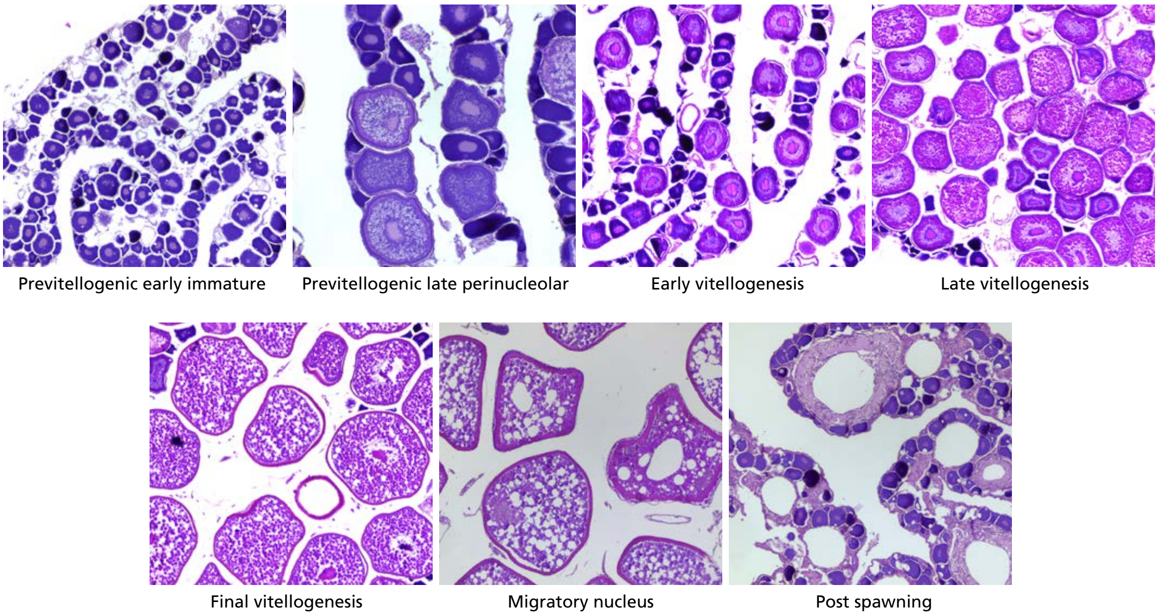
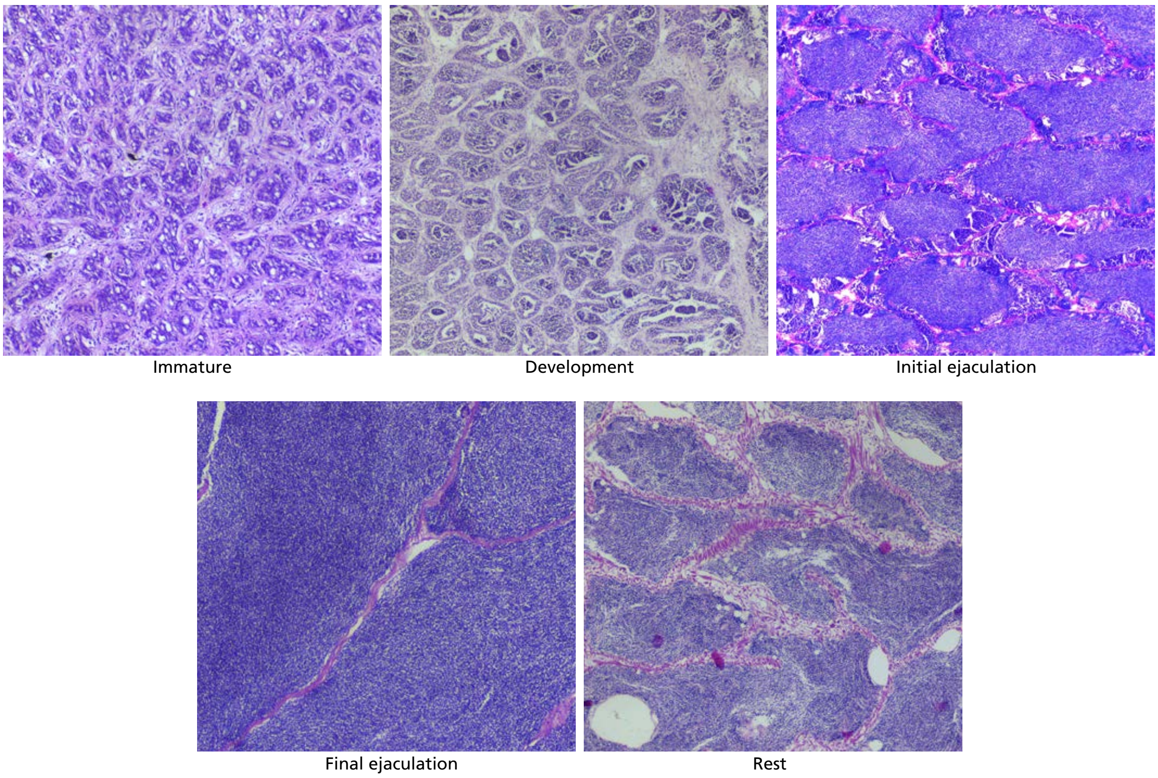


FIGURE 1.11
Histological sections of male *Mugil cephalus* gonads across developmental stages



1.7 LARVAL ONTOGENY

1.7.1 Digestive system

From hatching until mouth opening, larval nutrition in *M. cephalus* is sustained by endogenous reserves, consisting of the yolk and the oil globule, which are gradually absorbed through the syncytial layer. During this endogenous feeding phase, the digestive tract of newly hatched larvae appears as a closed, straight, and undifferentiated tube positioned between the notochord and the yolk sac (Figure 1.12a).

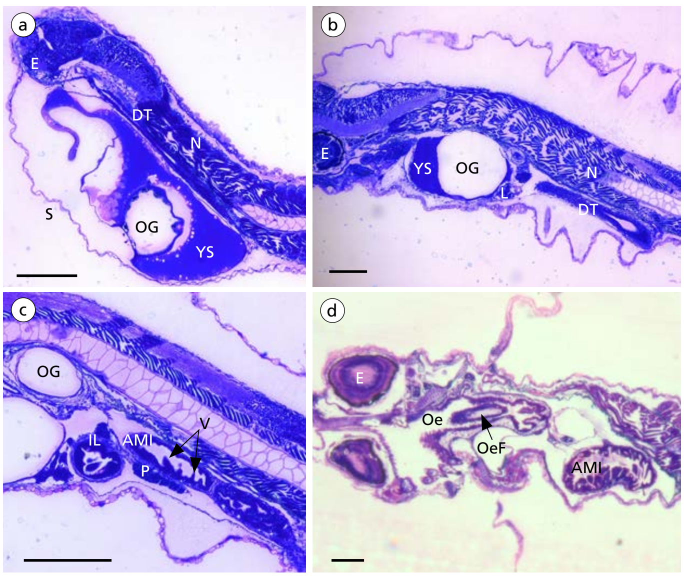
The liver is the first accessory digestive gland to develop, becoming visible at 1 day post-hatch (DPH) when larvae reach a total length (TL) of 2.86 ± 0.09 mm (44 degree-days). Located posterior to the yolk sac, the liver rapidly expands to partially surround the anterior portion of the intestine (Figure 1.12b). Functionally, it plays a central role in nutrient metabolism, lipid storage, and the conversion and transport of metabolites to peripheral tissues, supporting the early energetic demands of larval development.

By 3 DPH (TL 2.79 ± 0.08 mm; 88 degree-days), the mouth and anus open simultaneously, marking the transition from endogenous to exogenous feeding. At this stage, the intestine elongates and begins to coil, forming a loop that accommodates its increasing size within the visceral cavity (Figure 1.12c). The intestinal wall displays a few villi with absorptive functions, which progressively increase in number and size as feeding activity intensifies.

FIGURE 1.12

Ontogenesis of the larval digestive system (I)

(a) Newly hatched larvae, with undifferentiated digestive tube; the mouth is closed, and the sustenance depends on the absorption of nutritive reserves by the yolk. (b) Liver develops at 1 DPH behind the yolk sac, and it is involved in the nutrient metabolism and lipid storage. (c) Intestine coils at 3 DPH due to its increasing size and is now divided into antero-median and posterior intestine by the ileo-rectal valve; absorptive villi are present in the intestinal wall, while the pancreas appears around the middle intestine. (d) From 5 DPH, in response to food intake, the oesophagus expanded with the formation of longitudinal folds



E: eye; **S:** syncytium layer; **DT:** digestive tube; **N:** notochord; **OG:** oil globule; **YS:** yolk sac; **L:** liver; **SB:** swim bladder; **IL:** intestinal loop; **AMI:** antero-median intestine; **P:** pancreas; **V:** villi; **IRV:** ileo-rectal valve; **PI:** posterior intestine; **Oe:** oesophagus; **OeF:** oesophageal fold. Histological sections are stained with Methylene blue/Azure II/Basic Fuchsin.

Scale bar: 100 µm

Concurrent with the onset of exogenous feeding, intestinal segmentation becomes evident through the formation of the ileo-rectal valve, a sphincter that separates the antero-median and posterior intestine. This structure helps retain digestive enzymes in the anterior section for reuse, improving digestive efficiency (Figure 1.12c). The antero-median intestine serves as the primary site for lipid absorption, whereas the posterior intestine is mainly involved in protein absorption.

At the same developmental stage (3 DPH), the pancreas begins to differentiate near the mid-intestinal region, just behind the liver. Its early formation allows for the production of digestive enzymes, such as trypsin and amylase, enabling larvae to efficiently process exogenous food sources (Figure 1.12c).

By 5 DPH (TL 2.80 ± 0.04 mm; 132 degree-days), longitudinal folds appear in the oesophageal mucosa, providing the elasticity necessary for food passage during ingestion (Figure 1.12d). The increasing structural complexity of the digestive tract at this stage reflects the rapid functional maturation that supports the transition to active feeding and growth.

At 7 DPH, when larvae reach a TL of 2.95 ± 0.08 mm (176 degree-days), they acquire the sense of taste with the formation of the first taste buds in the lower region of the mouth (Figure 1.13a). The development of taste buds plays a crucial role in food recognition and selection, allowing larvae to discriminate between edible and non-edible particles. At the same time, the stomach begins to differentiate, marked by the appearance of the cardiac and pyloric sphincters, which separate it from the oesophagus and intestine, respectively (Figure 1.13b). These structural changes signal the early formation of a functional stomach chamber.

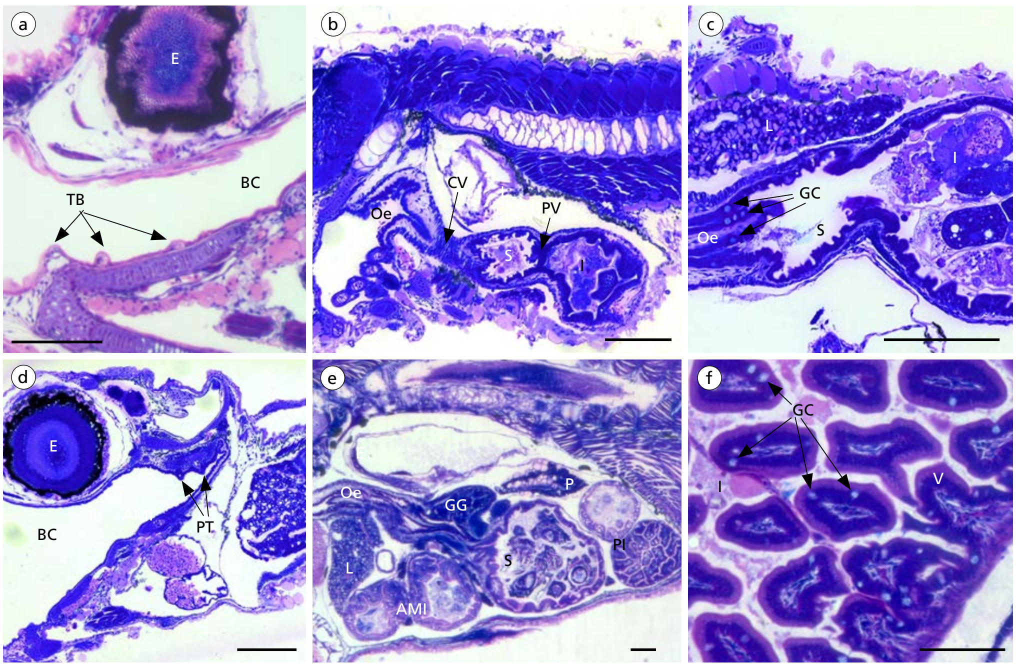
By 9 DPH (TL 3.14 ± 0.32 mm; 220 degree-days), goblet cells become visible within the oesophageal epithelium (Figure 1.13c). These mucus-secreting cells facilitate food ingestion by lubricating the oesophageal lining and providing a protective barrier against mechanical abrasion and microbial invasion, contributing to the maintenance of epithelial integrity during the initial feeding period.

At 13 DPH (TL 4.24 ± 0.30 mm; 307 degree-days), the first pharyngeal teeth emerge (Figure 1.13d), indicating that larvae have developed the ability to mechanically process live prey. This development, combined with the ongoing secretion of digestive enzymes from the pancreas, significantly enhances digestive efficiency even before the full formation of gastric glands. The appearance of pharyngeal teeth thus represents a critical step in the transition toward active predation and the digestion of more complex food items.

FIGURE 1.13

Ontogenesis of the larval digestive system (II)

(a) Taste buds develop by 7 DPH in the buccal cavity to provide larvae with the sense of taste. (b) The cardiac and pyloric sphincters separate the stomach from the oesophagus and the intestine. (c) The first oesophageal goblet cells appear at 9 DPH and produce mucous substances that facilitate the swallowing of food. (d) The pharyngeal teeth enable the 13-DPH larva to process live prey mechanically. (e) The gut differentiation can be considered accomplished at 17-DPH, after the formation of the gastric glands all around the stomach; from now on, the larva can digest complex foods. (f) The intestinal goblet cells facilitate the absorption of nutrients and lubricate faeces



E: eye; **TB:** taste buds; **BC:** buccal cavity; **Oe:** oesophagus; **CV:** cardiac valve; **S:** stomach; **PV:** pyloric valve; **I:** intestine; **GC:** goblet cells; **L:** liver; **PT:** pharyngeal teeth; **AMI:** antero-median intestine; **GG:** gastric glands; **P:** pancreas; **PI:** posterior intestine; **V:** villi. Histological sections are stained with Methylene blue/Azure II/Basic Fuchsin.

Scale bar: 100 μm

The final step of digestive tract differentiation occurs at 17 DPH (TL 5.98 ± 0.40 mm; 395 degree-days) with the formation of gastric glands and the onset of acidic secretion in the stomach (Figure 1.13e). From this stage onward, the stomach begins producing hydrochloric acid (HCl) and digestive enzymes such as pepsin, enabling larvae to efficiently digest a wider variety of prey and utilize complex or inert food substrates. This marks the establishment of a fully functional digestive system.

By 22 DPH (TL 7.81 ± 0.33 mm; 502 degree-days), goblet cells within the intestinal mucosa are abundant and clearly visible (Figure 1.13f). These cells secrete mucous substances rich in glycoproteins, which not only protect the intestinal lining but also facilitate nutrient absorption and faecal lubrication. Their proliferation reflects the maturation of intestinal function and the larva's increasing capacity for sustained exogenous feeding and nutrient assimilation.

1.7.2 Visual system

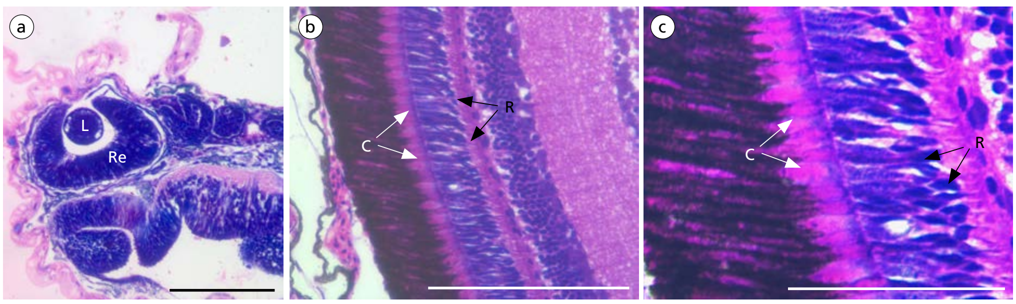
At hatching, *M. cephalus* larvae possess undeveloped eyes. In the central region of the eye, the lens appears as a simple spherical structure surrounded by the retina, which at this stage consists of an undifferentiated neuroepithelium (Figure 1.14a).

Between 1 and 2 DPH, the initial pigmentation of the eyes becomes evident, marking the onset of retinal differentiation and the early formation of the morphological structures involved in vision. This pigmentation process signals the beginning of functional visual development, an essential prerequisite for the transition to exogenous feeding.

At 3 DPH (TL 2.79 ± 0.08 mm; 88 degree-days), coinciding with mouth opening and the onset of external feeding, the first photoreceptor cells (cones) appear and gradually increase in number (Figure 1.14b and Figure 1.14c). These cones are responsible for photopic (daylight) vision, enabling larvae to detect and capture prey under illuminated conditions, thus supporting the shift to active feeding behaviour.

By 17 DPH (TL 6.59 ± 0.57 mm; 437 degree-days), the first rod cell nuclei become visible within the retina (Figure 1.14b and Figure 1.14c). The appearance of rods, which mediate scotopic (low-light) vision, marks an important functional advancement in the visual system, allowing larvae to detect and respond to food and environmental stimuli under dim light conditions. The coexistence of cones and rods from this stage onwards provides larvae with a broader visual capacity suited to both diurnal and crepuscular activity.

FIGURE 1.14
Ontogenesis of the larval visual system
(a) Newly hatched larvae have an undeveloped eye, with a central lens surrounded by undifferentiated retina. (b) The cones ensure the diurnal vision ability by 3 DPH, while by 17 DPH larvae can detect food items in low light conditions with the appearance of the rods. (c) Magnification of cone and rode cells



L: lens; **Re:** retina; **C:** cone cells; **R:** rod cells. Histological sections are stained with Methylene blue/Azure II/Basic Fuchsin. Scale bar: 50 µm

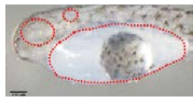
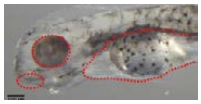
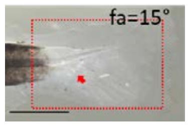
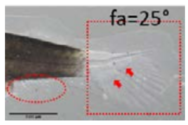
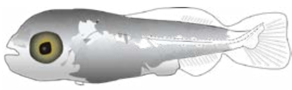
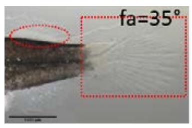
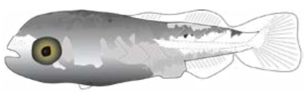
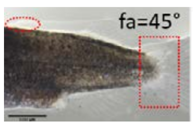
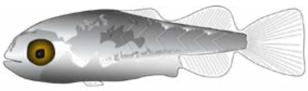
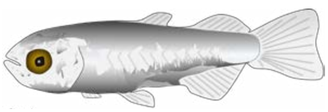
1.7.3 Fins and axial spine

The skeletal development of *M. cephalus* larvae progresses through ten distinct stages, labelled A to J, as illustrated in Figure 1.15. Newly hatched, Stage A grey mullet larvae have a fin fold that surrounds the body. The pectoral fins first appear at Stage C. As the larvae grow to Stage D, the fin fold starts to shrink back to the upper back part of the body. By Stage E, the caudal fin begins to take shape, with small rays reaching downward and the notochord (a flexible rod that supports the body) starts to bend (defined as flexion), marking the onset of metamorphosis.

FIGURE 1.15

Skeletal development and metamorphosis of flathead grey mullet larvae

Important anatomical or morphological features are indicated in red in the images on the right-hand side

Stage	Stage defining features	Images	
A	Round and smooth head Large yolk sac with pigmented oil globule		
B	Beginning of eye pigmentation Mouth formation Yolk sac size decrease		
C	Mouth opening and dentary formed Fully developed and pigmented eyes		
D	Oil globule completely absorbed No rayed fins		
E	Beginning of flexion (7-15°) Beginning of caudal fin rays formation		
F	Progression of flexion (15-30°) Beginning of anal fin rays		
G	Progression of flexion (30-45°) Beginning of posterior dorsal fin rays		
H	Anterior dorsal fin rays form, but still attached to posterior dorsal fin with fin fold Urostyle and hypural plate nearly invisible		
I	Complete separation of anterior and posterior dorsal fins Urostyle and hypural plate completely disappear		
J	Fully rayed and developed fins Prominent silvery and shiny full body pigmentation		

Fa: Flexion angle

Scale bars: stages A–E: 100 µm; stages F–J: 500 µm

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As the larvae continue to develop, horizontal rays develop in the caudal fin, and the anal fin develops rays as shown in Stage F. At this stage rays develop in the middle of the caudal fin and in the posterior dorsal fin. The pelvic fins, which are near the lower middle part of the body, also begin to appear (Stage H). This whole process of fin growth ends with the anterior dorsal fin becoming distinct and separate (Stages H–J). At Stage J individuals are fully covered by scales and the transition to juvenile stage is complete.

Table 1.2 summarises the developmental progression of flathead grey mullet larvae from hatching to the juvenile stage, describing changes in morphology, organ formation, pigmentation, and behaviour in relation to days after hatching and total length. The table highlights key developmental milestones, including yolk-sac absorption, mouth opening, fin and scale formation, and shifts in swimming behaviour, phototaxis, and habitat use within the water column. Two critical periods characterised by elevated larval mortality are also identified, emphasising the close relationship between physiological development, behaviour, and survival during early ontogeny.

TABLE 1.2
Development of flathead grey mullet larvae and their behaviour

Stage	Days after hatching	Total length (mm)	Larval development and behaviour
A	1	2.56–3.52	Newly hatched larvae have a large yolk and oil globule. The front part of notochord is curved along the yolk sac. The degree of curvature of the notochord is related to the duration of hatching. At low temperatures, the duration of hatching is longer, and the curvature of the notochord is greater. Weak swimming activity is observed with the posture of the belly up and head down, sometimes with a jerky motion slightly up and down. Pigmentation is variable between individuals. The eyes are not pigmented. The mouth is not developed. The digestive tube is not well-developed.
B	2	2.64–3.28	Formation of organs is in progress. The pigmentation of the eyes and body has increased from 1 DPH. The total larval length is shorter than 1 DPH. The mouth begins to develop. The buds of the pectoral fins are visible. The nostrils are visible.
C	3–4	3.11–3.53	The mouth opens. The upper and lower jaws develop. Irregular peristalsis of the stomach and intestines initiates. Yolk has diminished to a ¼ of its original size. The oil globule has also reduced. This is the first critical period in survival and is accompanied by high larval mortalities. Gill clefts appear. The larvae are attracted to light and tend to concentrate in areas with 600–1 400 lux. Larvae are distributed in the upper water level during the night.
C	5–7	3.06–3.40	The digestive tube is well developed. Larvae move up and down in the water column both during day and night. Feeding is easy to observe, but larvae only feed during the day. Formation of abnormalities in the bladder and swelling of hyoid can be reduced by keeping good water parameters. The formation of the stomach, intestine, gall bladder, pancreas and swim bladder are visible and accompanied by the continued reduction of oil globule.
D	8–9	3.35–3.80	The oil globule has completely disappeared. Formation of the gill filaments is visible. Growth rate starts to significantly increase.
D	10–13	3.45–5.10	The fin fold has moved backwards. The gill filaments are well developed. The body surface has a dark colour. Strong phototaxis of the larvae is evident. The formation of hypural bone is visible. This is the second critical period with high larval mortalities.
E	14–15	3.85–5.70	Larvae begin to swim in schools. The formation of the urostyle is visible. The anal and second dorsal fin form 7–9 ray bases. Gill lamellae form on the gill filaments.
F	16–19	5.40–6.60	Seventeen soft rays are visible in the caudal fin. Black spots are visible scattered over the whole larval body. A shiny silver white complexion appeared from gill cover along ventral part to the anus. The larvae show schooling in the upper and mid water levels.
F	20–21	6.00–7.65	The larvae show positive phototaxis during the day while floating in mid water during the night. Larvae appear to have a brown or silver/green colour with a high colouration variability in healthy larvae. Four soft rays are visible on the first dorsal fin.
G	22–24	8.25–10.9	The formation of 20 soft rays is visible in the caudal fin, 11 rays in the anal fin, 6 rays in the pelvic fins and 15 rays in the pectoral fins. The fin membrane of the dorsal fin and the pelvic fins is almost entirely degenerated. The appearance of scales with 1–3 ridged circles are visible. Largest scales can reach 400 × 250 µm. Silver-white complexion is visible to the body. During the day, larvae swim in upper water levels in schools against aeration and water flow. During the night, larvae float scattered on the water´s surface.
H	25–28	8.80–15.0	All scales and fin rays are well formed. Silver-green complexion is visible to the body. Teeth are visible. Two separate nostrils are visible.

TABLE 1.2 (CONTINUED)

Stage	Days after hatching	Total length (mm)	Larval development and behaviour
H	29–32	16.6–20.7	Larvae appear to be very sensitive. The larvae gather in small schools. During the day, larvae swim in middle and lower water levels, while at night the larvae float at the surface and are easily startled.
I	34–35	22.2–26.2	During the day, larvae swim in large schools around the circumference of the rearing tank in the middle and lower water column. During the night, larvae float at the surface. The larvae have a grass-green body colour, sometimes silver-white at the dorsal part. Some diseased larvae were found with "pop-eye" symptom.
I	37–40	23.1–29.3	Changes are observed in the feeding behaviour, with feeding in the late afternoon. Larvae are sensitive to light but are no longer attracted to light.
J	45	27.5–32.8	The transition from larvae to juvenile stage is complete. At this stage they are robust, resistant to changes in environmental parameters and suitable for stocking in grow-out facilities.

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1.7.4 Pigmentation

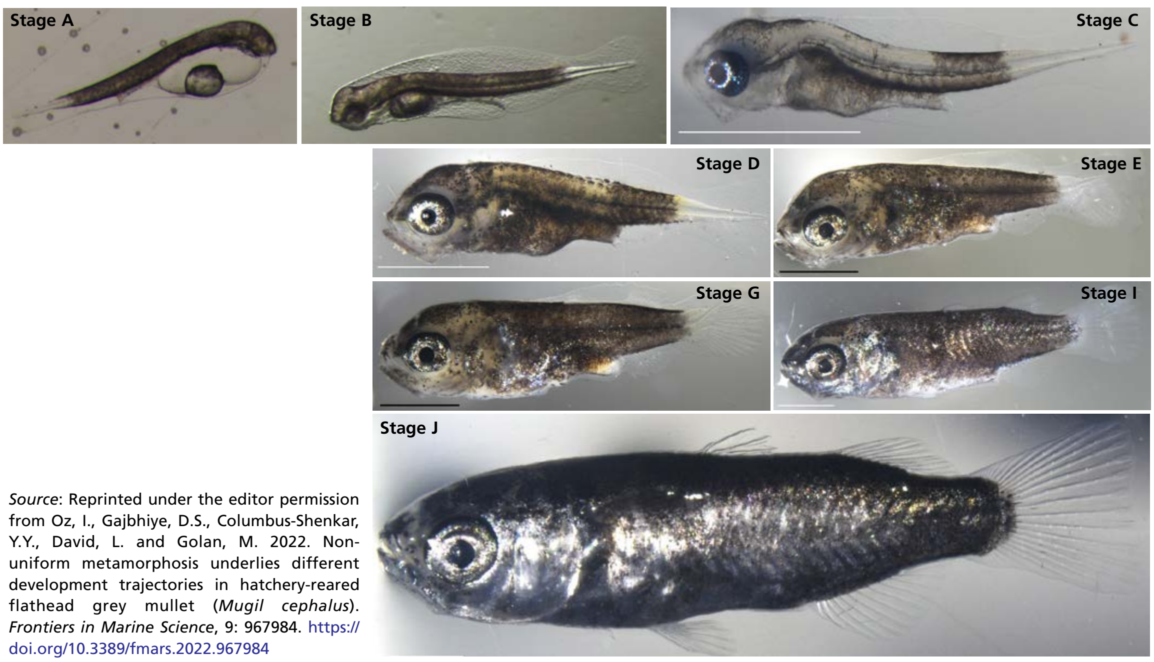
Upon hatching, flathead grey mullet larvae exhibit a distinctive pattern of star-shaped pigmented cells, known as melanophores, which are distributed over the oil globule and body, except for the posterior region (Figure 1.16a). As the larvae grow, melanophores begin to appear across the dorsal portion of the head and trunk (Figure 1.16c), marking the gradual progression of pigmentation during early development.

The transformation into juvenile fish, or metamorphosis, is characterized by the appearance of reflective, silver-hued cells called iridophores around the mid-body region (Figure 1.16e). Iridophores continue to expand, spreading along the larval body and reaching maximum density at Stage J, at which point the larvae display a fully iridescent body. This stage coincides with the completion of scale formation, providing a visible and practical marker of metamorphosis.

FIGURE 1.16

Development of pigmentation in flathead grey mullet in larvae and early juvenile stages

Stages A–C are largely transparent. Dark pigmentation covers the body except for the caudal region from Stage D-G. Silver iridophores first appear at Stage E and gradually cover the body until the attainment of a full silver body at Stage J



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The overall change in pigmentation and the emergence of iridophores are easily observed in rearing tanks, offering a clear visual indication of the transition from larva to juvenile in this species.

1.7.5 Skeletogenesis

The schematic illustration in Figure 1.17 shows the development of the axial skeleton of hatchery reared flathead grey mullet during the first 25 DPH.

A 10 DPH – The first elements of the vertebral column, the neural arches (na), emerge and undergo direct ossification (red) dorsal to the notochord (grey).

B & C 14 DPH – Additional skeletal elements, including vertebral centra (vc) and haemal arches (ha), develop sequentially from anterior to posterior. During this stage, the notochord begins to flex dorsally, and elements of the caudal fin skeleton, namely the hypurals (hyp) and parhypural (ph), appear as cartilaginous precursors (blue).

D & E 18–19 DPH – Further vertebral elements, such as the ribs (ri), form, alongside caudal fin components including epurals (eu), ural centra (uc), and accessory neural (ans) and haemal spines (ahs). Cartilaginous preformed elements begin ossification. The pterygiophores of the anal (ap) and first and second dorsal fins (d1p and d2p) develop as cartilaginous precursors, with the first fin rays emerging. Additional structures, such as supraneurals (sn) and interdorsal pterygiophores (ip), also form.

F 25 DPH – Vertebral centra attain their characteristic hourglass shape, while neural and haemal arches and spines continue to grow. Intermuscular bones, including the epipleurals (ep), begin development. Preformed caudal fin elements ossify, and the final elements, such as the uroneural (ur), emerge. Some elements (e.g. uc1 and uc2) fuse to form compound structures (cc). Elements of the anal and dorsal fins, along with supraneurals and interdorsal pterygiophores, also complete ossification.

Skeletal malformations are frequently reported in hatchery-reared juveniles compared to their wild conspecifics. These deformities can result from multiple factors, including:

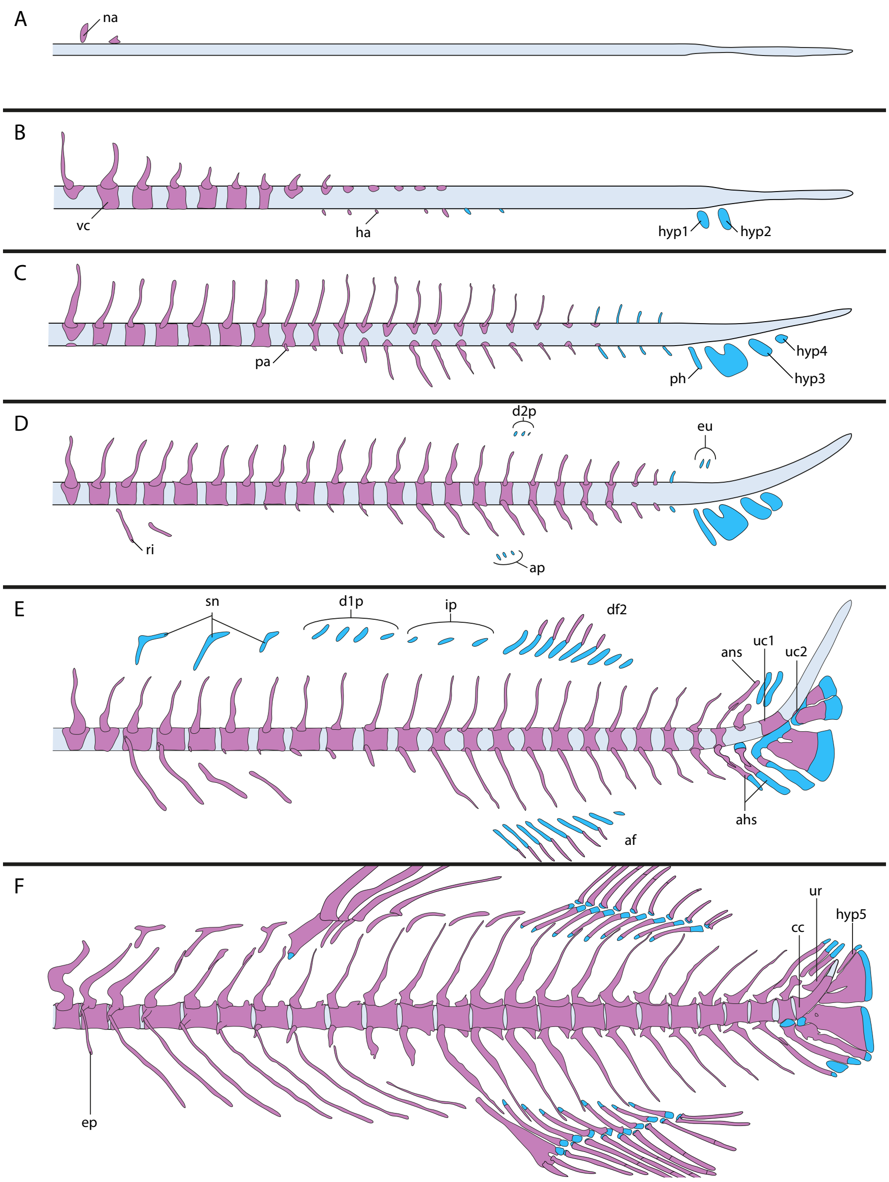
- *Nutritional imbalances*, particularly deficiencies in essential fatty acids, phosphorus, or vitamin D_3.
- *Water quality stress*, such as ammonia spikes or low dissolved oxygen levels.
- *High larval densities*, which reduce swimming space and may impair muscle and bone development.

A more detailed discussion of juvenile skeletal deformities is provided in Section 8.7.

FIGURE 1.17

Schematic illustration of the development of the axial skeleton of the flathead grey mullet, *Mugil cephalus*, during the first 25 days post-hatch

(Details of sub-figures and labels are in Section 1.7.5)



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2. Hatchery type and design

2.1 CULTURE SYSTEMS

The flathead grey mullet (*Mugil cephalus*) has been cultured in a wide range of aquaculture systems, from extensive coastal ponds to semi-intensive and intensive operations (open, semi-closed, and closed systems). However, fingerlings produced in marine fish hatcheries are reared in semi-closed or closed systems to enable optimal control of water parameters, environmental conditions and biosecurity. Nevertheless, all three system types remain relevant to *M. cephalus* hatchery operations, as broodstock typically attain advanced gametogenic development – specifically oocyte maturation and spermiation – under open system conditions, whereas such progression may be limited in more controlled environments. Consequently, the incorporation of open systems may be necessary to ensure a consistent and reliable supply of fertilized eggs for hatchery production.

2.1.1 Open system

An open system refers to the practice of culturing organisms in natural bodies of water, where environmental conditions are largely beyond human control. In these systems, water temperature fluctuates naturally with seasonal and climatic changes, and water exchange is driven by tides, coastal currents or freshwater inflows.

Two main types of open culture systems that can be used for farming the flathead grey mullet:

<u>Coastal and estuarine lagoons</u>: these have a long history of use in mullet farming. These lagoons often have narrow channels communicating with the sea, which can be managed using net barriers to limit the migration of fish species or physical barriers to regulate water exchange. Such systems are particularly valuable as sources of mature broodstock for hatcheries. Given the challenges associated with maintaining a reliable egg supply, the availability of mature breeders remains a key consideration when selecting a hatchery site for *M. cephalus*.

Sea cages: are less commonly used in mullet culture. However, owing to the species' high adaptability to a wide range of rearing environments, cage culture represents a viable and effective system for *M. cephalus* farming.

2.1.2 Semi-closed system

A semi-closed system, also referred to as a flow-through system, involves the continuous supply of water to enclosed tanks, raceways or ponds. Within these holding units, key farming parameters – including water quality, oxygen concentration, lighting and disturbance levels – can be regulated. The water is typically used once and then discharged, although in some semi-closed designs, it may be reused without treatment, flowing sequentially from one rearing unit to another. For example, in a raceway cascade system, solids wastes are removed from the water before it enters the next raceway.

As water moves through the culture units, key parameters are frequently monitored to ensure that conditions remain suitable for maintaining fish health and welfare. The major advantage of single-pass semi-closed systems is that, when located at an appropriate site, water quality remains consistently high. Flow rate, oxygenation, feed utilization and waste removal can all be effectively managed, resulting in production levels that may exceed those of extensive open systems by several hundred- to thousand-fold.

However, semi-closed systems also present challenges. Temperature regulation remains challenging, and high operational costs arise due to the large volumes of water required, leading to substantial intake and pumping demands. Furthermore, compared with open systems, the initial capital investment for constructing semi-closed facilities is higher. Despite these limitations, semi-closed systems form an essential component of *M. cephalus* hatchery operations.

2.1.3 Closed system

Closed systems treat and reuse water in a continuous cycle and are more commonly known as recirculating aquaculture systems (RAS). RAS technology enables near-complete control over both biological and environmental parameters, maintaining a stable, high-quality culture environment through continuous filtration and water reuse. The level of system complexity depends primarily on the degree of water renewal required. Even in fully closed RAS, approximately 5 percent of the water volume must be replaced daily – an amount that can still be significant in large-scale operations.

Closed RAS offer several advantages over semi-closed systems, including reduced water consumption, lower dependence on external clean-water sources, enhanced biosecurity, and increased productivity through high-density stocking under controlled conditions. However, these systems also have notable drawbacks, such as high setup and operational costs (particularly energy consumption), the need for skilled personnel, and potentially higher greenhouse gas emissions.

A functional RAS comprises several essential components (Figure 2.1). Pumps circulate water throughout the system and ideally feature variable flow rates to accommodate changing operational requirements. Solid removal is typically the first stage of water treatment and is accomplished using sand filters or mechanical filters (e.g. drum filters), which require regular backwashing to remove accumulated solids. Biological filtration is then employed to detoxify nitrogenous compounds, with biofilters providing a large surface area for nitrifying bacteria that convert ammonia to nitrite and subsequently nitrate. Nitrates, being less toxic, can be further reduced by denitrifying bacteria or partial water replacement.

Before water re-enters the rearing units, ultraviolet (UV) disinfection is commonly applied to reduce bacterial load. It is critical to ensure that flow rates match the UV system's specifications and that bulbs are replaced according to manufacturer guidelines to maintain disinfection efficiency. Degassing and oxygenation are also essential and can be achieved through cascade aeration, mechanical aerators, or oxygen cones that efficiently inject oxygen into the water. Protein skimmers may be used to remove dissolved organic compounds and excess carbon dioxide.

Additional components can be integrated to achieve finer control over water parameters. Temperature regulation is critical for optimal growth and reproduction; heat pumps are among the most energy-efficient options, capable of both heating and cooling via refrigeration-based systems. Due to metabolic and bacterial activity, pH tends to decrease over time; ideal levels (pH 6.8–7.2) can be maintained by adding sodium bicarbonate, either manually or via automated dosing systems integrated into RAS designs. For finer filtration, especially in larval rearing units, cartridge filters (down to 1 µm) and activated carbon filters can be employed to remove fine particles and chemical contaminants.

Modern RAS are increasingly automated to enhance performance, reliability and operational safety. Automation enables continuous monitoring and adjustment of key parameters such as flow rate, temperature, dissolved oxygen and pH; it can trigger alarms when parameters deviate from preset thresholds; provide maintenance alerts (e.g. for pump or UV lamp servicing); and increasingly leverage artificial intelligence (AI) to perform predictive adjustments for improved stability and efficiency. Given the complexity and high capital investment associated with RAS, automation provides substantial benefits by minimizing human error, improving response time, and preventing costly system failures.

FIGURE 2.1
The IRTAmar® 50 recirculation aquaculture system module provides a complete RAS system for 50 kg biomass of aquatic organisms
The module is mounted on a pallet for easy transport and flexible use. The visible RAS components include: (a) pump; (b) heat exchanger; (c) sand filter; (d) biofilter; (e) reservoir for water renewal and level maintenance; (f) cartridge filter housing; (g) automated control unit; (h) UV disinfection unit; and (i) the visual traffic light alarm system

2.2 SITE SELECTION

Site selection for a *M. cephalus* hatchery must consider biological, environmental and technical factors, as well as legal, social and economic aspects. Biological, environmental and technical considerations are specific to the requirements of the cultured fish species, whereas legal, social and economic factors are more general and relevant to any business operation.

2.2.1 Biological, environmental and technical considerations

Selecting a site that meets the biological requirements of the flathead grey mullet can significantly enhance the viability of a hatchery project and reduce operational costs. Water supply is the most critical factor in site selection. An ideal site provides water of appropriate temperature and quality to meet the species' biological needs. Obtaining eggs for an *M. cephalus* hatchery has historically been challenging and represents a bottleneck in the species' aquaculture development. Therefore, selecting a site that facilitates access to eggs or mature breeders is essential and may be a determining factor depending on the strategy for supplying eggs and larvae.

The water supply at the selected site is a critical determinant of hatchery success. Water quality should approximate pristine marine conditions, supporting all procedures related to spawning and larval rearing. An optimal temperature range of 20–25 °C is recommended to support spawning and larval development while minimizing the cost of heating or cooling. Full-strength seawater is required for egg incubation and larval rearing. These parameters provide a solid foundation to satisfy the species' biological needs and support hatchery success.

Sites that fail to meet these optimal conditions should be avoided, despite the robustness of *M. cephalus* in tolerating a wide range of temperatures and salinities and the availability of RAS for water control. Sites with high variability or extreme environmental conditions, including contaminants should be avoided. Similarly, exclude areas exposed to strong sea conditions, large waves, or extreme storm events, whether from the sea or from terrestrial runoff during heavy rainfall. Sites affected by contamination from municipal, industrial or agricultural sources (e.g. herbicides, pesticides or fertilizers) should also be avoided, as should estuaries with highly variable salinity or other physical parameters. Freshwater availability is also essential for controlling salinity, performing cleaning operations, and treating certain diseases.

Proximity to areas where *M. cephalus* naturally reaches sexual maturity and reproduces is advantageous, as it facilitates the acquisition of mature breeders and supports larval and juvenile rearing. Depending on the egg-supply strategy, sites that include open systems or large extensive ponds in which *M. cephalus* naturally completes sexual maturation and spawning should be strongly considered.

2.2.2 Legal, social and economic considerations

Legal, social and economic aspects are important considerations in site selection of a marine fish hatchery. Legal issues and permits must be considered to understand the constraints for the construction, operation and future development of the hatchery. Potential restrictions due to wildlife protection, natural parks, historical sites, military bases and future local developments should be examined. Building regulations (e.g. height, materials), land use for fish farming, and use and discharge of water must also be considered.

Modern high-technology approach to marine fish hatcheries requires good technical support from services and employees to operate efficiently. The goods and services available in the area must meet the requirements of the hatchery. The hatchery and equipment must be maintained and specialist contractors that are not part of the workforce should be readily accessible to address maintenance and repair work. Likewise, the availability of a skilled workforce is essential and the availability of professionals in the area should be assessed. Local facilities, schools, shops and amenities should provide for the needs of the work force. If local resources are insufficient, provision must be made for workshops, facilities and specialized personnel. Strategies for training or attracting skilled workers should also be considered.

Efficient communication networks are essential to ensure smooth information exchange and effective coordination of all hatchery operations. Reliable internet, phone, and data transfer systems facilitate remote monitoring of water quality, environmental controls, and automated systems, as well as timely consultation with technical experts or contractors. In addition, well-established communication channels are crucial for coordinating the delivery of supplies, maintenance services, and emergency response, minimizing delays that could affect fish health or production schedules.

Significant cost savings and operational efficiency can be achieved when critical inputs – such as equipment, consumables, feeds and treatment materials – are sourced locally. Local sourcing reduces transportation time and costs, ensures timely availability, and lowers the risk of supply chain disruptions. Efficient planning, including optimized delivery routes, storage facilities, and inventory management, further enhances hatchery performance. Moreover, sourcing materials from nearby suppliers allows for rapid replacement or repair of essential components, reducing downtime and maintaining continuous production.

The market for the juveniles produced by the hatchery must also be considered. If juveniles are to be sold, the following questions need to be considered and answered. Where are the *M. cephalus* producers or clients? How and when will juveniles be transported and over what distances? A thorough market assessment will be essential. If the company plans to operate vertically, controlling the entire production cycle, site selection must also account for grow-out facilities and access to markets for harvested fish and value-added products must be considered.

2.3 HATCHERY DESIGN

2.3.1 Seawater system

The seawater supply is a critical aspect of both site selection and hatchery design. The seawater system must address the construction of the seawater intake, water treatment, delivery and use within the hatchery, and the discharge of used water. These components collectively determine the water quality available for producing healthy fingerlings. A seawater system is composed of several units, including the seawater intake, pumping station, storage or holding tanks, primary water treatment system, secondary storage tanks, water distribution network, secondary water treatment before delivery to the rearing units, and wastewater collection and treatment system prior to discharge back to the environment.

The design of the seawater intake is strongly influenced by site selection, which determines the type of coastline and the dynamics of the coastal waters at the intake location. Ideally, sites avoid high-energy exposed coasts, estuaries, and areas affected by contamination from human populations, industry or agriculture. Optimal conditions for a seawater intake include low wave and wind exposure, a preferably rocky coastline with low sediment loads, and some natural protection from the open sea. Most importantly, the intake must provide reliable, high-quality seawater within the optimal temperature range for hatchery operations, while ensuring construction, maintenance, and operational costs remain economical.

Two main seawater sources are typically considered: (a) marine wells and (b) direct pumping from the sea. Marine wells, which draw seawater from aquifers, offer a secure supply unaffected by weather or sea conditions. However, their feasibility depends on specific geological conditions that must be carefully assessed and characterized. It is essential to ensure the groundwater is free from contamination by metals or other geological substances, and that the temperature is suitable for hatchery operations. While groundwater often maintains a stable, optimal temperature for larval and juvenile rearing, it may be less suitable for broodstock, which require natural temperature fluctuations to trigger reproductive development.

Direct seawater intakes are more common and present their own challenges. The intake must be protected from physical damage caused by waves or storms and must avoid clogging from sediments or fouling. Civil engineering structures may be required to ensure stability and functionality. Construction costs increase in dynamic coastal environments, highlighting the advantage of selecting sheltered bays or inlets with good seawater quality. Various engineering solutions are available depending on the coastal setting, including breakwaters to protect the intake, coarse substrate filtration, anchoring or routing the intake through rocks, burying intake pipes in sand or heavy rocks, or constructing a coastal lagoon, pond, or tank connected to the open sea via pipes or filtration systems (for detailed guidance, see FAO *Manual on Hatchery Production of Seabass and Gilthead Seabream*, Volume 2. See Reference and further readings).

The intake construction project should be designed and executed by experienced professionals. The intake must be positioned relative to the hatchery discharge to prevent re-circulation of effluent back into the system. It is also considered good practice to install two or more intake pipes. This allows continuous water supply during maintenance or in case of pipe failure, minimizing interruptions to hatchery operations. Periodic cleaning and maintenance of the intake pipes are necessary to ensure consistent water flow and system reliability.

Figure 2.2 and Figure 2.3 illustrate two seawater intake scenarios.

A sheltered bay – Figure 2.2 shows an intake in a calm bay with small tides and low wave activity. A high-density polyethylene (HDPE) pipe runs under coastal sediments and over the seabed to depths exceeding six metres, with the intake positioned at an intermediate depth within the water column. A buoy marks the intake for vessels and maintenance. On land, the pipe connects to an underground tank below seawater level beneath the pumping room. Water flows by gravity into the tank and is then pumped to the hatchery via submersible or dry pumps.

An open, dynamic beach – Figure 2.3 depicts an intake on a dynamic sandy coast with large tides and waves. The HDPE pipe is buried to the low tide mark and terminates in a PVC structure with multiple vertical pipes extending three meters into the sand. The lower meter of these pipes, below low tide level, has fine openings that filter out sediment. The pipe connects to a watertight pumping room below sea level, with the pump house located above the high tide mark. From there, seawater is pumped to the hatchery.

The pumping station must be designed with respect to the seawater level. Ideally, the pump should be located at or below sea level to allow gravity-fed water delivery, which maximizes efficiency and reduces mechanical stress. Pumps positioned above the water level must operate under suction, increasing wear and the risk of air entrainment, which

FIGURE 2.2
Example of a seawater intake used for a calm bay

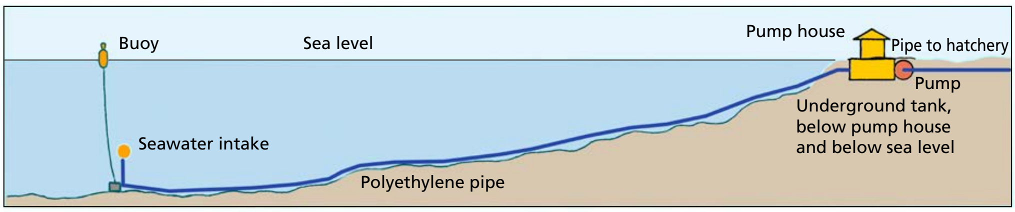
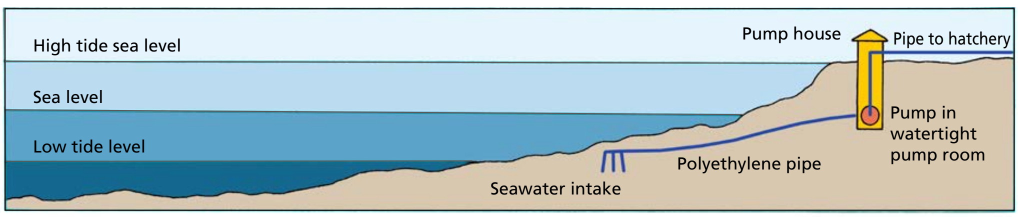


FIGURE 2.3
Example of a seawater intake used on a dynamic coast that is affected by constant wave and tidal action



can lead to priming failures. For below-sea-level installations, submersible pumps or a watertight pump room are essential to prevent flooding and protect equipment. A sump pump can be incorporated to maintain dry conditions in case of leaks from the pipework or pump.

From the pumping station, seawater is delivered to a storage tank or reservoir to ensure an adequate volume is available for hatchery operations. Initial sedimentation occurs in the storage tank, followed by coarse filtration to remove solids in the 25–100 µm range. Large sand filters with automated backwash are recommended for primary filtration. After coarse filtration, water may either be directed to secondary storage tanks feeding the culture units by gravity or pumped directly to the culture systems. Prior to entering the culture units, finer filtration is applied (1–20 µm depending on stock life stage). Ultraviolet sterilization can be incorporated to reduce bacterial load and activated carbon filters may be used to remove dissolved chemical contaminants.

Culture systems may operate as semi-closed flow-through units or closed RAS. Both generate effluent that must be collected, treated, and discharged in compliance with local regulations, ensuring no contamination of intake water. Effluent from multiple culture units can be routed through a common collection pipeline to sedimentation tanks or ponds with residence times of at least one day. Sedimentation ponds may incorporate artificial wetlands for bioremediation, and more advanced treatments can include solids removal and biofilters to eliminate nitrogenous waste. The discharge point must be located downstream or down-current from the intake to prevent re-entry of treated effluent into the facility.

For small *M. cephalus* hatcheries, beach wells offer a low-technology, cost-effective seawater source. Water is naturally filtered through sand and gravel, removing suspended solids, plankton, pathogens, and organic matter while maintaining stable temperature and salinity with fewer fluctuations than direct surface intake. Beach wells reduce fouling and clogging, maintain consistent water quality, lower operating costs, and minimize reliance on large-scale filtration.

Design and construction of a beach well require careful site selection close to shore while avoiding flood or contamination risks. Well depth typically ranges from 3–10 m, depending on local geology, with PVC or HDPE casing and screened sections. Pumps should be submersible or low-lift and sized for peak demand. Water passes through sand or multimedia filters followed by 5–50 µm cartridge filters. Redundant systems, such as a second well or direct seawater intake, are recommended to ensure uninterrupted supply. Limitations include seasonal fluctuations in water yield and salinity, potential iron or manganese leaching in certain soils, and unsuitability in rocky or clay-rich coastal areas.

2.4 LIVE FEED

The live feed unit is a vital component of a flathead grey mullet (*M. cephalus*) hatchery, responsible for the continuous production of microalgae, rotifers and *Artemia*. These live feeds are indispensable during the larval and early post-larval stages, as they provide essential nutrition that directly influences larval survival, growth, and development. The efficiency and reliability of this unit determine the overall success of the hatchery's larval rearing operations.

The live feed unit should be strategically located adjacent to the larval rearing unit to facilitate the rapid and hygienic transfer of live feeds. Nevertheless, each of its components must remain functionally independent to prevent cross-contamination among cultures. The unit is typically organized into distinct sections dedicated to microalgal culture, rotifer production, and *Artemia* hatching and enrichment, as well as a small laboratory and a cold storage room for the preservation of enrichment products and feed materials.

Each section of the unit should be spacious, well ventilated, and designed for easy cleaning and disinfection. Walls and floors should be constructed from smooth, non-porous materials, preferably coated with epoxy paint or tiles, and floors should slope gently toward a central drain to ensure proper wastewater removal. The working environment must maintain stable temperature and humidity levels, with a reliable supply of filtered seawater and freshwater, sterile air for aeration, safe electrical connections, and adequate lighting for monitoring and routine operations.

A support laboratory should be integrated within or located adjacent to the live feed unit. This laboratory is essential for preparing and sterilizing media, examining live feed organisms, and maintaining aseptic working conditions. It should contain analytical balances for weighing reagents, compound and stereomicroscopes for observing microalgae and zooplankton, an autoclave for sterilization, and a sink with freshwater and drainage for cleaning vessels. A refrigerator or freezer is required for storing reagents, algal concentrates, and enrichment products, while sturdy workbenches and storage cabinets should be available for materials and consumables. A separate cold room maintained at 4–10 °C is recommended for storing concentrated algae, emulsified enrichment products, and preserved feeds used in live feed preparation.

2.4.1 Algal culture facilities

Microalgae play a vital role as they serve both as the primary feed for rotifers and as a "green water" source in larval tanks. The scale and design of the algal culture facilities depend on the hatchery's production capacity and the daily live feed demand.

In some hatcheries, live or concentrated microalgae are purchased from commercial suppliers, reducing the need for on-site production. This practice can be effective if a consistent and reliable supply chain is available. However, most hatcheries prefer to produce microalgae on site to ensure continuous supply, quality control, and cost-effectiveness. Microalgal culture systems are commonly classified into batch systems and continuous systems such as photobioreactors. This manual will focus on the batch system, as it is the most widely used in hatcheries around the world.

FIGURE 2.4
Pure algal strain room, with controlled environment (air-conditioned), walls and floor that can be disinfected, slopped floor with central drain, shelving with culture vessels and back lighting

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Pure algal strain room

The foundation of all algal culture operations is the maintenance of pure, monospecific microalgal master cultures. These strains can be isolated from natural seawater or obtained from recognized culture collections such as the Culture Collection of Algae and Protozoa (CCAP) or from the University of Texas at Austin (UTEX). The species obtained from the CCAP in Oban, Scotland, are typically supplied in small 50 ml test tubes, which are then transferred to 100–250 ml Erlenmeyer flasks containing sterilized culture medium (Figure 2.4 and Figure 2.5). The flasks are loosely capped with cotton or aluminium foil to allow gas exchange while preventing contamination. Because flasks support larger inoculum volumes than test tubes, they are preferred for laboratory-scale maintenance of stock cultures. These cultures are stored in an incubator under controlled light and temperature conditions to avoid any contamination (Figure 2.6).

The pure algal strain room must be climate-controlled and designed to provide sterile working conditions. The optimal temperature range is between 18 and 24 °C, although temperatures between 16 and 27 °C are acceptable. A typical room has a floor area of approximately 10 m^2, though this can be adapted to the hatchery's production needs. The walls and floors should have smooth, washable surfaces finished with tiles or epoxy paint, and the floor should slope toward a central drain for efficient cleaning. Shelving should be installed along the walls to support culture vessels of diverse sizes, including test tubes, conical flasks of 250–500 ml and larger 5–10 L carboys. Backlighting or fluorescent lamps should be arranged to provide a light intensity of approximately 1 000–6 000 lux to promote algal growth.

A sterile air supply is essential to keep algae in suspension and to provide both oxygen and carbon dioxide. Industrial-grade carbon dioxide at about 1 percent can be mixed into the air stream to enhance algal productivity. The air should pass through sterilizing filters before entering the culture vessels to avoid contamination, and sterile plastic tubing fitted with glass diffusers should be used for aeration. A direct water connection is not required in the pure strain room because all water and culture media are prepared and sterilized in the laboratory before inoculation.

The laboratory should be located next to the pure algal strain room and equipped for all preparation and sterilization procedures. It must include a sink with freshwater for washing vessels, which are subsequently sterilized in an autoclave. The seawater used for algal cultures should have a salinity between 12–40 ppt, with an optimum range of 20–24 ppt and must be filtered to one µm and sterilized either by UV treatment or autoclaving. Nutrients should be

FIGURE 2.5
Algal culture vessels, conical flasks (250–500 ml) and 5–10 L flasks or carboys on shelve with back lighting

FIGURE 2.6
Stock microalgae culture flasks stored in an incubator

added to the culture water to promote algal growth, following standard formulations such as Guillard's f/2 or Walne's medium. All materials required for media preparation and culture maintenance should be readily available within the laboratory.

Essential equipment for the laboratory includes an autoclave, refrigerator, compound microscope, workbenches with adequate lighting, and cupboards for storage of chemicals and glassware. Glass culture vessels with tightly fitting caps, pipettes, Petri dishes, microscope slides, and reagents should also be available. All media, culture vessels, and seawater must be sterilized before use, and stock cultures should be regularly examined microscopically to detect and prevent contamination. Periodic renewal of master cultures is recommended to maintain their health, genetic stability, and productivity.

Algal production sub-unit

The algae production sub-unit is specifically designed to produce large volumes of concentrated microalgae required for larval culture. The algae serve a dual purpose: they provide "green water" in larval tanks, which improves larval feeding efficiency by stimulating prey recognition and foraging behaviour, and they are used as a feed source for rotifers, which are an intermediate live feed for larvae. To optimize operational efficiency, the algae production sub-unit should be located near both the pure algal strain room and the larval rearing unit, allowing rapid and hygienic transfer of algal cultures. All surfaces within the unit should be smooth, non-porous, and easy to clean and disinfect. The floor should be slightly sloped toward a central drain to facilitate wastewater removal. Continuous access to filtered seawater, freshwater, sterile air, and CO_2 is required. A dedicated sink and disinfection station, including a disinfection bath for equipment, should be available, along with waterproof electrical outlets and strategically placed lighting to ensure safe operation and maintenance.

Algae are typically cultured in either transparent plastic culture bags or transparent plastic or fiberglass cylinders with volumes ranging from 50–400 L (Figure 2.7 and

FIGURE 2.7
Algal culture bags with back lighting
Bags are made from a plastic tube that is doubled and tied off to make a watertight seal. A concave base is used to support the bag in the mesh cylinder

Figure 2.8). Sterile plastic culture bags, 0.2–0.3 mm thick, offer practical advantages because they do not require the rigorous disinfection procedures needed for cylinders. For structural support, these bags are often placed inside protective plastic-coated wire mesh cylinders with a concave base that distributes the bag's weight evenly. The bottom of each bag is either heat-sealed or tied off and secured with zip ties to prevent leakage.

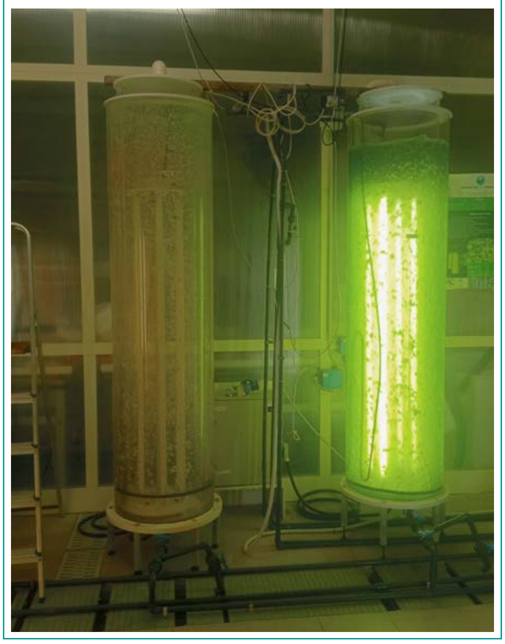
Each container, whether a bag or a cylinder, is filled with sterilized seawater to which the appropriate nutrients are added to support algal growth. The containers are then inoculated with one or two flasks or carboys (5–10 L) of concentrated microalgae and are continuously aerated with sterile air enriched with CO_2 to maintain the algae in suspension and support optimal photosynthetic activity. Air is delivered to the bottom of the containers through sterile plastic tubing (5–6 mm internal diameter), often weighted or attached to a ridged pipe to ensure even dispersion. Illumination for the containers should be set between 4 000 and 10 000 lux, using natural daylight when available or artificial light sources such as fluorescent or LED lamps. Supplemental lighting may be required during nighttime periods to maintain continuous growth.

Temperature control is critical for optimal algal productivity. The culture environment should be maintained between 16–27 °C, with an optimum range of 18–24 °C, particularly when using transparent walls that can amplify heat fluctuations. Each container requires approximately 0.25 m² of floor space and should be arranged along a wall to optimize lighting exposure. A working space of 1–2 m in front of the containers must be maintained for safe handling, maintenance and sampling. The total area required for the algae production sub-unit is determined by the hatchery's production targets and microalgae growth cycles. For instance, if two containers are required per day and the algal growth period is two weeks, the unit must accommodate at least 28 containers, along with sufficient space for working, transferring and monitoring the cultures.

Large volumes of microalgae can also be produced in 120–500 L custom-built photobioreactors (Figure 2.9). These photobioreactors are constructed from concentric Plexiglas® cylinders glued to a Plexiglas® base, forming a cavity to contain the algal culture. The cavity is fitted with a lower drain valve for harvesting and a perforated PVC cover that allows the introduction of air and CO_2 for aeration and pH regulation, as well as the placement of sensors to monitor temperature, pH, dissolved oxygen (O_2), and CO_2 concentrations within the culture.

FIGURE 2.8
Algal culture fiberglass cylinders with back lighting

FIGURE 2.9
Annular photobioreactors equipped with system for controlling temperature, pH, and CO_2 supply
The left reactor is sterilizing seawater in preparation for medium addition and microalgae inoculation, while the right reactor is actively producing microalgae under controlled conditions



Mixing of the microalgal culture is achieved through the diffusion of the air-CO_2 mixture, delivered via a circular perforated tube positioned at the bottom of the reactor. The airflow is provided by a linear membrane blower capable of delivering up to 70 N L/min. The flow to each reactor is regulated by individual flow meters, while solenoid valves control the entry of gases. The CO_2 flow is similarly regulated with dedicated flow meters, and its injection is automatically controlled by the system based on pre-set pH values.

The lighting system consists of six fluorescent tube lamps of the LUMILUX Cool Daylight type, with a light shade according to EN 12364-1 and with a nominal power of 58 W. The lamps are mounted on a structure ("castle") positioned within the internal cylinder, ensuring uniform light distribution throughout the culture volume.

The cultivation medium filtration system consists of a series of cartridge filters with porosities ranging from 10 to 0.6 µm, ensuring the removal of particulates prior to inoculation. Culture temperature is controlled by a heat pump that circulates fluid through two coils located within each reactor cavity, enabling precise thermal regulation.

A dedicated control system, consisting of a remote unit with software implemented on a Windows platform, monitors and regulates all critical parameters, including pH and temperature. This system also records operational data, enabling continuous observation and control of the photobioreactor conditions to ensure optimal microalgal growth.

2.4.2 Zooplankton culture facilities

Rotifer production sub-unit

The rotifer production sub-unit is designed to supply large quantities of enriched rotifers for *M. cephalus* larval rearing. The sub-unit should be located in close proximity to the larval culture area to ensure rapid transfer and minimize handling stress. A dedicated temperature-controlled cabinet or enclosed area is recommended for the maintenance of pure rotifer strains. Access to a laboratory, or the provision of a dry workbench equipped with a microscope, is required for routine enumeration, health assessment, and observation of rotifer activity. To prevent cross-contamination, the rotifer production sub-unit must be physically separated from algal culture sub-units and must not allow direct access between these areas.

All walls and floors within the rotifer sub-unit must be finished with non-porous, chemically resistant materials suitable for frequent cleaning and disinfection, such as ceramic tiles or epoxy coatings. Floors must be sloped toward appropriately sized drainage channels to allow rapid evacuation of large volumes of water generated during vessel cleaning and rotifer washing procedures.

The sub-unit must be equipped with dedicated supplies of seawater, freshwater, air, and oxygen, each delivered through flexible, easily detachable hoses to facilitate cleaning and maintenance. Seawater, air, and oxygen are used exclusively for rotifer culture operations, while freshwater is reserved for cleaning and sanitation. A sink and designated disinfection area, including a disinfection bath, must be provided. Electrical outlets must be water-resistant, and adequate general lighting must be installed to ensure safe and efficient operation.

The sub-unit must be supplied with all essential equipment and consumables, including buckets, funnels, graduated cylinders, beakers, containers for chemical storage, glassware for culture monitoring, thermometers, and dissolved oxygen meters for routine monitoring. Mobile trolleys are recommended for the safe transport of large volumes of rotifer cultures. Fine-mesh rotifer filter bags, typically with a mesh size of approximately 60 µm (adjusted according to the rotifer strain), are required for harvesting and cleaning operations.

FIGURE 2.10
Rotifer culture tanks that are circular made from plastic that is easily disinfected with a conical bottom and valve for purging and harvesting the rotifers

Rotifer mass culture is conducted in circular tanks with working volumes ranging from 1 000–4 000 L, allowing culture densities to exceed 1 000 ind./ml. Tanks must be constructed from food-grade fiberglass or plastic and finished with smooth gel-coat or rigid plastic surfaces to facilitate effective cleaning and disinfection. Each tank must be fitted with a conical bottom and equipped with a suitably sized drain and valve to enable efficient harvesting and removal of settled organic debris (Figure 2.10). Prior to inoculation, tanks must be filled with sterile seawater.

Aeration is provided using air stones positioned approximately 15 cm above the conical bottom to generate sufficient turbulence to maintain rotifers in suspension. An airflow rate of 2–3 m^3/h/1 000 L of culture volume is required. At high culture densities, supplemental oxygen may be supplied via fine-bubble ceramic aeration stones positioned near the tank bottom. Culture temperature must be maintained at approximately 25 °C using submersible electric water heaters installed in each tank.

A raised walkway located behind or between culture tanks must be provided to allow safe installation and adjustment of aeration devices and heaters, as well as routine sampling and monitoring activities. Each tank requires approximately 1 m^2 of floor space. A minimum operational area of 8 m^2 must be allocated for cleaning, harvesting, enrichment and handling procedures. The total number of tanks and overall floor area required depend on projected hatchery production capacity, peak daily rotifer demand for larval feeding, reinoculation requirements, target harvest density, and the growth rate or culture duration necessary to reach harvest density. In most operations, a 4–5 day batch culture cycle is implemented for each tank, with an additional safety margin incorporated to account for potential culture losses or system failures.

For high-density continuous production, a recirculating aquaculture system (RAS) incorporating circulation pumps, biofilters, protein skimmers with ozonation capability, and UV sterilizers should be installed. Continuous flow-through systems with adjustable flow rates and appropriate filtration units – such as biofilters or foam fractionators equipped with 1 µm filter cartridges – are used to effectively remove metabolic wastes and suspended solids. Seawater heaters and automated temperature controllers must be installed to maintain culture temperature within a narrow tolerance range, typically around ±1 °C.

As in the batch production system, to maintain oxygen saturation, a dedicated air distribution system must be installed, with air stones suspended approximately 15 cm above the tank bottom and distributed both along the tank periphery and centrally. Supplemental oxygen may also be delivered through ceramic fine-bubble aeration stones as required. Culture conditions must be maintained within the following operational ranges: pH 7.5–8.5, salinity 20–30 ppt, and total ammonia (NH_3) concentrations below 1 mg/L. Moderate and uniform turbulence must be maintained throughout the culture volume. A lighting system must be installed; however, illumination is required only during periods of feeding with microalgae.

Artemia production sub-unit

The *Artemia* production sub-unit is designed to supply large quantities of *Artemia* nauplii and metanauplii for the larval rearing of *M. cephalus*. The sub-unit should be located in close proximity to the larval culture area to facilitate rapid transfer and minimize handling time. It may also be situated adjacent to the rotifer production sub-unit, as all three units can share common seawater, air, and freshwater supply systems.

All walls and floors within the *Artemia* sub-unit must be finished with smooth, non-porous materials suitable for frequent cleaning and disinfection, such as ceramic tiles or epoxy coatings. Floors must be sloped toward appropriately sized drainage channels to allow rapid outflow of the large volumes of water generated during harvesting and cleaning operations.

The sub-unit must be equipped with dedicated supplies of seawater and air for *Artemia* culture and freshwater for cleaning and sanitation. A sink and a designated disinfection area, including a disinfection bath, must be provided. Electrical outlets must be water-resistant. General lighting should be installed throughout the unit; however, additional lighting must be provided above the *Artemia* culture tanks to deliver approximately 2 000 lux at the water surface.

As with other production areas, this sub-unit must be equipped with all essential materials and consumables, including buckets, funnels, graduated cylinders, beakers, containers for chemical storage, glassware for culture monitoring, thermometers and dissolved oxygen meters for routine checks. Mobile trolleys are recommended for the safe transport of large containers filled with *Artemia* cultures.

Artemia culture is conducted in circular tanks typically with volumes ranging from 500–2 000 L and fitted with conical bottoms (Figure 2.11). Tanks must be constructed from food-grade fiberglass or plastic and finished with smooth interior surfaces to facilitate effective cleaning and disinfection. Each tank must be equipped with a conical bottom fitted with an adequately sized drain and valve to enable efficient harvesting and removal of settled debris. A transparent viewing window located near the drain at the base of the conical bottom may be installed to introduce light, thereby concentrating nauplii and facilitating harvesting. To further enhance harvesting efficiency, the use of the SEP–Art® system is recommended. This system separates unhatched cysts and empty shells from *Artemia* nauplii using magnetic separation, retaining cysts and shells while allowing nauplii to be collected (Figure 2.12).

To initiate *Artemia* culture, tanks must be filled with sterile seawater and inoculated with decapsulated cysts. Strong aeration must be provided using open pipes or air stones to generate sufficient turbulence and maintain *Artemia* in suspension. An aeration rate of 6–8 $m^3/h/m^3$ of culture volume is required. Culture temperature must be maintained within the range of 25–30 °C using submersible electric water heaters installed in each tank.

Each culture tank requires approximately 1 m^2 of floor space. A minimum operational area of 8 m^2 must be allocated for cleaning, harvesting, and enrichment procedures. The total number of tanks and overall space requirements depend on projected hatchery production capacity, peak daily demand for nauplii and enriched

FIGURE 2.11
Examples of *Artemia* culture tanks made from plastic that is easily disinfected with a conical bottom and valve for purging and harvesting the *Artemia*

FIGURE 2.12
***Artemia* harvesting with the PVC SEP–Art® separation tool**
The inner tube containing circular magnets is inserted into the outer tube (a), which is connected to the tank outlet (b). During tank drainage, unhatched cysts and empty shells are retained on the magnets, while hatched *Artemia* nauplii pass through the tool and are collected in a mesh bag suspended in a bucket

metanauplii, average production density per tank, and the duration of the production cycle. Typical production times include approximately 24 h for nauplii incubation, followed by 12–24 h for metanauplii enrichment.

2.5 EGG INCUBATION AND LARVAL CULTURE AREA

Eggs can be incubated either in specialized hatching tanks or directly in larval rearing tanks; however, the use of separate hatching tanks is preferred due to several advantages. Once eggs hatch, only the larvae are transferred to clean larval tanks, while the hatching tanks can be quickly emptied, cleaned, disinfected and prepared for the next batch of eggs. This approach also simplifies the management of batches with low hatching success, as the smaller tank size allows easier handling.

Egg incubation typically takes place in circular plastic or fiberglass tanks with conical bottoms and capacities ranging from 100–250 L. The cylindroconical design promotes efficient water circulation when aeration is applied near the cone tip and facilitates the removal of non-viable eggs and hatching debris. Tanks must feature smooth interior surfaces to minimize damage to eggs and newly hatched larvae.

Before introducing eggs, tanks must undergo thorough cleaning and disinfection using a hypochlorite solution, following the same procedures used for rotifer and *Artemia* tanks. Inlet and outlet pipes, as well as submerged aeration devices such as diffusers and tubes, must also be sanitized. Tanks are then filled with filtered and sterilized seawater, ensuring that temperature and salinity match those of the spawning tank from which the eggs were collected.

The incubation system typically operates as a flow-through setup, where outgoing water is not recycled to prevent the accumulation of hatching by-products and potentially harmful microorganisms. Water exchange rates are adjusted according to egg density to maintain full oxygen saturation without forcing eggs against the outlet screen. Outlets are fitted with removable 400 µm nylon mesh screens to retain eggs and larvae. Continuous aeration creates gentle water movement around the screen, preventing clogging, while bottom aeration keeps eggs and larvae suspended and avoids stratification. The photoperiod aligns with that of the larval rearing area. Recommended stocking densities range from 10 000–15 000 eggs/L. Water turnover is maintained at one full renewal per hour during incubation and increased to two renewals per hour during and immediately after hatching.

The larval rearing unit serves as the core production area of the hatchery, where eggs are hatched and larvae reared using both live and inert feeds to produce early-stage juveniles. The size of the unit – number of tanks and total occupied area – depends on projected hatchery production. The unit should be located close to live feed sub-units (microalgae, rotifers and *Artemia*) to facilitate efficient transport and feeding.

The unit must include a refrigerator or large cool boxes for cold storage of enriched rotifers, *Artemia* nauplii and enriched metanauplii. Walls and floors must be finished with materials that are easy to disinfect, such as epoxy paint, with floors sloped to direct drainage into collection channels. A sink and a designated disinfection area, including a disinfection bath, must be available for cleaning equipment and utensils. Electrical outlets must be water-resistant. General lighting is standard throughout the area, with additional adjustable lighting above larval tanks to provide specific light intensities at the water surface. The unit must be supplied with seawater, freshwater, air, and oxygen. Seawater must be fine-filtered and UV-disinfected before entering the larval rearing systems. Freshwater is used for cleaning and must be available at multiple locations via flexible hoses. Air and oxygen provide gentle aeration to maintain high dissolved oxygen levels (>6 mg/L).

The larval rearing unit must be equipped with all necessary materials, including buckets, funnels, graduated cylinders, beakers, glassware for culture monitoring, thermometers, and oxygen meters for routine checks. The unit may be divided into sections: one for egg incubation and, depending on capacity, additional sections for rearing multiple cohorts of larvae from different spawnings.

Small circular tanks of approximately 500 L are typically used for egg incubation (Figure 2.13a). These tanks have conical bottoms made from fiberglass or plastic and are easily cleaned and disinfected. They are supplied with seawater, air and oxygen. Low-level lighting is used to improve hatching rates. Bottom and side outlets are fitted with mesh screens (350–500 µm) to retain eggs, with the bottom outlet incorporating a valve for purging debris and concentrating larvae for transfer. These tanks may operate as either flow-through or RAS, as water requirements are low. Ideally, temperature and oxygen sensors are monitored automatically, with alarms for critical deviations.

FIGURE 2.13
Examples of tanks for larval rearing of *Mugil cephalus*
500 L tanks used for egg incubation and larval rearing fitted with side and bottom central outlets and conical bottom (a); 2 000 L tanks used for larval and early juvenile rearing fitted with side and bottom central outlets and sloped bottom (b)

Larger larval rearing tanks are constructed from the same materials and feature gently sloped bottoms leading to a central drain (Figure 2.13b). Walls are typically black to enhance larval feeding efficiency, while lighter-coloured bottoms facilitate cleaning. Smaller tanks provide greater biosecurity by isolating potential diseases, particularly bacterial infections, but require more labour and may result in less stable culture conditions. Current trends favour larger tanks, typically 5–10 m^3, for improved production efficiency. Tanks are fitted with central and side outlets and mesh screens of varying sizes according to larval stage.

Key considerations when selecting larval rearing tanks include:

- Visibility of larvae throughout the water column.
- Ease of cleaning the tank bottom.
- Uniform feed distribution across the surface.
- Accessibility to tank outlets.
- Efficient drainage and harvesting.
- Good water circulation without dead zones.
- Tank design that facilitates larval swimming and distribution.
- Cost and availability.
- Optimal use of hatchery space.
- Labour requirements for maintenance.

Seawater is introduced at the tank periphery to create a gentle circular flow, regulated via ball valves. As larvae grow, the frequency of water exchanges is increased. Static water may be maintained during daytime feeding, with exchanges at night to remove uneaten feed. Systems may operate as either flow-through or RAS, with RAS preferred to maintain optimal temperatures of 20–25 °C.

Space requirements for the larval rearing unit depend on production targets and tank design. At least 75 percent of each tank's circumference should be accessible for effective feeding and cleaning, with full accessibility preferred. Tank volume requirements depend on the number of juveniles to be produced, desired final density, and the duration of the larval rearing period. The larval and post-larval period concludes when fish can feed on formulated micro-diets, marking the transition to the juvenile culture stage, commonly referred to as weaning.

2.6 JUVENILE CULTURE AREA

The juvenile culture unit is designed to rear juveniles until they reach a size and weight suitable for transfer to grow-out facilities, typically between 2–10 g. The unit should be located close to the larval rearing area to facilitate efficient juvenile transfer. It must be easily accessible, with large doors opening onto a paved area to allow lorries to deliver supplies and collect juveniles. This external space serves as a loading and unloading zone for equipment, feed and juvenile transport.

Inside the unit, large tanks are used for juvenile rearing, with ample workspace for routine tasks such as grading and health inspection. Floors must be sloped toward drainage channels to collect spilled water and cleaning runoff efficiently. A sink and designated disinfection area, including a disinfection bath, must be provided for equipment and utensil cleaning. Electrical outlets must be water-resistant, and adequate general lighting is required to support safe and effective operations.

The unit must be supplied with seawater, freshwater, air, and oxygen. Seawater must be finely filtered and UV-disinfected before use. Freshwater is required for cleaning and should be available at multiple points via flexible hoses that can be easily disconnected for maintenance. Air and oxygen are used to maintain optimal dissolved oxygen levels above 6 mg/L in the rearing tanks.

Rearing tanks must be equipped with temperature and dissolved oxygen sensors, continuously monitored by an automated system with alarms for any deviations. The unit must also be stocked with essential tools and materials, including buckets, funnels, nets, thermometers, and oxygen meters for routine checks and maintenance.

Juvenile tanks are typically large, ranging from 10–30 m^3, constructed from fiberglass with smooth gel-coat surfaces for ease of cleaning and disinfection. Tanks may be circular or raceways. Circular tanks feature gently sloping bottoms that direct water to a central outlet, while raceways are generally constructed from concrete and sloped lengthwise toward the outlet.

Aeration is provided to prevent dead zones, and mesh screens are installed to retain juveniles during water exchanges. Water supply may operate as either flow-through or RAS, with the latter preferred for optimized water quality and environmental control.

The space required for the juvenile culture unit depends on projected hatchery production. Tank design, volume, target juvenile density at harvest, and the planned rearing period (typically 150–200 days) are key factors in determining floor space and layout.

2.7 BROODSTOCK HOLDING AND SPAWNING AREA

The broodstock holding and spawning area is essential for producing the quantity and quality of *M. cephalus* eggs required for successful hatchery operations. This area must include a quarantine unit for incoming broodstock, long-term holding facilities to maintain adult breeders and meet egg production targets, and spawning tanks specifically designed for *M. cephalus* spawning and egg collection. Ensuring a consistent supply of high-quality eggs has historically been a significant bottleneck in the development of *M. cephalus* aquaculture, making the effective design and operation of the broodstock area critical to hatchery success.

Broodstock held in captivity typically exhibit two types of reproductive dysfunctions. Early-stage reproductive arrest usually occurs in semi-closed or closed aquaculture systems, where fish fail to initiate or progress through gonad development, often due to inadequate environmental cues such as temperature, photoperiod or water quality. Late-stage reproductive failure generally affects fish captured from extensive ponds or open water systems; these broodstock may develop mature gonads but fail to complete final maturation or spawning, often because the stress of capture and confinement disrupts hormonal control. Each type of dysfunction is linked to specific environmental and management conditions. To overcome these challenges, hormone induction protocols are used to stimulate final gonadal development and spawning. Detailed procedures are provided in Chapter 4 (Broodstock management).

Given the complexity of managing reproduction in *M. cephalus*, broodstock facilities must be carefully planned from the earliest stages of hatchery design. This planning includes defining the spawning strategy to be used, whether relying on wild or captive broodstock, ensuring that the appropriate infrastructure is available to address either early- or late-stage reproductive dysfunctions, and providing access to external environments, such as extensive ponds or open natural systems, when required.

Successful implementation of spawning induction protocols also depends on skilled personnel. Qualified staff are essential to ensure that hormonal treatments and broodstock management practices are properly carried out. The hatchery's ability to reliably produce eggs and larvae depends on a combination of the correct infrastructure, access to suitable environmental conditions, and expert knowledge in broodstock management.

2.8 SEMI-CLOSED AND CLOSED BROODSTOCK HOLDING SYSTEMS

Hatchery broodstock holding and spawning areas must be kept separate from live feed, algal, larval, and juvenile units. Strict biosecurity and quarantine measures are essential, including changing clothing and/or disinfecting when moving between the broodstock area and other parts of the hatchery. The broodstock area is composed of three functional units: quarantine, broodstock holding and spawning. While these units can have similar construction and design, quarantine procedures are particularly critical in the first unit.

Mugil cephalus broodstock have requirements similar to most marine farmed fish species and thrive in a variety of holding conditions. Tanks for broodstock can range from 5–60 m^3, constructed from fiberglass or concrete with smooth, easily disinfected surfaces. Quarantine and spawning units are preferably housed indoors, while broodstock holding units can be outdoors. Ponds ranging from 50–200 m^3 may also be used for broodstock holding, provided they are designed to retain water, using clay or pond liners where necessary. Ponds must be above the water table, fully drainable, and ideally equipped with gravity-fed inlets and outlets. Walls or dykes should have a gradient between 1:1 and 1:2, depending on the substrate, with pond bottoms sloping 2–5 percent from inlet to outlet for complete drainage. Minimum water depth should be one meter to limit weed growth, and ponds should have firm surrounding ground for vehicle or forklift access, preferably along the dyke tops.

All tanks and ponds should receive coarse-filtered seawater and be equipped with air and oxygen supply systems. Freshwater must be available for cleaning and treating disease or parasite infections. Alarms to monitor oxygen, temperature and water flow are recommended. Daily water exchange should range from 400–600 percent for tanks and 50–100 percent for ponds, depending on pond volume. Tanks must be covered with 2–4 cm mesh netting to prevent fish from jumping and protect against predation. Shade netting is recommended for outdoor tanks and ponds, as *M. cephalus* breeders prefer low light. Floating structures can provide shade in ponds but must not trap jumping fish. Stocking densities should not exceed 5 kg/m^3 in tanks or 1 kg/m^3 in ponds. Water

FIGURE 2.14
The IRTAmar® 200 recirculation aquaculture system module
The module includes a complete recirculating aquaculture system designed to support up to 200 kg of marine aquatic biomass. The RAS is linked to two 10 m³ tanks located downstream, which are suitable for holding broodstock or for on-growing juveniles

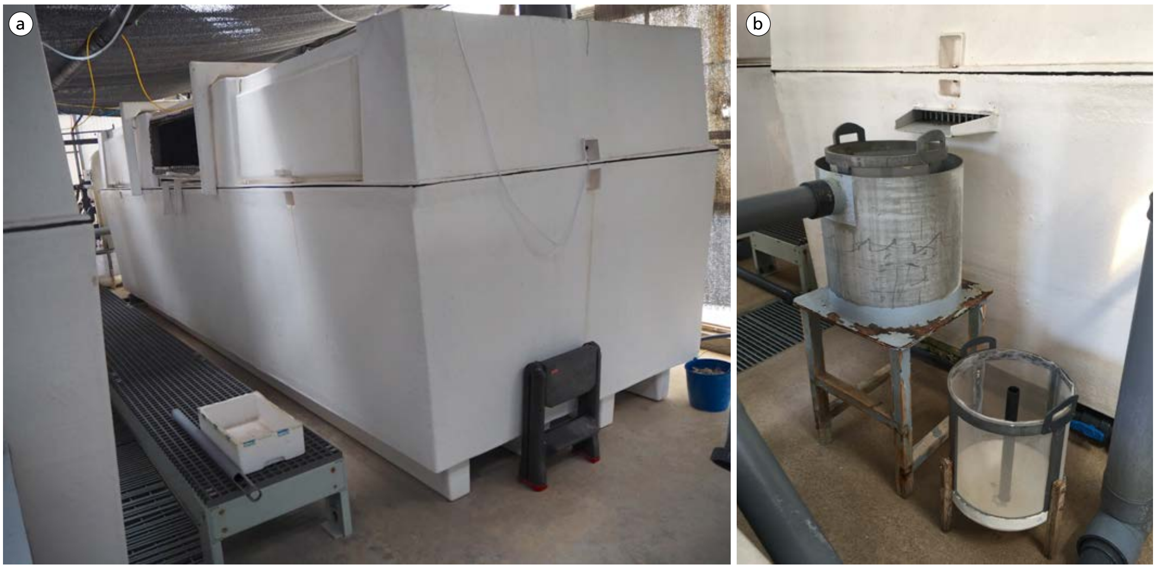
systems may be semi-closed flow-through or RAS, with RAS preferred for tanks requiring temperature control for preconditioning and spawning (Figure 2.14). For more details see Section 4.2 on Captive broodstock management.

The quarantine unit should have smaller tanks of 5–10 m^3 to facilitate treatments to minimize disease risk. Broodstock holding units should use larger tanks or ponds capable of holding significant numbers of breeders. Spawning units should consist of medium-sized tanks, approximately 10–20 m^3, equipped with surface egg collectors and dual outlets: one at the bottom and one at the surface (Figure 2.15 and Figure 2.16). Outlet flows must be adjustable for efficient egg collection. Preconditioning tanks in the holding unit should match the spawning tank design to acclimate breeders prior to transfer, but natural spawning will not occur in these tanks. Hormone-induced spawning protocols are required to overcome early-stage reproductive dysfunction.

Hormone induction involves weekly injections administered in preconditioning tanks. It is recommended to use two tanks per broodstock group to minimize stress and prevent jumping injuries. One tank remains temporarily empty while breeders are held in the second. Fish are individually captured from this second tank, anesthetized, examined, and injected before transfer to the empty tank to recover. Lowering water levels during capture further reduces jumping risk. Injected breeders are placed in a quiet, net-covered tank before transfer to spawning tanks. Eggs are collected using mesh baskets at the surface, counted, disinfected, and moved to the larval rearing unit.

The size of the broodstock area, number of tanks, and total water volume should reflect projected juvenile production. Planning must consider juvenile requirements, survival rates at each life stage, egg production per female, male-to-female ratios, and stocking densities for holding, preconditioning and spawning. It is advisable to overestimate breeder numbers by approximately 50 percent to account for mortalities during the 3–5 y required for maturation. Females typically spawn once per year,

FIGURE 2.15
Example of a 10 m³ tank used for holding broodstock or on-growing juveniles (a) and a surface water egg collector (b)
The surface water exits the tank and passes through the mesh basket to collect all the eggs. An example of the mesh basket is seen on the floor beside the egg collector



while males may participate in multiple spawnings over a 4–6-week period. A ratio of two to three males per female is effective, though a 1:1 ratio can suffice as males fertilize multiple females. Hormone induction allows for out-of-season spawning, enabling year-round use of facilities. For example, a hatchery targeting six million juveniles per year could conduct spawning every two months, producing one million juveniles per cycle, rather than building facilities large enough to manage a single large production event.

FIGURE 2.16
Interior view of a 10 m³ tank, used for holding broodstock or rearing juveniles

2.9 PONDS AND OPEN SYSTEMS FOR BROODSTOCK MATURATION

Breeders in open natural systems or semi-extensive aquaculture ponds may undergo gametogenesis, reach sexual maturity, and sometimes spawn naturally. However, these environments are not suitable for controlled spawning or reliable egg collection. Breeders may fail to spawn or attempt to migrate to their natural spawning grounds. For these reasons, once mature, *M. cephalus* breeders must be captured and transferred to controlled spawning tanks for egg collection.

Stress from capture and confinement often triggers late-stage reproductive dysfunction. In females, oocyte maturation may stall or regress into atresia, while spermiation in males can be reduced to negligible levels. To ensure successful spawning, mature breeders must be hormonally induced according to the protocols described in the Section 4.1 on Wild broodstock management.

Hatcheries relying on mature breeders must ensure consistent access to mature fish. This can be achieved by forming agreements to access and capture breeders from nearby extensive ponds/natural systems or by incorporating such systems into the hatchery site. Selected ponds or natural systems should have a proven history of supporting abundant *M. cephalus* populations. These are often former coastal lagoons with long aquaculture histories and water flow control systems, either tidal or pump-driven.

In the Mediterranean, coastal lagoons and estuaries – such as Italy's *lavorieri* – use nets to manage fish migration and capture mature breeders. Ideally, capture sites should be within 30 minutes' transport of the hatchery to minimize stress-related spawning failure. Long transport times increase the risk of poor egg quality or failed spawning. If nearby capture is not possible, a small spawning facility should be established at the capture site, where breeders can be hormonally induced and spawned with minimal stress. Fertilized eggs, which are easier to transport than live fish, can then be moved to the hatchery (see Section 6.4 on Egg transport).

Because wild broodstock may introduce diseases, all spawning tanks receiving wild fish must be treated as quarantine areas, with a minimum recommended quarantine period of 3–4 weeks. Hatcheries should not rely exclusively on wild-caught breeders. Dependence on wild fish is unpredictable and unsustainable due to seasonal availability, legal restrictions, environmental fluctuations, and potential declines in natural stocks. For example, in Italy, mature fish can be obtained from *lavorieri* for the *bottarga* industry (see Appendix I), whereas in some Spanish regions, fish capture during the spawning season is prohibited.

Exclusive reliance on wild fish also prevents hatcheries from implementing selective breeding programmes to improve key traits such as growth, disease resistance and product quality. By contrast, structured breeding programmes can increase growth rates by 10–20 percent per generation, providing a significant business advantage. Where large extensive ponds are available, they can eventually be stocked with hatchery-produced fish, allowing gradual genetic management and improvement of broodstock traits over successive generations.

Calculations for broodstock requirements – whether from wild sources or ponds – follow the same principles outlined previously, considering factors such as the number of juveniles needed, survival at each culture stage, egg production per kilogram of female, male-to-female biomass ratios, and stocking density for spawning.

It is important to note that relying solely on natural spawning limits egg and larval production to the 1–2 months/y when *M. cephalus* naturally spawn. Consequently, all eggs required for the entire year must be produced during this brief period, creating a seasonal bottleneck and leaving much of the hatchery infrastructure underutilized. For example, a hatchery aiming to produce six million juveniles annually would need to produce all eggs during a two-month window. Larval rearing facilities would then be active for approximately four months, remaining idle for the remainder of the year.

2.10 OTHER SPACE REQUIREMENTS

Proper planning of hatchery spaces is essential for efficient operations, biosecurity and overall productivity. In addition to production units, hatcheries require administrative, technical and logistical areas to support daily activities. Below is a comprehensive breakdown of the essential spaces and supporting facilities needed for a fully functional hatchery.

Administrative and staff areas

Office space is needed for administration, record-keeping, and operational planning. Meeting rooms support staff meetings, training, and client consultations, with presentation tools for technical discussions. Staff facilities should include changing rooms for hygiene and biosecurity, a kitchen or dining area, and rest spaces for extended shifts.

Technical and operational facilities

- Computer room: Centralizes system monitoring (temperature, oxygen, pH), manages alarms, and provides data access.
- Diagnostics laboratory: Isolated space for examining diseased fish or testing water quality, equipped with microscopes, testing kits, and sterilization tools.
- Feed and chemical storage: Organized, labelled storage with climate control if needed to preserve feed quality.
- Dead fish management: Safe handling and disposal through silage or temporary freezer storage, complying with environmental and biosecurity regulations.

Support and maintenance rooms

- Workshop: Storage and repair of equipment such as pumps, nets, and aerators; includes workbenches and tools for quick maintenance.
- Packing and dispatch: Space for grading, counting, and packing fry or fingerlings with direct access to transport vehicles; equipped with scales and packaging tools.
- Power backup: Uninterruptible power supply (UPS) or generators to maintain aeration, circulation, and temperature control; room should be ventilated and accessible.

Water storage and distribution

- Head tanks/reservoirs: Provide gravity-fed water supply with initial filtration and sedimentation.
- Freshwater storage: For treatments, salinity adjustments, and system cleaning; must be separate from seawater.

Wastewater treatment

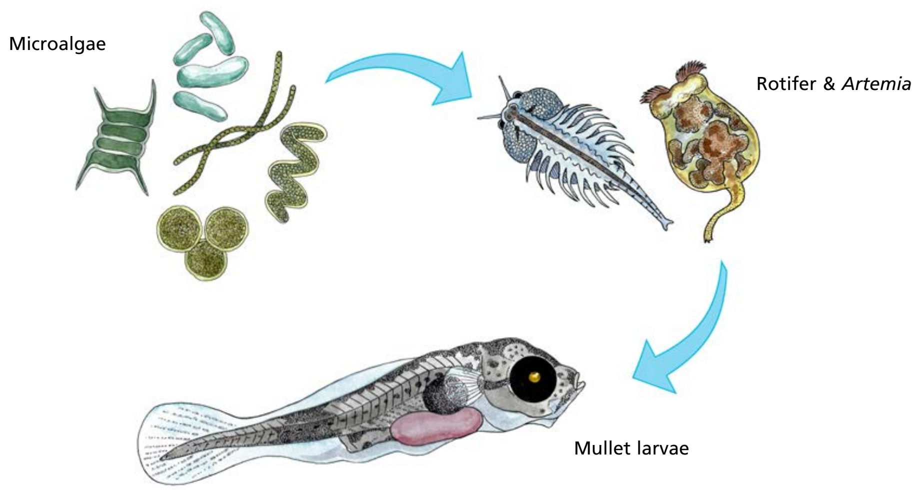
Located downstream to avoid contamination of intake water. Includes effluent settling tanks, biofilters or constructed wetlands for nutrient removal, and discharge canals for safe environmental release. Compliance with local regulations is mandatory.

3. Hatchery operations

3.1 CULTURE OF MICROALGAE

Phytoplankton forms the basis of the marine food chain; consequently, microalgae are indispensable in the commercial culture of marine animals. They serve as the primary food source for all developmental stages of bivalve molluscs, certain crustacean larvae, and numerous marine fish species during their earliest stages of growth. Figure 3.1 provides a schematic representation of the role of microalgae in the larval rearing of *Mugil cephalus*.

FIGURE 3.1
Microalgae are used both as a direct feed and to enrich zooplankton, which is then provided as live prey for the larvae of *Mugil cephalus*



The selection of suitable microalgal species is based on criteria such as cell size, digestibility, mass culture potential, and the nutritional requirements of the target animals. Microalgae are highly productive photosynthetic organisms, capable of generating large amounts of biomass and accumulating substantial lipid content, ranging from 16–75 percent of dry weight depending on the species. Certain species can exceed 60 percent lipid content under stress conditions such as nitrogen or phosphate deprivation, osmotic stress, or variations in light, pH or temperature. In addition to providing energy and essential nutrients for cultured animals, microalgae generate oxygen through photosynthesis, which supports fish respiration and helps reduce ammonia and carbon dioxide accumulation in the culture environment.

Microalgae serve as a direct food source for bivalve molluscs and certain crustacean larvae at all developmental stages, supplying the energy and nutrients necessary for growth and survival. For fish larvae, microalgae are typically provided indirectly through live feeds such as rotifers (*Brachionus* spp.). These tiny metazoans, ranging from 0.1–0.5 mm in length, are essential live food for fish larvae, as well as for filter-feeding invertebrates. Rotifers are commonly enriched with microalgae, which allows the incorporation of essential nutrients such as vitamins and minerals through bioencapsulation. When consumed by the fish larvae, these nutrients are directly transferred, enhancing growth, development and survival.

Flagellate species of microalgae, such as *Tetraselmis suecica* and *Isochrysis galbana*, are particularly useful for this purpose, while non-flagellate species like *Nannochloropsis oculata* are often used to feed rotifers and enrich the water environment for fish larvae, including *M. cephalus*. Beyond nutrition, microalgae contribute to improving the immune system of larvae and increasing their resilience to stress and disease.

Through careful selection of species and the application of controlled stressors, microalgae can be induced to produce high lipid content, enhancing their value as a feed source. These strategies maximize the nutritional quality of both the microalgae and the organisms that consume them, supporting sustainable and efficient aquaculture production.

3.1.1 Flagellate and non-flagellate microalgae

Tetraselmis suecica (Chlorophyta, Chlorodendrophyceae) has been used for decades as a feed in aquaculture due to its numerous advantages. This species is easy to produce using low-cost methods, and its relatively large cells, measuring 12–15 µm, make them less accessible to undesired grazers. Cultures of *T. suecica* typically produce substantial biomass and accumulate significant amounts of lipids. The total lipid content can vary widely from 8–50 percent of dry weight, depending on the growth stage and environmental conditions. The lipid profile is dominated by C16 and C18 fatty acids and includes eicosapentaenoic acid (EPA), a fatty acid of considerable nutritional importance for cultured animals.

Isochrysis galbana is widely recognized for its high nutritional value and is frequently used in aquaculture to feed early larval stages of fish. These small, motile, naked flagellates, measuring 5–7 µm in diameter, are easily ingested and efficiently digested by larvae. *Isochrysis galbana* is particularly notable for synthesizing and accumulating substantial amounts of polyunsaturated fatty acids, especially docosahexaenoic acid (DHA, 22:6 [n-3]), an essential n-3 fatty acid for larval development. In addition, its high ascorbic acid content is considered an important nutritional attribute, further enhancing its value as a live feed in aquaculture.

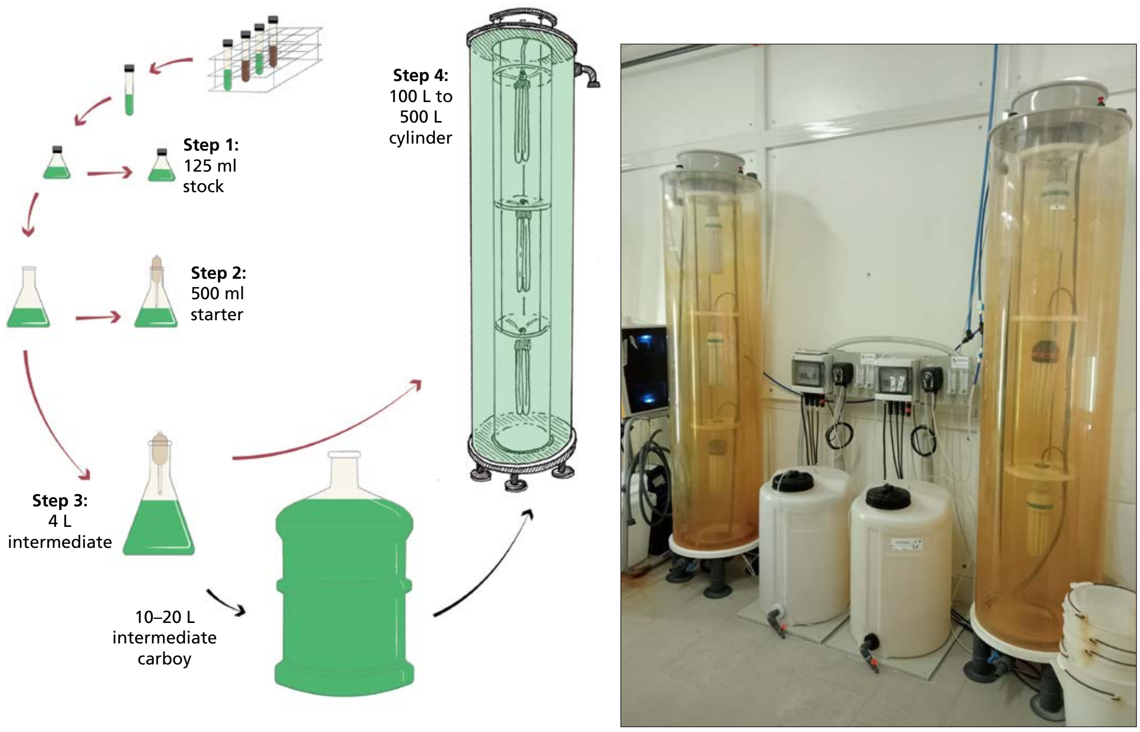
Nannochloropsis oculata is a genus of unicellular, planktonic algae, subspherical in shape with a diameter of 2–4 µm, predominantly found in marine environments. This species has been used extensively as live feed in mariculture due to its high concentrations of EPA acid (20:5 [n-3]), a polyunsaturated fatty acid essential for the healthy growth and survival of many marine animals during larval and juvenile stages.

Throughout the production cycle, a variety of microalgal species are cultivated to satisfy specific requirements regarding cell size, digestibility, culture characteristics and nutritional composition. The combination of *T. suecica*, *I. galbana*, and *N. oculata* provides all the essential nutrients needed for the optimal development of *M. cephalus* larvae. Consequently, algal culture facilities must maintain large-scale production of these three microalgae species to ensure a consistent and balanced food supply.

3.1.2 Microalgal culture overview

The progressive batch culture method involves cultivating algae to harvestable quantities by sequentially transferring them through a series of increasingly larger culture containers. Initially, small volumes of concentrated algae inoculum are introduced into larger volumes of nutrient-enriched, treated seawater (Figure 3.2). The smaller cultures, such as stock starters and intermediate stages, are typically maintained as batch or static cultures, while the larger volumes may be managed as semi-continuous or continuous cultures.

FIGURE 3.2
Progressive microalgae batch culture process



3.1.3 Stock cultures

Stock cultures represent the foundational source of microalgal material in a culture system and are critical for maintaining consistent, high-quality inoculum for all downstream production stages. These cultures are maintained in Erlenmeyer flasks containing sterilized seawater enriched with Guillard F/2 medium (see Appendix II for preparation), under constant illumination at 23 °C. Stock cultures are generally maintained under controlled, stable conditions to minimize stress, prevent contamination, and preserve the physiological and nutritional quality of the cells.

The primary function of stock cultures is to serve as a reliable and continuous source of inoculum for starter cultures. They ensure that microalgal material is

FIGURE 3.3
Microalgae starter cultures

available at all times, even if intermediate or large-scale cultures fail. To maintain their viability and productivity, stock cultures are routinely monitored for cell density, growth rate, and contamination. Monthly subsampling and re-inoculation are performed to refresh the cultures and maintain their health and vigour.

3.1.4 Starter cultures

Starter cultures are established using stock cultures as inoculum to initiate intermediate cultures in flasks of 500–2 000 ml. Starter cultures are usually aerated to keep cells suspended and provide sufficient carbon dioxide (1 % CO_2) to maintain pH balance, ensuring carbon availability for photosynthesis as cell density increases. Starter cultures are maintained in 2 L Pyrex Erlenmeyer flasks, closed with cotton plugs and covered with aluminium foil. Autoclaved natural seawater enriched with Guillard F/2 medium serves as the growth medium. Cultures are exposed to constant illumination (155 µmol/s/m²) from fluorescent lamps (OSRAM type Natura), and continuous aeration is supplied at 3 L/min via a peristaltic pump (ECOH Air Pump). Temperature is maintained at 23 °C through air conditioning (Figure 3.3).

3.1.5 Intermediate culture

Intermediate cultures, grown in 5 L flasks or 6 L lab-scale photobioreactors, are inoculated with starter cultures (Figure 3.4). These cultures are aerated and typically reach densities averaging 4×10^4 cells/ml, with maximum densities up to 5×10^5 cells/ml. Growth conditions are maintained at pH 7.60 ± 0.2 and 22 °C, with volumetric flow rates of 0.2 L/min CO_2 and 2 L/min air.

FIGURE 3.4
Microalgae intermediate cultures

3.1.6 Large-scale culture

Large-scale cultures are initiated from intermediate cultures and can be managed as batch or semi-continuous systems. In batch cultures, microalgal biomass is harvested 4–5 days after inoculation. In semi-continuous systems, cultures are maintained for 15–20 days; upon harvesting down to one-quarter of the vessel volume, fresh sterile seawater and nutrients are added to restore full culture volume. Continuous cultures can be established in fiberglass cylinders (Figure 3.5), receiving a constant supply of seawater and nutrients and allowing daily harvesting once suitable densities are reached.

Algal culture monitoring is essential and this should be integrated into an automated daily check. A sample must be taken daily in order to guarantee the availability of healthy and clean algal cultures free of ciliates or other undesirable microorganisms for high quality microalgae biomass production. All steps in the process of preparing and inoculating algal flasks or photobioreactors follow the same methodology and microbiological techniques must be used meticulously at all times.

FIGURE 3.5

Continuous culture of microalgae in cylindrical fiberglass photobioreactors used for live-feed production

(a) Nutrient and seawater reservoirs (1) are pumped by a peristaltic pump with adjustable flow rate (2) and continuously fed into the photobioreactors (3), enabling daily harvesting once optimal cell densities are achieved. Excess culture overflows through the top outlet (4) to a phytoplankton collection tank or directly to zooplankton cultures (e.g., rotifers); (b) Peristaltic pump with adjustable flow rate (red arrow) delivers seawater and nutrients from the reservoir through the IN line and into the photobioreactor via the OUT line

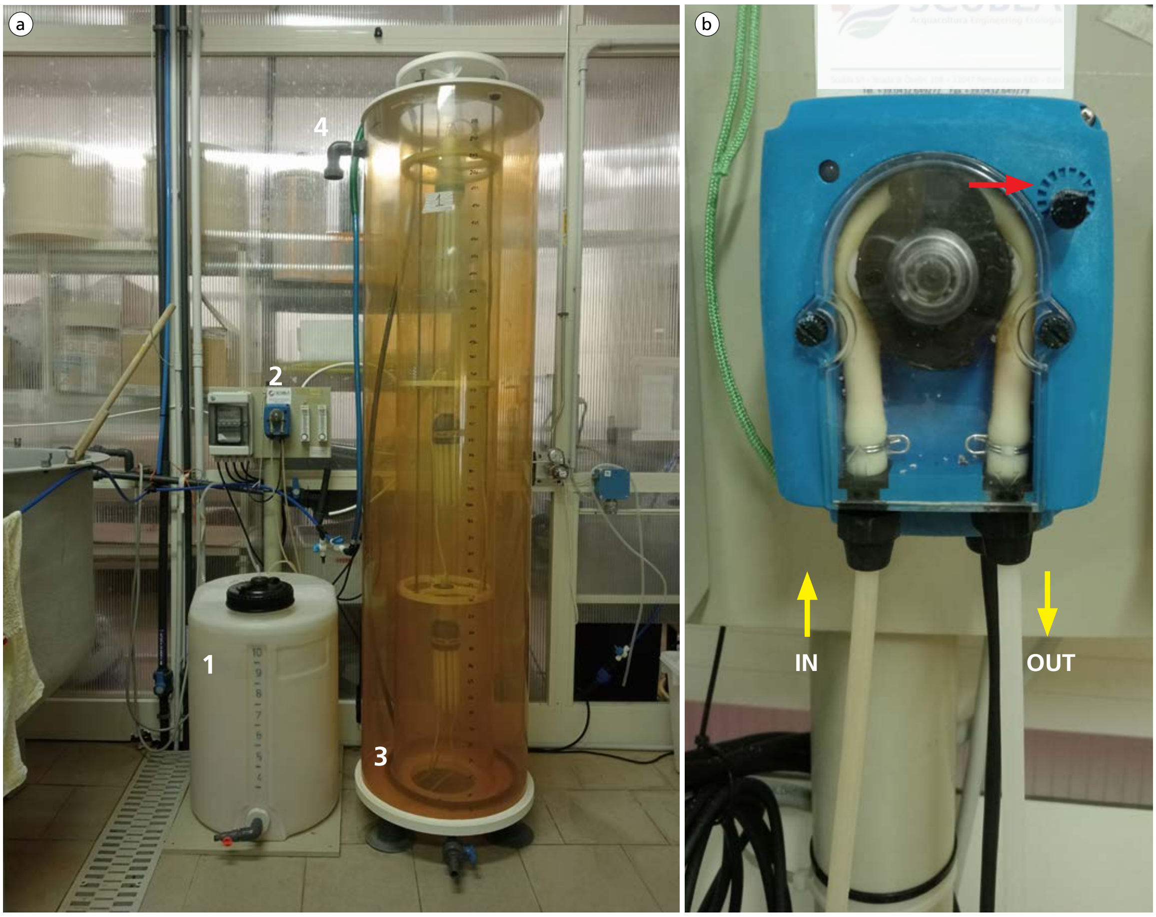


FIGURE 3.6
Example of Neubauer counting chamber

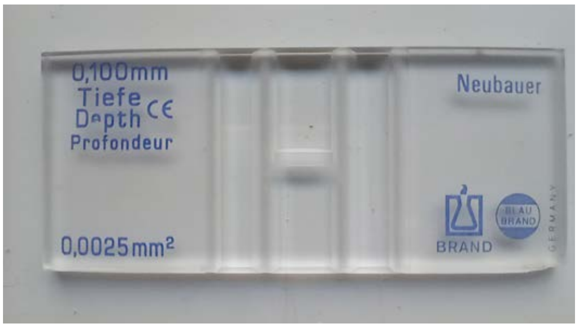


FIGURE 3.7
Example of viable cell count (a) and the absorbance growth curve of *Nannochloropsis oculata* (b)

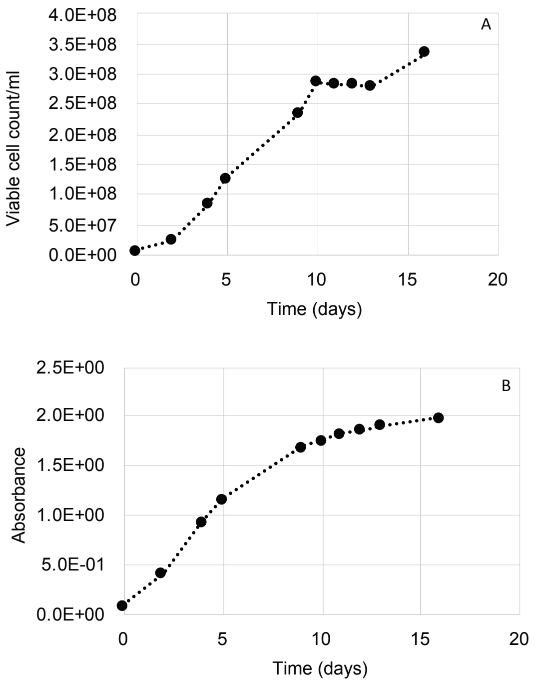
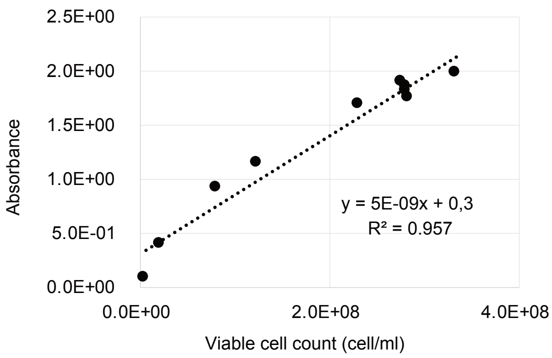


FIGURE 3.8
Example of the correlation between absorbance and viable cell counting of *Nannochloropsis oculata*



3.1.7 Determination of microalgal culture density

The growth of microalgae can be assessed by monitoring changes in cell number or cell biomass over time. Several methods are available to quantify microalgal density. Subsamples of algal cultures prepared for harvesting are examined microscopically to verify the absence of ciliates and other unwanted microorganisms, ensuring that the cultures are clean and suitable for use in aquaculture applications.

For green water systems and rotifer feeding, it is critical to maintain daily monitoring of microalgal cultures. Cell densities are typically determined using a haemocytometer, which allows direct counting of individual cells under a microscope (Figure 3.6).

Figure 3.7a shows an example of a microalgal growth curve obtained through daily cell counts performed using the hemocytometer. Alternatively, optical density measurements using a spectrophotometer provide a rapid method for estimating culture density. Optical density data is obtained with a dual beam spectrophotometer (UV-VIS Jasco V-530) set to read at 750 nm wavelengths. Absorbance is directly related to cell counts and can be used to monitor the growth of the microalgae (Figure 3.7b). To quantify the number of cells in relation to absorbance, it is necessary to prepare a linear correlation curve for each microalga under study (Figure 3.8). The correlation curve plots spectrophotometer absorbance of a sample against the number of cells obtained by direct viable cell counting under the microscope and is used to determine the number of cells in a culture from an optical density absorbance measurement.

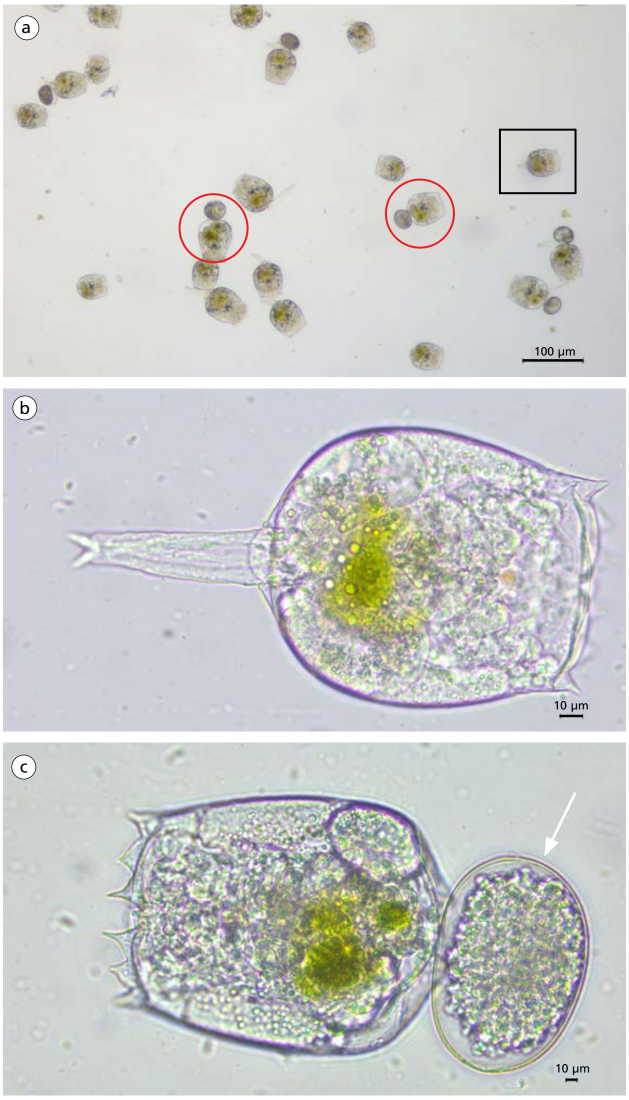
Regular monitoring of the microalgal production cycle is critical for ensuring the availability of healthy, contaminant-free cultures. This practice enables the accurate calculation of food rations for live feed organisms, such as rotifers, which are subsequently used to feed larval stages. Additionally, precise control of microalgal density is required to maintain the appropriate green water conditions for *M. cephalus* larvae. By systematically tracking cell density, aquaculture practitioners can optimize harvest timing, enhance culture productivity, and ensure high-quality biomass for both live feed and green water applications.

3.2 CULTURE OF ROTIFERS

Live feeds play a vital role in the rearing of larval stages in the culture of many marine finfish species. The success of rotifers as culture organisms is due to their planktonic nature, tolerance to wide range of environmental conditions, high reproduction rate, suitability for mass culture at very high densities, and that the nutritional composition can be quickly enhanced with targeted enrichment diets. Additionally, their small size and low swimming velocity make them a suitable prey for fish larvae that have just resorbed their yolk sac and cannot yet ingest the larger *Artemia* nauplii.

In aquaculture, only a few species within the genus *Brachionus* are commonly used as live feed (Figure 3.9). Rotifers used in aquaculture are classified into two distinct morphotypes (or strains), which can be readily distinguished by their morphological characteristics: *Brachionus plicatilis* is commonly referred to as large (L-type) rotifers, while *Brachionus rotundiformis* represents the small (S-type) rotifer.

FIGURE 3.9
Light microscopic view of rotifers
(a) General view of rotifers, rotifer with egg circled and rotifer with no egg boxed; (b) close up of rotifer with green indigested feed; (c) close up of rotifer with egg, arrow indicates egg attached to the rotifer



The lorica length, which is the total length of the rotifer's external protective shell, varies between these morphotypes. For the L-type, lorica length typically ranges from 130–340 µm, with an average of approximately 239 µm. In contrast, the S-type length falls between 100–210 µm, averaging 160 µm. Additionally, a smaller strain of *B. rotundiformis*, referred to as the super small (SS-type) rotifer, has been developed and is particularly beneficial for tropical marine fish larvae with mouth openings smaller than 100 µm during their initial feeding stages. The smaller size of the SS-type facilitates ingestion by larvae, improving feeding efficiency, growth, and survival during the critical early developmental period.

The L- and S-morphotypes of rotifers exhibit distinct differences in their optimal growth temperatures. The L-type achieves maximal growth at 22–28 °C, whereas the S-type grows optimally between 28–35 °C. When rotifers are exposed to temperatures approaching their upper or lower tolerance limits, their multiplication rates decrease, which can facilitate the selective dominance of a desired morphotype. Within the optimal temperature range, increasing temperature generally enhances reproductive activity. However, higher temperatures may also increase food consumption, necessitating more frequent and smaller feedings, which can raise operational costs.

Good growth and reproduction of rotifers require sufficient dissolved oxygen, ideally greater than 5 mg/L. Oxygen availability in the culture water is influenced by temperature, salinity, rotifer density, and the type and quantity of food provided.

Rotifers naturally tolerate pH levels above 6.6, but in controlled culture conditions, optimal growth and reproduction are generally achieved at pH values above 7.5.

High concentrations of un-ionized ammonia (NH_3) are toxic to rotifers. Culture systems should maintain NH_3 levels below 1 mg/L to ensure safe rearing conditions. The NH_3/NH_4^+ ratio is affected by both pH and temperature, highlighting the need for careful monitoring and control of water chemistry.

The following sections provide an overview of the procedures involved in rotifer production, including stock maintenance, feeding, and monitoring to ensure optimal growth and quality for use as live feed in aquaculture systems.

3.2.1 Population dynamics

Rotifers reproduce both sexually (mictic reproduction) and asexually (amictic reproduction), depending on environmental conditions. In hatcheries, asexual reproduction is mainly used to produce high quantities of organisms needed for feeding of fish larvae. The dynamics of rotifer populations under mass rearing conditions are like those observed in microalgae cultures:

- The lag-phase starts immediately after inoculation, during which rotifers begin consuming phytoplankton. In this phase, there is an increase in the number of individuals with eggs and in the quantity of amictic eggs.
- The log-phase, or exponential phase, is characterized by rapid reproduction and exponential population growth.
- The transitional phase, or declining growth phase, in which there is a less prevalence in the number of individuals with eggs.
- The decline phase is characterised by a rapid decrease in the population as death rates surpass growth rates, leaving predominantly old rotifers without eggs.

For effective inoculation, the rotifer population should ideally be in its log-phase, with a fertility rate of at least 20 percent (measured as the percentage of eggs over total rotifers). Populations in the final declining phase should be discarded. A rotifer population can reach its harvesting density within 4–5 days.

3.2.2 Culture methods

Three primary methods are commonly employed for culturing rotifers:

1. **Batch culture:** In this method, a defined volume of culture water is added or exchanged daily, and the culture is restarted at regular intervals. This approach allows for controlled growth over a short period but requires frequent culture renewal to maintain high-quality rotifer populations.
2. **Semi-continuous culture:** This method maintains rotifer cultures over an extended period by periodically harvesting a portion of the population while replenishing the culture with fresh water and food. Semi-continuous culture allows for more stable rotifer densities and reduces the frequency of culture restarts compared to batch systems.
3. **Continuous culture:** Continuous culture typically employs recirculation or flow-through systems to maintain high rotifer densities over prolonged periods. This method minimizes the need for culture restarts and allows for a steady and predictable supply of rotifers, making it suitable for large-scale aquaculture operations.

General culture requirements:

- pH: 7.5–8.5
- Dissolved oxygen (DO): >5 mg/L
- Salinity: 20–30 ppt. The L-type strain (*B. plicatilis*) thrives best at salinity of 30 ppt, while the S-type strain (*B. rotundiformis*) exhibits optimal growth at 18–20 ppt

- Un-ionized ammonia (NH_3): <1 mg/L
- Total Ammonia Nitrogen (TAN): ≤5 mg/L

Stock cultures of rotifers are commercially available from a variety of aquaculture supply companies, either as live animals or as desiccated cysts. When acquiring rotifers to initiate larviculture, it is essential to select the appropriate size strain for the target cultured species. Fish Larvae with small mouth openings may be unable to ingest rotifers that are too large, which can negatively affect survival and growth.

For the establishment of a new culture, it is recommended to use initial stocking densities of ≥200 rotifers/ml of culture water. Lower densities may prolong culture start-up, reduce growth efficiency, and increase the risk of contamination by unwanted microorganisms.

To minimize stress during the transfer of rotifers from shipping bags to culture vessels, water quality parameters should be carefully equilibrated. Temperature, pH, and salinity should be measured and adjusted as necessary to match the culture system. Gradually mixing approximately 10 percent of the shipping water with the culture medium can further reduce transfer shock and improve survival rates.

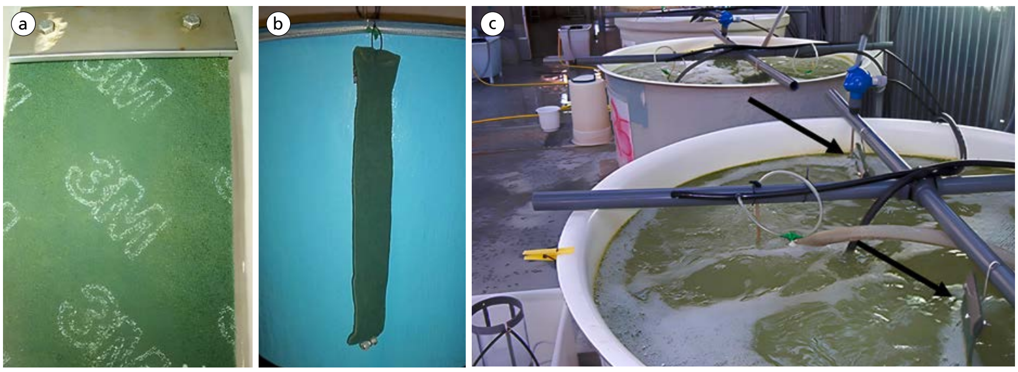
Under optimal conditions, rotifer populations may achieve a daily growth rate of up to 50 percent, although longer-term averages typically range between 20–30 percent/day. Careful management of stocking densities, water quality, and feeding regimes is critical to maintaining robust rotifer cultures and ensuring a consistent supply of live feed for larval fish or other aquaculture organisms.

Batch culture

Batch culture is a prevalent method for rotifer production in marine fish hatcheries due to its simplicity. This approach involves maintaining a constant culture volume with increasing rotifer density or vice versa. In a traditional batch culture system, a 4–5-day culture rotation is typically followed. This method involves inoculating a tank with rotifers on day 1, followed by daily feeding and adjusting the culture volume to accommodate rotifer growth. Rotifer densities achieved using this approach usually range around 500 rotifers/ml. At the end of the cycle, most of the rotifers are harvested and used as feed for larval fish, while a portion is retained for the next tank inoculation.

Tanks for batch culture vary in size, ranging from 500–1 000 L plastic tanks to up to 10 t concrete tanks. To extend the cycle duration, regular water exchanges can be performed once a terminal density is reached. Removing 10–30 percent of the water volume daily helps maintain water quality within desired parameters, although eventually, the culture will need to be restarted due to the accumulation of uneaten feed. To control protozoans and trap suspended matter, "floc traps/mats" (Figure 3.10)

FIGURE 3.10
Floc traps/mats used in rotifer culture
Close up of mat (a); mat suspended outside of tank (b); and mat suspended in rotifer culture, arrows indicate mats (c)



made from commercially available scrubbing pads can be submerged in the tank. These traps require daily cleaning and disinfection.

- Low density in large tanks (3–10 m^3): Inoculate at 50 ind./ml increasing to 250 ind./ml
- Medium density in tanks (2–5 m^3): Inoculate at 200 ind./ml increasing to 800 ind./ml
- High density in tanks (2–3 m^3): Inoculate at 500 ind./ml increasing to 2 000 ind./ml
- Ultra-high density in tanks (1–3 m^3): Inoculate at 1 000 ind./ml increasing to 3 500–10 000 ind./ml

Semi-continuous cultures

Semi-continuous cultures, also referred to as thinning cultures, maintain a constant rotifer density through periodic harvesting. Unlike batch cultures, this approach involves long-term culture at low densities for 7–14 days without water renewal. Typically, larger culture tanks are used compared to batch cultures (Figure 3.11). The initial density ranges from 50–200 ind./ml. With microalgae or baker's yeast as food, rotifer density can increase from 300 to over 1 000 ind./ml within 3–7 days.

FIGURE 3.11
Rotifer culture tanks of 1 000 L

Continuous culture

For a continuous culture, recirculating aquaculture technology is used. A typical rotifer recirculating system has a standpipe screened with 55 µm mesh situated within the culture tank. This standpipe permits uneaten feed and ciliated protozoans to exit the culture tank while retaining the rotifers inside. The waste stream then passes through a biological filter and a foam fractionator before being reintroduced into the culture vessel. Flow rates in such systems typically range from two to five tank turnovers per day through the recirculating system. Routine maintenance involves cleaning the screened standpipe and floc traps/mats, as well as purging settled materials from the tank bottom. Additionally, ozone injection and UV sterilization can be utilized to further regulate water quality parameters.

It is advisable to incorporate a daily water exchange of 20–30 percent of the culture volume, either harvested for feed or simply purged to a waste line. Rotifer densities in a continuous system can reach 1 000/ml.

3.2.3 Mass culture facilities and systems

Rotifer mass culture is typically conducted in large tanks with volumes ranging from 1–10 m³, designed to support high densities that often exceed 1 000 ind./ml. These tanks are constructed from food-grade fiberglass or plastic and feature rounded corners and a conical bottom to facilitate cleaning, reduce biofouling and minimize particle sedimentation.

For high-density, continuous production, a recirculation system is essential. This system incorporates pumps for water circulation, biofilters and protein skimmers – often paired with an ozone generator – and UV sterilizers to maintain optimal water quality. Alternatively, continuous flow-through systems with adjustable flow rates can be employed. Filtration is critical in both setups, with biofilters or foam fractionators equipped with 1 μm filter elements to efficiently remove waste and suspended particles.

To maintain optimal culture conditions:

- **Temperature:** Seawater heaters and temperature controllers are used to maintain a stable temperature, typically within ±1 °C of the target range, ensuring consistent rotifer metabolism and growth.
- **Oxygenation:** Dissolved oxygen is maintained using an air distribution system, with air stones positioned approximately 15 cm above the tank bottom along the periphery and centre. Ceramic fine-bubble aeration stones can also be used for precise and uniform oxygen delivery.
- **pH and salinity:** pH should be stabilized between 7.5 and 8.5, while salinity maintained within 20–30 ppt to provide optimal physiological conditions.
- **Ammonia and water movement:** Un-ionized ammonia (NH_3) levels must remain below 1 mg/L, and moderate turbulence applied to enhance rotifer health, prevent sedimentation and improve food distribution.

A lighting system is included, but illumination is generally only necessary during microalgae feeding to enhance rotifer nutrition. This integrated design supports efficient, high-yield rotifer production while maintaining water quality, culture stability, and optimal growth conditions for continuous or semi-continuous aquaculture operations.

3.2.4 Enrichment

Rotifers have limited nutritional value for marine finfish larvae; enriching rotifers with specially formulated feed products before feeding them to fish larvae is needed to enhance the rotifers nutritional content. Enrichment involves providing diets with polyunsaturated fatty acids (PUFAs), vitamins, and minerals crucial for the proper development and survival of marine fish.

Traditionally, mass culturing of rotifers in Mediterranean hatcheries involved feeding them a combination of algae and baker's yeast, such as *Saccharomyces cerevisiae*, a common, easily available and cost-effective food. Compared to artificial diets, this approach yields lower densities at harvest (rarely exceeds 450 rotifers/ml, with an average daily increase ranging from 19–33 percent) and requires additional time, typically an extra day. To compensate for the poor nutritional quality of yeast-fed rotifers, they are enriched with high levels of (n-3) HUFA and vitamins before distribution to fish larvae.

Enrichment procedures usually involve feeding rotifers with microalgae rich in PUFA and vitamins, such as *Chlorella* sp., *Nannochloropsis* sp. and *Isochrysis* sp. Alternatively, commercial products like oil emulsions or powder formulations formulated from algae rich in fatty acids (e.g. Easy Selco, INVE, Multigrain, Algamac 3050) are used for enrichment. In recent years, there has been a notable increase in the use of concentrated algal-based enrichment products due to their consistency and ease of application.

Concentrated enrichment products, such as Easy Selco, are commonly used to enrich rotifers prior to feeding to larval fish. The required volume of enrichment solution can be calculated as follows:

Calculate the volume of rotifer culture required for enrichment:

$$V_E = \frac{\text{Desired number of rotifers}}{\text{Rotifer density (ind./ml)}}$$

Where:
- V_E = Volume of rotifer culture required for enrichment (ml)
- Desired number of rotifers = total rotifers to be enriched
- Rotifer density = current rotifer density in the culture (ind./ml)

Calculate the amount of enrichment product to be added, such as Easy Selco:

$$\text{Mass of Easy Selco (g)} = V_E \times \text{concentration of Easy Selco (g/L)}$$

Where:
- V_E = volume of enrichment solution (L)
- Concentration of Easy Selco = recommended grams per litre of enrichment solution

This procedure ensures that rotifers receive the correct dosage of enrichment, providing essential nutrients such as fatty acids, vitamins, and minerals for optimal larval growth and survival.

Another modern alternative to traditional methods is the use of a totally artificial diet such as the Culture Selco® (CS) introduced by INVE SA, which is a dry and complete rotifer diet. This compound feed eliminates the need for algae and serves as an effective enrichment medium. Its composition includes proteins (>35%), lipids (>15%, including 23% PUFA), carbohydrates (30%), carotenoids, and other micronutrients such as minerals and vitamins A, D3, F and C. Rotifers fed on CS consistently exhibit average daily production ranging from 45–60 percent of the initial rotifer density. Additionally, they are enriched with high levels of essential n-3 PUFAs and vitamins.

Enrichment can be conducted either within the mass culture tanks or post-harvest in dedicated enrichment tanks. When enrichment is performed within the mass culture tanks, the enrichment media can be added directly to the rotifers at specific times, typically 8–24 h before harvest, or continuously throughout the culture period. The last 12–24 h of feeding are the most critical in determining the nutritional value of the rotifers. Continuous enrichment helps maintain stable fatty acid reserves, which are less prone to rapid degradation during periods of starvation.

When rotifers are harvested, rinsed, and transferred to a separate enrichment tank, only short-term gut enrichment is achieved. In this approach, the enrichment media is added to the concentrated rotifers for a brief period prior to feeding them to the fish larvae. In either method, the dosage of enrichment media should follow the manufacturer's recommendations to ensure optimal nutritional quality.

3.2.5 Monitoring rotifer populations

Check all rotifer cultures daily for both qualitative and quantitative evaluations. Rotifers exhibit phototaxis and can move vertically through the water column; therefore, thorough mixing of the culture is essential before taking samples. Healthy rotifers should exhibit high activity levels. Reduced activity may indicate suboptimal water quality, requiring corrective measures. While the presence of some ciliated protozoans in rotifer cultures is normal, they can compete with rotifers for oxygen and food resources.

To count rotifers, follow these steps:

1. After observing the live sample, extract a 1 ml subsample using a pipette and load it onto a Sedgwick-Rafter counting chamber or a reticulated Petri dish, both of which are gridded.
2. Add one to two drops of Lugol's solution to immobilize the rotifers.
   Place the Sedgwick-Rafter slide under a microscope at 40× magnification.
3. Count the rotifers in the entire grid or in the central two grids, then multiply to obtain the total number of rotifers per millilitre. The count obtained corresponds to 1 ml, which can then be scaled to estimate the total population in the culture tank.
4. Record both the total number of rotifers and the number of individuals carrying eggs.
5. Based on the rotifer concentration, calculate the daily harvest to maintain a fixed density, following the recommended specifications for each strain.

A population in which ≥30 percent of individuals carry eggs indicates a healthy culture. A decline to 15–20 percent is still acceptable but may hinder culture growth. A decrease <15 percent indicates suboptimal conditions in the culture tank, likely due to inadequate feeding or water quality, and requires immediate corrective action.

Managing rotifer concentrations:
Maintaining optimal rotifer population densities is critical for culture stability and production efficiency. Excessive densities can lead to culture collapse, whereas overly dilute conditions reduce productivity. For continuous culture systems, rotifers should generally be maintained at 150–300 ind./ml. Short-term enrichment or holding phases may temporarily support higher densities, ranging from 600–800 ind./ml.

- *Reducing rotifer density by adding water* – If rotifer concentration is high and water needs to be added, the required volume can be calculated as follows:

Initial rotifer concentration × Tank volume = Desired rotifer concentration × Y

Example:
- Current rotifer density = 180 ind./ml
- Tank volume = 50 L
- Desired rotifer density = 150 ind./ml

$$180 \times 50 = 150 \times Y$$

$$V_E = \frac{180 \times 50}{150} = 60 \text{ L}$$

Water to be added = 60 - 50 = 10 L

If the tank is already full, these 10 L can be removed as part of the daily harvest.

- *Increasing rotifer concentration by removing water* – If the rotifer concentration is lower than the desired level, the density can be increased by removing a calculated volume of water from the culture tank.

Initial rotifer density × Initial volume = Target rotifer density × Y

Example:
- Current rotifer density = 40 ind./ml
- Tank volume = 50 L
- Target rotifer density = 150 ind./ml

The required final volume is calculated as follows:

$$40 \times 50 = 150 \times Y$$

$$V_E = \frac{40 \times 50}{150} = 13.3 \text{ L}$$

This indicates that the culture should be reduced to a final volume of 13.3 L.

Water to be removed = 50 - 13.3 = 36.7 L

Therefore, 36.7 L of water must be removed to increase the rotifer density to the target level.

Managing high rotifer concentrations:

In rotifer aquaculture, maintaining optimal population densities is critical to both culture stability and production efficiency. Excessive densities can lead to culture collapse, while overly dilute conditions reduce productivity.

For hatchery best practices, rotifers should be maintained at densities of 150–300 ind./ml for continuous culture systems. Short-term enrichment or holding phases may temporarily support higher densities, ranging from 600–800 ind./ml.

To prevent excessive dilution of rotifer concentrations and to maintain stable culture conditions, the following management strategies should be applied:

Harvest prior to water addition

- Remove a portion of the rotifer population using a fine mesh net (typically 40–60 µm). This practice simultaneously reduces culture density and provides live feed for larval rearing.
- After harvesting, add treated seawater to restore the culture volume and maintain stability.

Re-concentration of rotifers after dilution

- If water addition results in excessive dilution, concentrate rotifers by filtering the culture water through a rotifer net and resuspending them in fresh, treated seawater.
- Alternatively, gentle airlift may be used, as commonly applied in hatchery operations.

Gradual water addition

- Avoid sudden water additions; instead, introduce seawater gradually by drip addition. Seawater should be filtered, sterilized, and adjusted to match the culture temperature and salinity. Gradual addition minimizes osmotic shock and dilution stress.
- Continue feeding microalgae or suitable algal substitutes (e.g. yeast or commercial concentrates) during this process.

Culture splitting

- When rotifer densities become excessively high (>500–700 ind./ml), divide the culture into two separate tanks.
- Top up each tank with treated seawater. This will maintain densities within the optimal range (150–300 ind./ml for routine rearing) and reduces the risk of culture collapse.

Maintenance of water quality

- High rotifer densities can rapidly deteriorate water quality due to increased ammonia, carbon dioxide and oxygen consumption.
- Ensure adequate aeration and, where available, biofiltration or gentle water exchange when adding water.
- Regularly monitor pH and dissolved oxygen (DO) to maintain optimal culture conditions.

3.2.6 Harvesting

Rotifers are harvested using 40 µm mesh sieves, thoroughly washed with filtered seawater, and rinsed for approximately one minute with freshwater to reduce the bacterial load carried by the rotifers prior to feeding to larvae. All operations are conducted using seawater and freshwater filtered to 10, 5 and 1 µm, passed through a carbon filter, and adjusted to the culture temperature (26 °C)

Harvesting and counting procedure:

1. *Suspend aeration*
   - Remove aeration from all tanks containing rotifer cultures.
   - Wash the aerators using pressurized freshwater.
   - Allow the culture to settle for approximately 3 minutes before purging.

2. *Restore aeration and record volume*
   - Reinstall the aerators in the tank.
   - Record the total culture volume (V, in litres) after purging.

3. *Sample collection*
   - Collect a representative sample using a beaker.
   - Gently homogenize the sample and withdraw 1 ml using a pipette.

4. *Microscopic observation*
   - Load the sample onto a Sedgwick–Rafter counting chamber or equivalent device.
   - Observe rotifer activity and general condition.
   - Add Lugol's solution to immobilize the rotifers for counting.

5. *Rotifer enumeration*
   - Count the total number of rotifers without eggs (R).
   - Separately count rotifers carrying one egg (R1*), two eggs (R2*), and three or more eggs (R3*).
   - If possible, perform two to three replicate counts and calculate the average.

   The rotifer density, total number of rotifers per millilitre (R^{ml}), is calculated as:

$$R^{ml} = R^{*} + R1^{*} + R2^{*} + R3^{*}$$

   The total number of rotifers in the culture tank (R^{T}) is then calculated as:

$$R^{T} = R^{ml} \times 1\,000 \text{ (ml/L)} \times \textit{Volume of the culture tank} \text{ (L)}$$

6. *Calculation of harvest volume*

   To obtain the required number of rotifers for feeding, calculate the volume to be harvested as follows:

$$\textit{Volume of culture to be harvested (L)} = \frac{\textit{Desidered number of rotifiers}}{R^{T}} \times \textit{Total culture volume (L)}$$

7. *Harvesting*
   - Fill a bucket with seawater tempered to 26 °C.
   - Place the sieves over the bucket and extract the calculated culture volume.
   - Rinse the rotifers thoroughly on the sieve using tempered, filtered seawater.

   Example: Calculation of daily harvest for larval feeding.
   To feed a 500 L larval tank at a rate of 10 rotifers/mL, the total number of rotifers required is calculated as:

   $$500\ 000 \text{ ml} \times 10 = 5\ 000\ 000 \text{ rotifers required}$$

   If the rotifer culture has a density of 170 rotifers/ml in 110 L of water, the total number of rotifers in the culture is:

   $$\text{Total rotifers} = 170 \times 110\ 000 = 18.7 \times 10^6$$

   The volume of rotifer culture required to meet the larval feeding demand is calculated as:

   $$\text{Harvest volume (L)} = \frac{5\ 000\ 000}{(170 \times 1\ 000)} = 29.4 \text{ L}$$

   Therefore, approximately 30 L of rotifer culture should be harvested to meet larval feeding requirements.

Post-harvest Handling

8. *Enrichment*
   - Depending on the diet used during culture, harvested rotifers may require enrichment to improve their nutritional value for fish larvae (see Section 3.2.4 on Enrichment).

9. *Storage and use*
   - Enriched rotifers may be fed directly to larval tanks or stored at approximately 4 °C (refrigerated or in insulated containers with ice) for use during the same day.
   - Rotifers rapidly lose enrichment and nutritional value if maintained at room temperature and should therefore be used as soon as possible.

3.2.7 Cleaning of culture tanks and equipment

Regular cleaning and disinfection of culture tanks and associated equipment are essential to maintain optimal water quality, prevent the accumulation of organic matter, and reduce the proliferation of pathogenic or competing microorganisms. Effective hygiene practices contribute to stable rotifer production, improved survival, and consistent nutritional quality. Cleaning procedures should be carried out systematically and with care to avoid stressing the cultured organisms.

Tank and equipment cleaning and disinfection

- Use mild, non-abrasive detergents to remove biofilms, organic residues, and mineral deposits from tanks and equipment.
- Periodically disassemble and inspect aeration systems, diffusers, and filters to prevent clogging and ensure efficient water circulation and gas exchange.
- Disinfect tanks and equipment using non-toxic disinfectants such as chlorine dioxide or hydrogen peroxide. After disinfection, rinse thoroughly with clean, filtered water to remove any residual disinfectant before reuse.

Removal of bottom sediments

- In circular tanks with conical bottoms, suspend aeration and allow sediments to settle for 10–15 minutes, while closely monitoring dissolved oxygen levels during this operation.
- Open the bottom drain valve briefly to discharge settled sediments until the outflowing water appears clear.
- Repeat this procedure twice daily, preferably in the morning and evening.
- Remove greasy or oily surface films that accumulate at the water–air interface using a sponge or disposable paper cloth. Direct hand contact with the culture water should be avoided.

Removal of particulate matter

- Particulate matter, including faeces and aggregates of uneaten feed, can rapidly degrade water quality, stimulate bacterial growth and reduce dissolved oxygen levels. Regular removal of these particles helps limit bacterial proliferation and increases oxygen availability for rotifers.
- Install particle traps or floc traps/mats to capture suspended particles, as continuous aeration partially prevents their natural settlement. These traps typically consist of floating or suspended mats made of coarse sponge-like material (e.g. Scotch-Brite™).
- In a 3 000 L tank, suspend three mats measuring approximately 15 × 100 cm vertically in the water column. This can be achieved by placing a support (e.g. a wooden or plastic rod) across the tank rim, with a small weight attached to the lower end of each mat to maintain vertical orientation.
- Water circulation directs particulate matter toward the traps, where particles adhere and accumulate, eventually causing clogging. These mats also provide a favourable substrate for Vorticella, a sessile ciliate that competes with rotifers for food.
- To maintain effectiveness and limit microbial growth, particle traps must be removed, cleaned, and disinfected at least twice daily before being reinstalled.

3.3 PRODUCTION OF THE BRINE SHRIMP

Artemia, a genus of brine shrimp, exhibits a high degree of adaptability to variable environmental conditions and can tolerate a wide range of temperatures (6–37 °C), salinities (15–35 ppt), and pH levels (8.0–8.5). Optimal reproductive performance is generally achieved at temperatures around 25 °C.

Artemia is of major importance in aquaculture as a live feed organism due to its high content of easily digestible protein and its appropriate size for the larvae of many marine and freshwater fish species. The following procedure outlines the key steps involved in *Artemia* production. For more detailed information, reference should be made to the FAO *Manual on Artemia production and use* (see Other FAO publications).

3.3.1 *Artemia* cyst strains

Preferably select and use *Artemia* cyst strains with high hatchability. Where possible, SEP–Art® cysts are recommended, as they do not require chemical decapsulation prior to use and are specifically designed for magnetic separation of shells (chorions) after hatching. These cysts are coated with a non-toxic magnetic material that does not affect hatching performance and facilitates efficient separation of nauplii from empty shells.

Cyst quality and viability should be verified through routine hatching assays. Hatching efficiency can be determined as follows:

1. Weigh and record the quantity of cysts required to meet the daily *Artemia* demand. For planning purposes, use a conservative hatching value of 150 000 nauplii/g of cysts to calculate the amount of cysts needed.

2. Dehydration (or re-dehydration) of *Artemia* cysts may be practiced when using traditional cysts. This procedure can improve hatching synchrony and prolong storage life after the container has been opened.
3. Hatch the *Artemia* cysts according to the supplier's recommended procedures and conditions.
4. Collect all hatched nauplii and perform a total count.
5. Calculate hatching efficiency using the following formula:

$$\text{Hatching efficiency (nauplii/g)} = \frac{\text{Number of nauplii counted}}{\text{Weight of cysts (g)}}$$

6. Determine the mean hatching efficiency from the first three to four hatching batches and use this average value as the reference for subsequent production planning.

3.3.2 Disinfection and decapsulation of brine shrimp cysts

Disinfect *Artemia* cysts by soaking them in a 0.5–1.0 percent sodium hypochlorite solution for 10–15 minutes, followed by thorough rinsing with sterile or filtered freshwater to remove residual disinfectant.

When decapsulation is required, treat the cysts with a 5–10 percent sodium hydroxide (NaOH) solution for 2–3 minutes, while continuously mixing to ensure uniform exposure. Immediately neutralize the reaction using an acidic buffer, such as citric acid, and rinse thoroughly with filtered freshwater until neutral pH is achieved.

3.3.3 Cysts incubation

The water used for incubation should be filtered through a series of mechanical filters arranged sequentially at 10, 5 and 1 µm. Incubation tanks must be thoroughly cleaned and disinfected using sodium hypochlorite at a concentration of 0.05 percent, then rinsed and allowed to dry completely before being filled with clean seawater. Transparent tanks with a capacity of 50–100 L may be used.

Decapsulated *Artemia* cysts should be incubated in well-aerated seawater at a temperature of 28–30 °C for 24 h. Water quality parameters, particularly dissolved oxygen and pH, should be monitored regularly to ensure optimal conditions for nauplii development.

In brief, the hatching procedure is as follows:

1. Prepare a hatching tank according to the quantity of cysts to be incubated, considering the required number of nauplii and the appropriate water volume. The following calculations may be used:
   - Required nauplii / Nauplii per gram = grams of cysts required
   - Grams of cysts required / (2–3 g/L) = volume of water required (L)
2. Weigh the calculated quantity of cysts and add them to the prepared hatching tank.

3.3.4 Harvesting of nauplii

After 24 h of incubation, remove the heater and install the SEP–Art® separation tool at the outlet of the hatching tank (Figure 3.12). The SEP–Art® separation tool retains unhatched cysts and empty shells, while allowing newly hatched *Artemia* nauplii to pass through.

The SEP–Art® separation tool should be connected via tubing between the hatching tank outlet and a collection bucket containing clean seawater. Drain the hatching tank through the SEP–Art® separation tool and collect the nauplii in the bucket using fine-mesh sieves (125–150 µm).

Rinse the nauplii retained in the sieve with sterile seawater to remove residual decapsulation chemicals and debris. Subsequently, transfer the nauplii to a container with a known volume of seawater, provide gentle aeration or oxygenation, and determine nauplii density using a counting chamber to accurately calculate feeding rates.

3.3.5 Counting and evaluating *Artemia* nauplii

Artemia nauplii should be counted under a dissecting microscope or stereomicroscope. A representative sample from the container of newly hatched nauplii should be diluted using a known dilution factor. To improve accuracy, counts should be repeated at least three times.

The counting procedure is as follows:

1. Take a 1 ml sample of the diluted nauplii suspension and transfer it to a Petri dish. Distribute the sample into several small drops across the surface of the dish.
2. Fix the nauplii and count them under the microscope.
3. Calculate the total number of harvested nauplii, considering the dilution factor used.

3.3.6 Enrichment and storage of nauplii

Prior to feeding, *Artemia* nauplii should be enriched with lipid-based emulsions or concentrated microalgae to improve their nutritional value. Enrichment protocols should be adjusted according to nauplii age and size to ensure homogeneous nutrient uptake.

FIGURE 3.12
SEP–Art® separation tool: hatched and unhatched magnetic cysts adhere to the device

The density (D) of the culture must be determined first to calculate the required quantities; when expressing the volume in Liters (L), a conversion factor of 1 000 must be applied to the counts obtained from 1 ml samples.

The nauplii density (nauplii/L) is calculated as:

$$D \text{ (nauplii/L)} = \frac{\text{Total number of nauplii}}{\text{Volume of sample evaluated (ml)}} \times 1\,000$$

The volume of nauplii suspension required to obtain the desired number of nauplii should be calculated as follows:

$$\text{Volume of nauplii suspension to be separted (L)} = \frac{\text{Number of nauplii required}}{D \text{ (nauplii/L)}}$$

The enrichment volume should then be determined based on the recommended enrichment stocking density (e.g. 300 000–500 000 nauplii/L):

$$\text{Enrichment volume (L)} = \frac{\text{Total number of nauplii}}{\text{Enrichment stocking density (nauplii/L)}}$$

The quantity of enrichment product to be added should be calculated according to the enrichment volume, following manufacturer's instructions. For an enrichment product that requires 0.25 g of enrichment to be added to each litre of *Artemia* nauplii (300 000–500 000 nauplii/L):

$$\text{Enrichment quantity (g)} = \text{Enrichment volume (L)} \times 0.25 \text{ (g/L)}$$

Transfer the *Artemia* into the calculated enrichment volume of seawater and provide aeration, oxygenation and temperature control using a heater. Weigh the daily calculated amount of enrichment product and homogenize it in seawater using a blender for approximately 5 minutes. Add half of the enrichment mixture to the enrichment tank.

Place the remaining half of the enrichment mixture in a cylindrical container fitted with an air tube and store it in a polystyrene box with cooling packs or in a refrigerator. Add this portion to the enrichment tank after 12 h.

After 24 h of enrichment, harvest the *Artemia* using a 150 μm sieve. Rinse thoroughly with seawater until the effluent runs clear, gently agitating the bottom of the mesh by hand. Immerse the sieve containing the *Artemia* in freshwater for 5 minutes to disinfect the nauplii. Finally, transfer the *Artemia* to a container with seawater, adjust to a known volume, and distribute them to the fish larvae.

4. Broodstock management

Advancement of sexual maturation in *Mugil cephalus* broodstock is negatively affected by capture and captive rearing conditions, which frequently result in the arrest of gonadal development prior to spawning. Two principal types of reproductive dysfunction have been described in *M. cephalus*:

(a) *Arrest at late stages of maturation*
Broodstock can reach advanced stages of gonadal development in systems that provide near-natural environmental conditions, such as managed coastal lagoons or semi-extensive systems (e.g. large extensive ponds). However, when these mature breeders are captured and transferred to intensive aquaculture systems, ovarian development in females is frequently arrested before oocyte maturation and ovulation, while spermiation in males is reduced or ceases entirely. If no corrective action is taken, gonadal regression occurs, resulting in ovarian atresia in females and low or absent spermiation in males. This condition is typically observed in wild broodstock captured from extensive open systems and transferred to intensive facilities. To obtain viable eggs, these breeders must be hormonally induced, as described in Section 4.1.

(b) *Arrest at early stages of gametogenesis*
This dysfunction is commonly observed in broodstock reared in captivity for both short and extended periods. Breeders maintained in intensive aquaculture systems (e.g. semi-closed tanks or RAS) often exhibit arrest at early stages of gametogenesis, with females remaining previtellogenic or in early vitellogenesis and males showing no spermiation. To obtain viable eggs, these breeders require long-term hormonal induction protocols, as described in Section 4.2.

Both types of reproductive dysfunction may be encountered in *M. cephalus* broodstock used for hatchery production and can be overcome through appropriate hormonal treatments. Although fish born and reared in captivity or maintained in captivity for extended periods (at least three years), may undergo partial domestication, they may achieve spontaneous maturation in captivity when appropriately conditioned, for example through controlled photoperiod and temperature manipulation.

4.1 WILD BROODSTOCK MANAGEMENT

4.1.1 Establishing broodstock from the wild

Only healthy individuals should be selected for broodstock purposes. Selected fish should display normal body conformation, with no skeletal deformities, skin lesions (e.g. wounds, haemorrhages, necrosis, or extensive scale loss), or signs of parasitic infestation. Normal swimming behaviour, stable buoyancy, and appropriate positioning within the water column are essential indicators of good physiological condition and welfare.

Mugil cephalus is a gonochoristic species with no external sexual dimorphism. It is an isochronal, single-batch spawner characterized by a high gonadosomatic index (GSI) and high fecundity, ranging from approximately 650–850 eggs/g of female body weight.

To establish an optimal broodstock pool, it is generally recommended to select females aged 2–4 years, weighing 1.3–2.5 kg, and males aged 1–2 years, weighing 0.5–1.5 kg. Minimum total lengths should be ≥42 cm for females and ≥38 cm for males.

4.1.2 Origin, capture and handling of wild broodstock

Ripe wild *M. cephalus* do not spawn spontaneously in captivity and require hormonal induction. Failure to spawn is largely attributed to capture and handling stress, which should be minimized to maximize spawning success.

During capture, stress can be reduced through the use of appropriate trapping systems. Taking advantage of the species' reproductive migration toward the sea, fish may be confined using fixed rigid barriers installed near lagoon sea inlets. These traditional fishing enclosures, known as *lavorieri* in Italy have been used for centuries in Mediterranean coastal lagoons (Figures 4.1). Their design allows juvenile recruits to enter the lagoon while preventing adults from returning to the sea during the breeding season. When the *lavorieri* are unavailable or cannot be set up, alternative methods for capturing adult *M. cephalus* can be employed (see Appendix III). These methods are applicable during the natural reproductive season as well as outside of it.

FIGURE 4.1
Traditional fish traps, *lavorieri*, in the Is Benas coastal lagoon in Sardinia, Italy

© A. Alvito

4.1.3 Capture site selection

Optimal sites for the installation of rigid fish barriers in coastal lagoons or river estuaries should meet the following criteria:

- Documented historical abundance of *M. cephalus*;
- Shallow water depth (generally <1.5 m);
- Low turbidity and limited muddy substrate, allowing visual observation of fish in the water column.

> *Water turbidity may vary daily or seasonally; therefore, capture operations should be conducted during periods when fish are clearly visible from above the water surface.*

4.1.4 Gamete maturity assessment in wild breeders

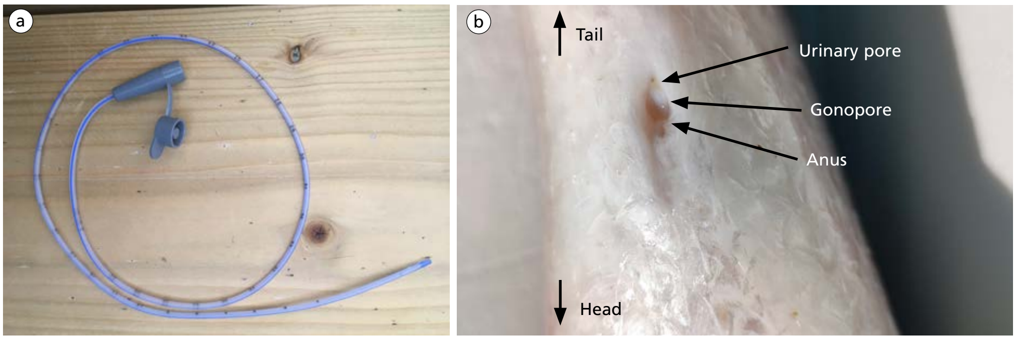
Assessment of gonadal maturation should be carried out on individuals belonging to the same cohort that are not intended for immediate use as broodstock, in order to minimize handling stress on selected breeders.

Fish are captured during their seaward reproductive migration and anesthetized in a dedicated tank using clove oil at a concentration of 0.08 percent.

Males are assessed for spermiation by applying gentle pressure to the abdomen. Spermiation is classified using a four-point scale: 0 = not fluent; 1 = fluent but no sperm sample obtained; 2 = fluent; 3 = very fluent, with little or no pressure required. Only males with spermiation indices of 2 or 3 should be selected for spawning.

Females are assessed by ovarian biopsy using a polyethylene catheter (outer diameter = 1.67 mm; 5 Charrière size; length = 500 mm) inserted into the gonopore to aspirate oocytes (Figures 4.2). The biopsy allows determination of ovarian developmental stage and measurement of oocyte diameter.

FIGURE 4.2
Polyethylene cannula used for ovarian biopsy (a); and ventral view of flathead grey mullet showing the anus, genital pore (gonopore) and urinary pore, indicating the insertion site for gonadal biopsy (b)



4.1.5 Ovarian biopsy procedures

All equipment must be prepared, cleaned and sterilized in advance. Catheters must be sterile; at least two catheters should be used alternately to allow cleaning between fish. Catheters may be sterilized using ethanol and subsequently rinsed thoroughly with distilled water flushed through the lumen using a syringe.

Fish should be handled carefully using latex gloves, avoiding damage to the urogenital papilla. Dissolved oxygen levels must be monitored continuously and maintained above 6.5 mg/L.

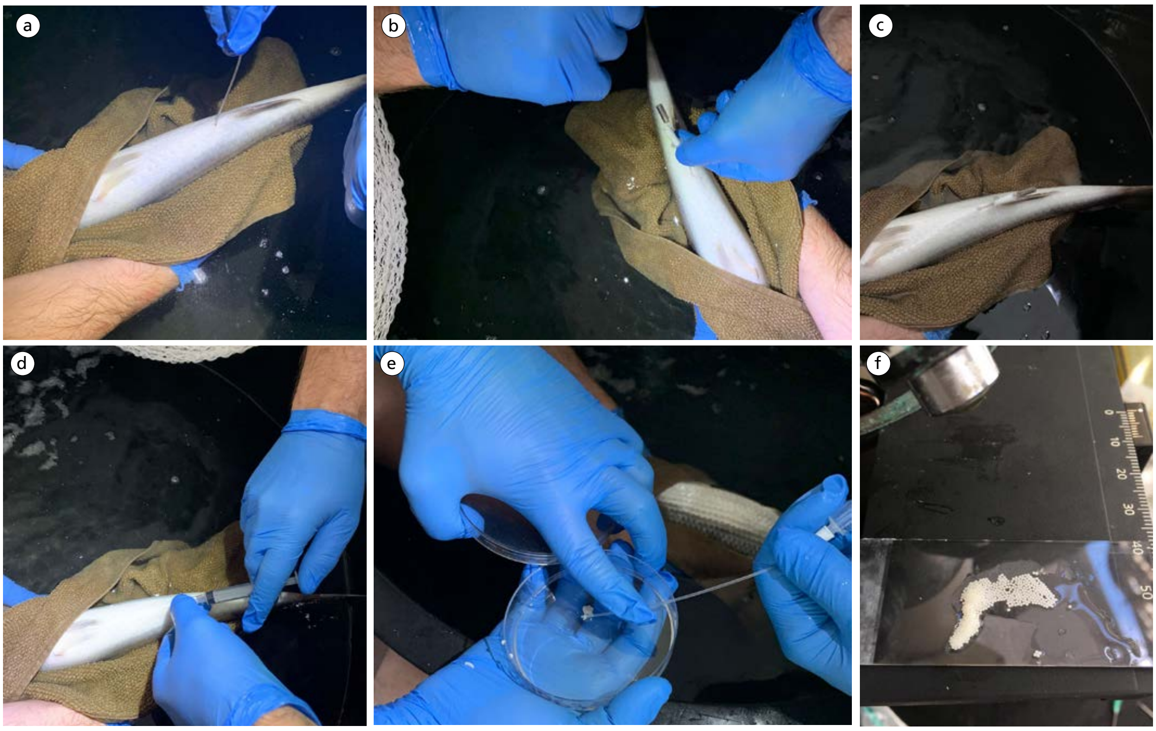
The biopsy procedure is as follows (Figure 4.3):

1. For captive-reared fish, withhold feed for 24 h prior to anaesthesia.
2. Close the water inlet and reduce the water level to 25–30 cm.
3. Add anaesthetic to obtain light sedation (clove oil 0.01 percent) and divide the tank into two compartments using a net barrier, with the fish confined to one side and the other side left empty.

4. Prepare all materials (glass slides, sterile catheters, Pasteur pipette, distilled water and alcohol).
5. Transfer one female at a time to a sedation tank (200 L) containing seawater at the same temperature and salinity as the holding system, with clove oil at 0.08 percent.
6. Once full sedation is achieved, gently insert the catheter through the gonopore into the oviduct and advance it several centimetres toward the ovary.
7. Apply gentle suction to collect a small oocyte sample.
8. Release the fish into the spawning tank for recovery.
9. Place the oocytes on a slide, add saline or clearing solution (6 ml ethanol, 3 ml formalin, 2 ml glycerol), and disperse tissue using teasing needles.
10. Examine the slide under a light microscope at 5× and 10× magnification.
11. Measure the diameter of the largest oocytes using a micrometric eyepiece and record results.
12. Clean the catheter in alcohol while sampling the next fish using a catheter.
13. Repeat the procedure, alternating catheters.

Oocyte diameter calibration should be performed for each magnification using a ruler or micrometre.

FIGURE 4.3
Ovarian biopsy procedure in flathead grey mullet
(a) When the animal is fully sedated, gently insert the cannula into the oviduct (gonopore); (b-c) carefully introduce the catheter up to the ovary for a few cm; (d) apply a slight suction and carefully withdraw a small sample of oocytes up into the catheter; (e) place the sample on a slide or on a Petri dish and add a few drops of saline solution or clearing solution; and (f) examine the slide under a light microscope at 5x and 10x magnifications and measure the diameter of the largest oocytes



An oocyte diameter of approximately 590 µm (late-secondary growth or full-grown secondary growth) represents the minimum size suitable for spawning induction (Figure 4.4). Hormonal induction of females with smaller oocytes frequently results in failure or atresia. Females with oocyte diameters ≥580–600 µm (late-secondary growth) respond reliably to hormonal stimulation, as vitellogenesis is complete and the oocytes are competent for germinal vesicle migration (GVM) and oocyte maturation.

Ripe females can be identified externally by their bulging abdomen and red protruded papilla (Figure 4.5). Migrating males are typically smaller than females and release fluid sperm under gentle abdominal pressure.

When more than 70 percent of males are fluent and more than 70 percent of females exhibit advanced vitellogenesis (oocyte diameter ≈590 µm), spawning induction may proceed without further assessment.

When fish are not at the same stage of gonadal maturation, the potential breeders are selected one by one through ovarian biopsy (females) and gentle pressure of the abdomen (males).

FIGURE 4.4
Fully grown secondary growth oocytes extracted from a flathead grey mullet female

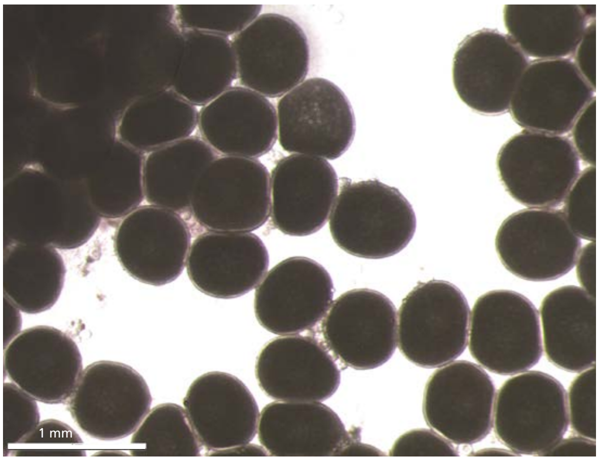


FIGURE 4.5
Ripe *Mugil cephalus* female with protruding genital papilla

4.1.6 Breeders capture and transport procedures

Capture operations of fish breeders must be conducted by at least two operators (Figure 4.6). One operator captures fish individually using a silicone net (mesh size = 2.5 cm; Figure 4.7), while the second operator handles labelled polyethylene transport bags (120 × 50 cm) filled to one-third of their volume (approximately 40–50 L) with lagoon water.

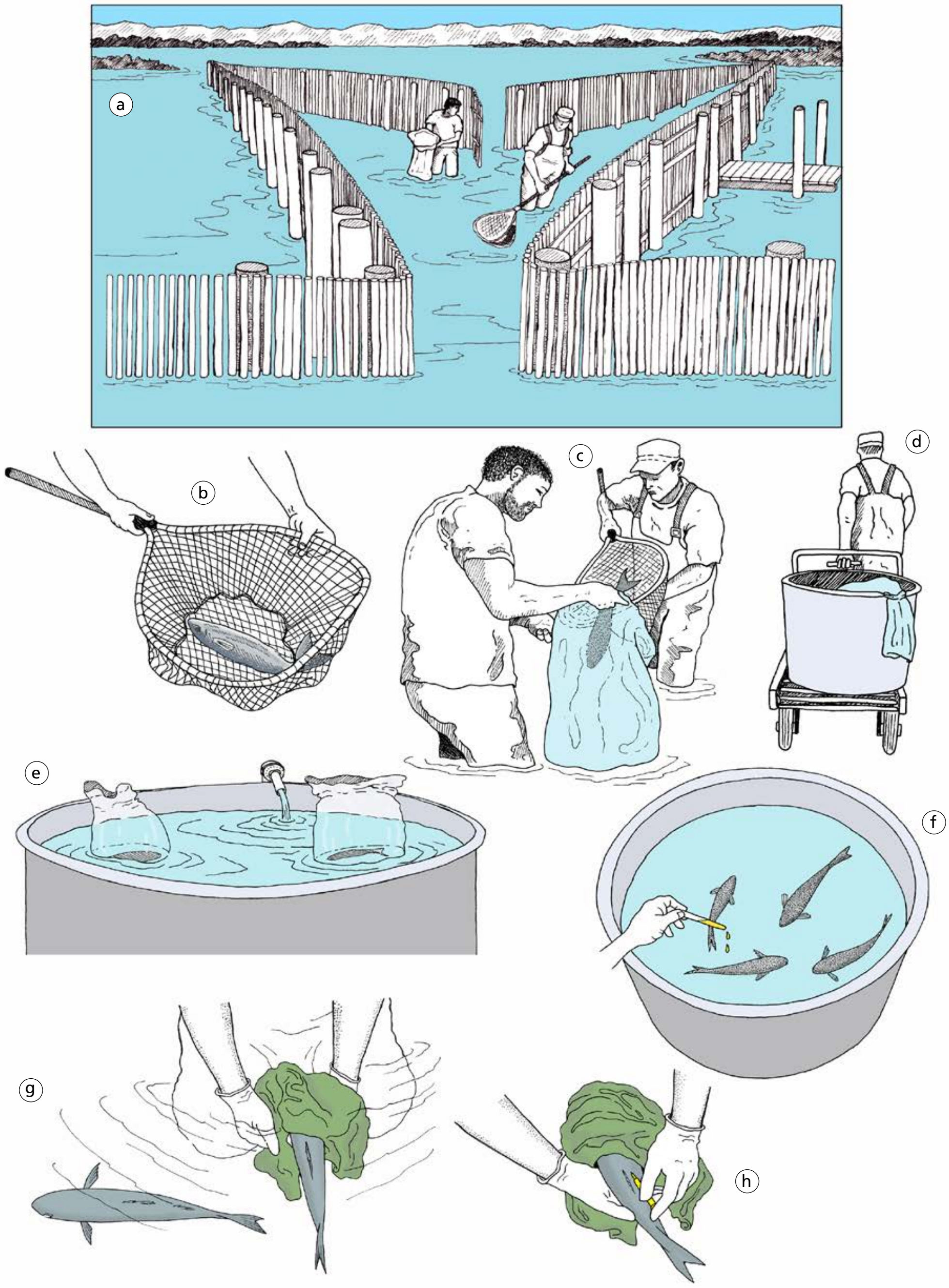
Captured fish are placed directly into the transport bags, which are sealed and left to drift within the enclosure until selection is complete. Each bag is weighed before and after fish insertion to determine individual body weight without direct handling, allowing accurate hormone dosage calculation. A scale with a capacity of 150 kg and 50 g precision is recommended.

Once selection is completed, broodstock must be transported promptly to the hatchery facilities. Transport duration should be minimized, as prolonged transport negatively affects spawning success. If transport exceeds 15 minutes, bags should be filled to two-thirds of their volume with pure oxygen, kept in darkness, and transported in a refrigerated vehicle at approximately 22 °C. Upon arrival, fish are gently released into designated broodstock tanks.

FIGURE 4.6

Capture of breeders and spawning induction procedure

(a) *Lavorieri*, operators preparing to capture breeders; (b–c) net captured fish placed into transport bags with 40–50 L of water from the capture site; (d–e) fish transported to broodstock tanks and released; (f) light sedation induced with 0.01% clove oil; (g) fish gently restrained with head and eyes covered by a wet towel; and (h) intramuscular GnRHa injection administered



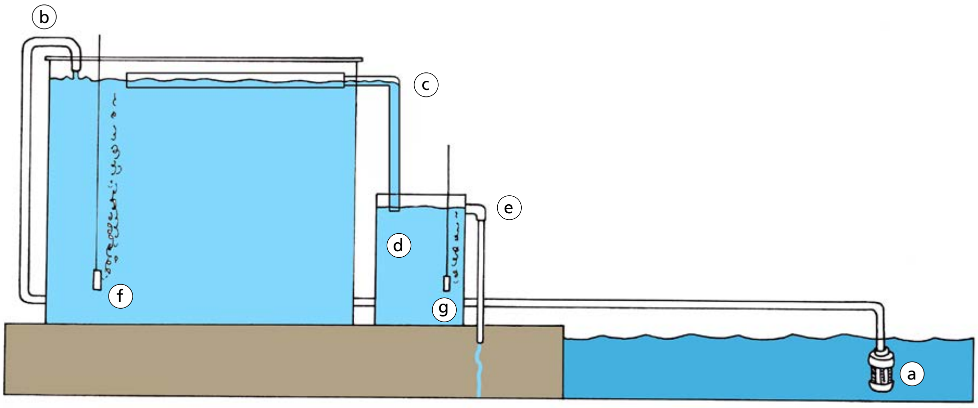
4.1.7 Breeding tank preparation and broodstock introduction

It is recommended to use a flow-through broodstock holding system located adjacent to the capture site, consisting of fiberglass tanks equipped with aeration and an overflow egg collector fitted with a 500 µm mesh (Figure 4.8).

An optimal male-to-female ratio of 3:1 and a maximum stocking density of 5 kg fish body weight (BW) per cubic metre should be maintained to ensure successful reproduction.

FIGURE 4.7
Silicon net to capture fish breeders (mesh size = 2.5 cm)

FIGURE 4.8
Spawning induction tank in an open system
A submersible pump (a) delivers lagoon water into the tank, with flow rate adjustable via a valve on the discharge side (b). An overflow siphon (c) directs floating eggs into a collection tank. The collection tank is equipped with an overflow pipe that serves as the system's outflow (e). Gentle aeration is provided to both tanks using porous stones (f and g) connected to a compressor or blower pump



The inlet water should be supplied from the lagoon via a submersible pump, with a renewal rate of 30 %/h. Once the tanks are prepared, fish should be gently released by opening the transport bags directly inside the tanks.

4.1.8 Induced spawning of wild breeders during their natural reproductive season

Spawning is induced using a slow-release gonadotropin-releasing hormone analogue (GnRHa), leuprorelin acetate (ENANTONE®, Takeda Italia Spa; $C_{59}H_{84}N_{16}O_{12}$; amino acid sequence H-Pyr-His-Trp-Ser-Tyr-D-Leu-Leu-Arg-Pro-NHEt).

At least one hour after the fish are released into the broodstock tanks, the water depth should be reduced to 25–30 cm and the inlet flow turned off. Fish are then partially anesthetized using a low dose of clove oil (0.01 percent) to achieve light sedation.

The hormone dose for each fish must be calculated in advance according to individual body weight, with recommended doses of 200 µg/kg BW for females and 100 µg/kg BW for males.

To calculate the quantity of hormone needed, you need the records of body weight of each individual breeder.

Hormone preparation and dosing

1. Transfer the total 1 ml ENANTONE® preparation from the manufacturer's syringe into a sterilized 1.5 ml microcentrifuge tube.
2. Prepare a numbered, sterilized 1.5 ml tube for each fish and add 1 ml of saline solution. Number the tubes starting from the heaviest female to the lightest male.
3. Use 200–1 000 µl micropipettes to withdraw the required hormone volume from the tube containing the ENANTONE® preparation.

Since 1 ml of ENANTONE® contains 3.75 mg of the drug, the injected volume is calculated as follows:

For females:

$$\mu l\ ENANTONE = \frac{1\,000\ \mu l\ \text{(total volume)} \times 200\ \mu g\ \text{(dose for 1 kg female)}}{3\,750\ \mu g/ml\ \text{(ENANTONE)}} \approx 53.3\ \mu l/kg\ BW$$

For males:

$$\mu l\ ENANTONE = \frac{1\,000\ \mu l\ \text{(total volume)} \times 100\ \mu g\ \text{(dose for 1 kg male)}}{3\,750\ \mu g/ml\ \text{(ENANTONE)}} \approx 26.7\ \mu l/kg\ BW$$

Collect 53.3 or 26.7 µL of ENANTONE® per kg BW for females and males, respectively. Dispense the calculated amount of ENANTONE® in each numbered tube.

Example

For a female weighing 1.7 kg:	53.3 µl/kg × 1.7 kg = 90.6 µl
For a male weighing 1.1 kg:	26. µl/kg × 1.1 kg = 29.4 µl

Sterile 2.5 ml syringes are used to inject each animal.

ENANTONE® *is a powder preparation that tends to settle at the bottom of the tubes; therefore it is necessary to re-suspend it frequently by shaking the test tube.*

Hormone administration

The initial step consists of the technician, wearing latex gloves and smooth rubber waders, kneeling at the bottom of the broodstock tank. Each fish is then gently restrained by covering its head with a wet towel while holding the body securely between the technician's knees, which helps keep the fish calm and motionless during handling (see Figure 4.6g).

A single intramuscular injection of the hormone is administered into the dorsal epaxial muscles just below the dorsal fin (see Figure 4.6h). To prevent mixing, hormone-treated fish can be separated from untreated individuals using a vertically held plastic net within the holding tank.

Post-treatment tank management

After hormone administration:

- Reactivate the submersible pump with a water renewal rate of 30%/h.

- Maintain dissolved oxygen at saturation (~8 mg/L).
- Keep water temperature below 27 °C (optimal range 21–24 °C).
- Provide gentle aeration using an airstone suspended approximately 10 cm above the centre of the egg collector tank.

Spawning success should be monitored for 42 h post-injection, as the likelihood of obtaining viable eggs decreases significantly after this period (Figure 4.9).

FIGURE 4.9
Fertilized eggs of flathead grey mullet in the collection vessel

FIGURE 4.10
Mature flathead grey mullet female with a visibly swollen abdomen

4.1.9 Dry stripping

Although viable fertilized eggs can be obtained through dry stripping, this method should not be considered the first option. It is, however, commonly used when some hormonally induced breeders – both females and males – fail to release their gametes spontaneously into the tank.

If females present an extremely swollen abdomen but do not spawn, it is recommended to perform an ovarian biopsy to verify whether ovulation has occurred (Figure 4.10; see Section 4.1.5 and Figure 4.3). If ovulation has not yet taken place, continue monitoring the females via cannulation every 1–2 h until ovulation is confirmed. While this monitoring method is highly stressful for the animals, it is currently the only reliable means of determining the precise timing of ovulation.

Prior to ovulation, during vitellogenic or early maturation stages, the eggs remain embedded in the ovarian tissue and cannot be stripped. Attempting to strip prematurely may cause severe damage to the ovaries, internal bleeding, or even death of the broodstock. Conversely, delaying the procedure too long can lead to egg overripening. Ovulated eggs retained in the ovary may undergo atresia – a degenerative process in which eggs are reabsorbed – reducing viability and compromising reproductive success. Accurate timing is therefore critical to safeguard both broodstock health and egg quality.

Only when females exhibit ovulated oocytes should dry stripping be performed, following these steps:

1. Prepare a few sterilized dry beakers (5 L) and three 100 L tanks equipped with aeration.
2. Turn off the water inlet and reduce the water column to 25–30 cm.
3. Partially anesthetize the female with a low dose of clove oil (0.01%) to achieve light sedation.
4. Collect 3–4 males and transfer them into a 100 L tank for full anaesthesia using clove oil (0.08%).

5. Once fully anesthetized, check males for sperm fluency by applying gentle pressure to the abdomen. If fluent, carefully dry the abdomen and collect sperm using a sterile 1 ml syringe or Pasteur pipette (Figure 4.11).
6. Fully anesthetize the female by transferring the specimen to the anesthetization tank (0.08 % clove oil). Once sedated, carefully clean the gonopore, cover the head with a wet towel, and apply gentle abdominal pressure to release eggs directly into a dry beaker, taking care to avoid contamination with urine or faeces.
7. Release 0.3–0.7 ml of collected sperm onto the eggs and gently stir the mixture by hand while wearing latex gloves.
8. Add 2–3 L of clean seawater, adjusted to the same temperature and salinity as the broodstock tank, and continue gentle stirring.
9. Allow fertilization to occur for 3–5 minutes, then filter the eggs through a 500 µm mesh to remove excess sperm and gently rinse.
10. Transfer the eggs into 15 L buckets equipped with gentle aeration.
11. After 40 minutes, check the eggs for viability and fertilization under a light microscope.

FIGURE 4.11
Sperm collection from a mature *Mugil cephalus*
After capturing a milting male and once fully sedated, carefully dry the gonopore area with absorbent paper and apply gentle abdominal pressure by sliding the fingers toward the tail as shown (a); collect the sperm released from the gonopore using a clean dry pipette (b); and store in a fridge (at 4 °C) the collected sperm in a sterile 2.5 ml vial (c)

4.1.10 Other hormone treatments

In addition to the slow-release GnRHa protocol described above, alternative hormonal induction strategies have been successfully tested for *M. cephalus* and may be applied when standard induction protocols result in inconsistent spawning or incomplete ovulation.

- One approach involves a two-step GnRHa–dopamine antagonist protocol, consisting of a priming injection followed by a resolving injection. In this protocol, females receive an initial priming dose of GnRHa at 10 µg/kg body weight combined with the dopamine antagonist metoclopramide at 15 mg/kg. This is followed, after an interval of 22.5 h, by a resolving injection consisting of GnRHa at 20 µg/kg BW and metoclopramide at 15 mg/kg. The inclusion of metoclopramide is intended to block dopaminergic inhibition of gonadotropin release, thereby enhancing the endocrine response to GnRHa and promoting oocyte maturation and ovulation. This protocol has been shown to improve spawning success in arrested females. For more details see Aizen *et al.*, 2005.

- An alternative induction method involves the use of human chorionic gonadotropin (hCG), either alone or in combination with GnRHa. In this protocol, a priming injection of hCG at 10 000 IU/kg female body weight is administered, followed by a resolving dose consisting of 10 000 IU hCG combined with GnRHa at 200 µg/kg female body weight. This strategy provides both a direct gonadotropic stimulus through hCG and a central pituitary stimulation of gonadotropin release through GnRHa, potentially increasing ovulatory response in females that do not respond adequately to GnRHa alone. For more details see Besbes *et al.*, 2020.

These alternative hormone treatments should be considered when broodstock exhibit partial responsiveness to standard induction protocols, particularly in cases of incomplete ovulation or delayed spawning. Careful monitoring of ovarian development and ovulation timing remains essential to maximize egg quality and spawning success.

4.2 CAPTIVE BROODSTOCK MANAGEMENT

4.2.1 Broodstock handling and quarantine

Upon arrival at the facility, breeders should be transferred to quarantine tanks to undergo preventive treatments aimed at minimizing the risk of introducing parasites, viral agents and bacterial pathogens. The choice of seawater or freshwater as the receiving medium depends on the origin and capture environment of the fish. Quarantine tanks should be covered with protective nets to prevent fish from jumping out.

Preventive treatments consist of alternating baths using hydrogen peroxide (H_2O_2) and formalin, repeated twice at two-week intervals (see Appendix IV). Fish must be closely monitored throughout the treatment period. If individuals exhibit signs of acute stress, such as sudden immobility, markedly reduced opercular movements, or loss of equilibrium (e.g. turning upside down), they should be immediately transferred to clean, well-aerated water.

When treatments are not being applied, continuous water circulation should be maintained in the quarantine tanks. Fish may be fed normally during periods when no preventive treatments are in progress.

At the end of the quarantine and treatment phase, broodstock should be transferred to the recirculating aquaculture system. This transition presents an appropriate opportunity to collect individual biometric data, including sex, total length and body weight, as well as to tag fish using passive integrated transponder (PIT) tags. For tagging procedures, fish must be anaesthetized; detailed instructions are provided in Appendix V.

Within the RAS, seawater with a salinity of approximately 34 ppt is recommended to support gonadal maturation. Broodstock may receive routine prophylactic treatments with freshwater or hydrogen peroxide when necessary. Fish should be maintained either under controlled photoperiod regimes or under natural lighting conditions, while water temperature should be regulated seasonally. During winter, temperatures should be maintained at or above 14 °C, and during summer, temperatures should not exceed 23 °C. At temperatures below 14 °C, broodstock typically reduce feed intake, whereas temperatures up to 30 °C have not been observed to produce adverse effects on adult breeders.

4.2.2 Broodstock nutrition

Broodstock should be fed daily, seven days per week, at a ration corresponding to approximately 1–1.5 percent BW. The daily ration should be divided into two to three meals to avoid overloading the digestive tract. A minimum conditioning period of 3–4 months of adequate nutrition is required before attempting hormonal induction of spawning.

In the absence of species-specific formulated diets, fish should be fed commercial marine fish broodstock feeds. Pellets with a diameter of 3–4 mm are recommended to facilitate ingestion, as larger pellets may be regurgitated. Dry feeds should contain essential nutritional components, including marine polyunsaturated fatty acids (n-3 PUFA), particularly eicosapentaenoic acid (EPA; 20:5 n-3) and docosahexaenoic acid (DHA; 20:6 n-3). Diets should also be supplemented with adequate levels of vitamins and antioxidants to support reproductive performance.

In addition to formulated feeds, fresh or frozen mussels and polychaetes may be offered twice per week as a partial substitute for pellets, contributing to dietary diversity and potentially enhancing broodstock conditioning.

4.2.3 Spawning in RAS systems and out-of-season

Flathead grey mullet maintained under intensive rearing conditions generally do not undergo spontaneous gonadal development. Females typically remain at the previtellogenic stage or exhibit only a limited number of vitellogenic oocytes, while males often fail to produce sperm or release only small quantities of viscous milt. Under these conditions, specific hormonal and environmental interventions are required to promote gametogenesis and enable spawning, particularly for out-of-season reproduction.

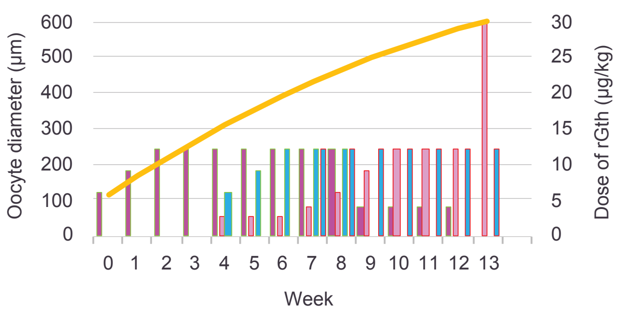
Several approaches have been described to advance gonadal development and induce spawning in captive broodstock.

Scenario A: Single hormonal treatment in females and slow-release implant in males

In this protocol, a single intramuscular injection of domperidone at 5 mg/kg BW is administered to females, while males receive an EVAc slow-release implant containing 17α-methyltestosterone at a dose of 4 mg/kg BW. This treatment is typically applied in July, approximately 99 days prior to the expected spawning period, to stimulate early gonadal development.

Spawning induction is carried out once females reach an oocyte diameter of approximately 570 µm. Induction consists of a priming injection of GnRHa at 10 µg/kg BW combined with metoclopramide at 15 mg/kg, followed 22.5 h later by a resolving injection of GnRHa at 20 µg/kg BW and metoclopramide at 15 mg/kg. Further methodological details and outcomes are reported in Aizen *et al*., 2005.

FIGURE 4.12
Recombinant gonadotropin (rGth) protocol used for *Mugil cephalus* and oocyte growth as increasing diameter (µm) (yellow line)
Columns represent the weekly dose (µg/kg) of rGth injected, where pink column fill represents females and blue represents males, a green column border represents recombinant follicle-stimulating hormone (rFsh) and a red border represents luteinizing hormones (rLh)



Source: Modified from Ramos-Júdez, S., Giménez, I., Gumbau-Pous, J., Arnold-Cruañes, L.S., Estévez, A. & Duncan, N. 2022. Recombinant FSH and LH therapy for spawning induction of previtellogenic and early spermatogenic arrested teleost, the flathead grey mullet (*Mugil cephalus*). *Scientific Reports*, 12: 6563.

Scenario B: Repeated hormonal treatments in females and males using recombinant gonadotropins

An alternative approach involves the administration of recombinant gonadotropins, specifically recombinant follicle-stimulating hormone (rFsh) and recombinant luteinizing hormone (rLh) (Rara Avis Biotec S.L., Spain), to both females and males (Figure 4.12).

In females, weekly injections are administered according to oocyte developmental stage. During the first four weeks, rFsh is administered at doses of 6, 9, 12 and 12 µg/kg BW, respectively. This is followed by five weeks during which rFsh is maintained at 12 µg/kg BW and combined with increasing doses of rLh (2.5, 2.5, 2.5, 4 and 6 µg/kg). In week 10, the rFsh dose is reduced to 4 µg/kg BW while rLh is increased to 9 µg/kg. Thereafter, 4 µg/kg rFsh and 12 µg/kg rLh are administered weekly until oocytes reach a diameter of approximately 600 µm.

Final spawning induction can then be achieved either by administering a priming dose of 30 µg/kg rLh followed 24 h later by a resolving dose of progesterone at 40 mg/kg BW (Prolutex®, IBSA Group, Italy), or by using both priming and resolving injections of rLh at 30 µg/kg BW.

Male treatment is initiated four weeks after the start of female treatment. During the first three weeks, males receive rFsh at doses of 6, 9 and 12 µg/kg BW, followed by two weeks of combined administration of rFsh and rLh at 12 µg/kg BW each. Subsequently, rLh is administered alone at 12 µg/kg BW for the remainder of the spawning period. Further details are provided in Ramos-Júdez *et al*., 2022.

Scenario C: Induction of gametogenesis through environmental control

Broodstock born and reared in captivity or maintained in captivity for extended periods (at least 2–3 years), may progressively undergo partial domestication. In such cases, precise control of environmental parameters – particularly photoperiod and temperature – can promote gonadal maturation and improve reproductive performance without reliance on prolonged hormonal treatments.

In the wild, *M. cephalus* initiates final gonadal development as day length decreases from late summer to autumn, with photoperiods shortening from approximately 14–11 h of light, accompanied by a gradual decline in water temperature. Hatcheries can mimic these natural cues through controlled photoperiod and temperature regimes.

During the conditioning phase (spring–summer), long photoperiods of 14–16 h of light support somatic growth and early gonadal development. As the maturation phase approaches (late summer–autumn), the photoperiod should be progressively reduced to 12:12 or 10:14 light–dark cycles. Light intensity should be maintained at moderate levels (200–500 lux at the water surface) to avoid stress or suppression of activity.

Water temperature plays a critical role in regulating gonadal development. Optimal temperatures range between 22 and 24 °C, supporting metabolic stability and oocyte growth. Temperatures below 18 °C slow gonadal development, while temperatures above 28 °C may induce stress and negatively affect egg quality. Temperature fluctuations greater than 2 °C per day should be avoided to prevent stress-induced endocrine disruption or oocyte resorption.

While natural light may contribute to maturation, it is often insufficient to ensure reliable reproductive outcomes in captivity. The use of programmable, dimmable lighting systems, including gradual dawn and dusk simulations, is therefore recommended. Hormonal induction using GnRHa or dopamine antagonists should only be applied once females reach advanced vitellogenesis, typically when oocyte diameter approaches 590 µm.

To maximize the likelihood of successful maturation and spawning under captive conditions, the following environmental parameters are recommended:

- *Tank volume*: Use tanks with a minimum volume >10 m^3 to provide sufficient space, reduce stress, and minimize stress-related inhibition of gonadal development.
- *Minimization of disturbances*: Avoid excessive noise, vibration, and frequent human activity around the tanks, as chronic disturbance elevates stress levels, disrupts endocrine regulation, and impairs reproductive performance.
- *Stocking density*: Maintain stocking densities <2 kg/m^3 to prevent overcrowding, which can increase cortisol levels, suppress somatic growth and inhibit gametogenesis.

- *Dissolved oxygen*: Maintain DO concentrations close to saturation (≥80–90% saturation, species-dependent). Hypoxic conditions negatively affect metabolic efficiency, oocyte development and spawning success.

Collectively, these environmental parameters promote a low-stress and physiologically supportive rearing environment, substantially increasing the likelihood of achieving complete gonadal maturation in captivity.

5. Production cycle of *Mugil cephalus*

This chapter provides a concise overview of the complete *Mugil cephalus* production cycle, from fertilized egg incubation through larval rearing, nursery, grow-out and broodstock selection (Table 5.1; Figure 5.1). Each production phase is described in detail in the subsequent chapters (see Chapters 6–9).

Fertilized eggs are incubated in dedicated incubation systems under controlled temperature and aeration until embryonic development reaches the eye stage, which occurs approximately 30–32 h post-fertilization at 23 °C. At this stage, embryos are transferred to larval rearing systems, where hatching takes place. Careful handling during transfer is essential to minimize mechanical stress and mortality.

Larvae are reared in these systems until the completion of metamorphosis, which typically occurs around 40 days post-hatching (DPH). Following metamorphosis, early juveniles are transferred to larger rearing tanks characterized by higher water flow rates and increased water exchange compared with larval systems. During this period, larvae undergo major morphological and physiological changes, including the transition from endogenous to exogenous feeding, swim bladder inflation, and progressive development of the digestive system. Juveniles are maintained in these nursery systems until they reach a BW of approximately 0.5–1.0 g.

At around 100 DPH, juveniles are counted either by biomass estimation or using automatic counting devices and subjected to size grading to reduce size heterogeneity. At this stage, fish can be gradually acclimated to a wide range of salinities, including freshwater, reflecting the strong euryhaline capacity of the species. Fish are then transferred to larger tanks (2 000–10 000 L) or nursery ponds (>5 000 L), depending on the production strategy.

TABLE 5.1
Overview of the main stages in the production cycle of *Mugil cephalus*

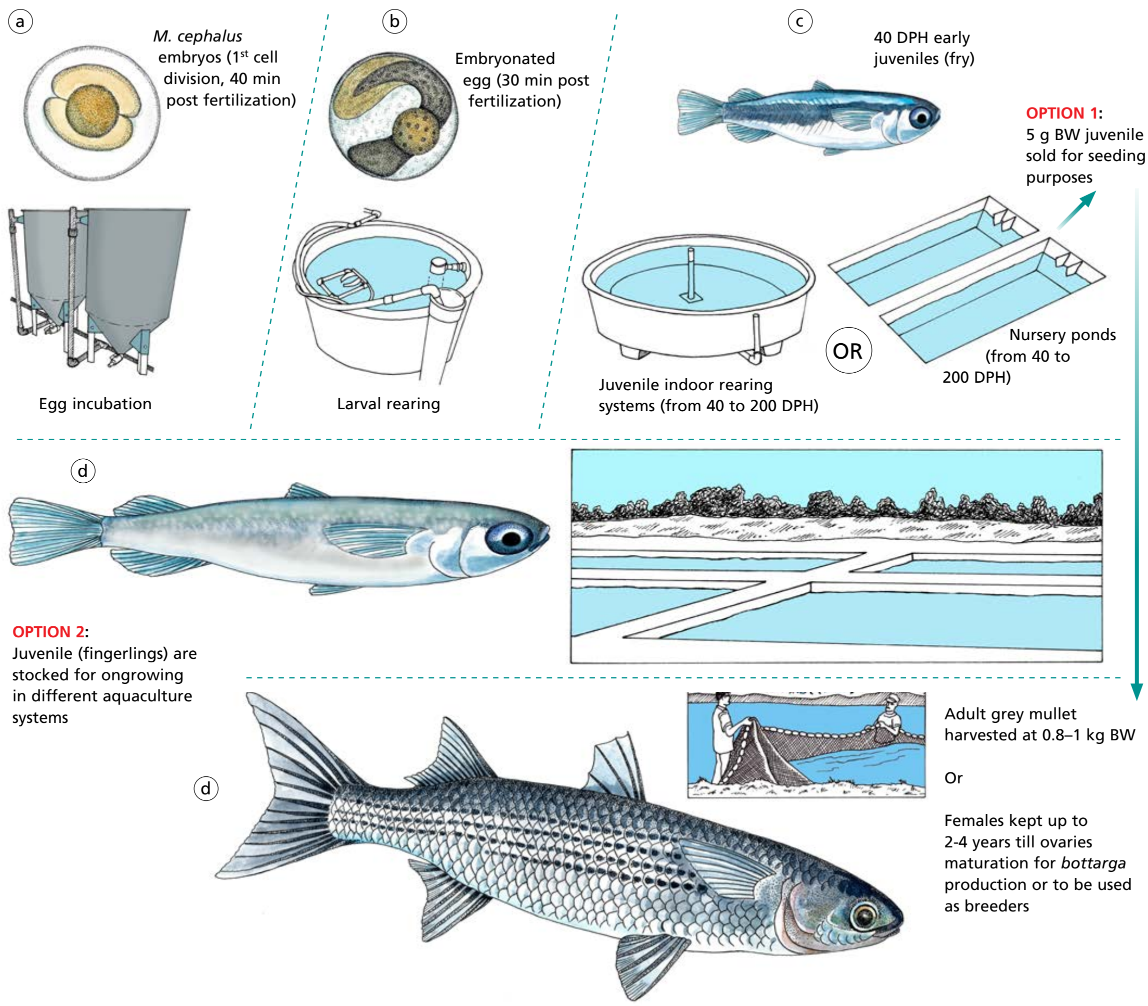
Stage	Timeframe/size	Process	Key considerations
a) Incubation	0–32 h post-fertilization	Fertilized eggs incubated at 23 °C under controlled temperature and aeration until eye stage.	Minimise mechanical stress during transfer to larval rearing systems.
b) Larval rearing	0–40 DPH	Larvae reared until completion of metamorphosis.	Major changes: transition to exogenous feeding, swim bladder inflation, digestive development.
c) Early juvenile	40 DPH 0.5–1.0 g BW	Juveniles transferred to larger tanks with higher water flow and exchange.	Gradual acclimation to various salinities possible.
d) Juvenile grading	~100 DPH/~200 DPH >5 g BW	**First grading (~100 DPH):** Counted, graded for size homogeneity; transferred to larger tanks or ponds. Can be acclimated to freshwater or other salinities. **Second grading (~200 DPH):** Size grading; juveniles sold or transferred to grow-out systems.	Can be acclimated to freshwater or other salinities. Stocking density and system choice depend on production strategy.
e) Grow-out/ broodstock selection	~200 DPH/market size (~1 kg)/adults	Reared in semi-intensive or intensive ponds.	**Option 1 – Grow-out:** Some females retained for *bottarga* production (2–4 y). **Option 2 – Broodstock:** Selected adults conditioned for spawning induction; ensures continuity of the hatchery production cycle.

At 200 DPH, a second grading is recommended. Individuals exceeding 5 g BW can be sold as juveniles for stocking purposes or transferred to outdoor grow-out systems, including semi-intensive or intensive ponds. In these systems, fish are reared to market size, typically 0.8–1.0 kg BW. A proportion of female fish can be retained for long-term rearing (2–4 yr) to allow full ovarian maturation for the production of *bottarga* (salted and dried mullet roe) (see Appendix I). For this purpose, a maximum stocking density of 5–10 kg fish/m³ is recommended.

Finally, a selected portion of adult fish can be conditioned and maintained as broodstock for spawning induction, enabling the production of new batches of fertilized eggs and ensuring continuity of the hatchery production cycle.

The productive cycle of *M. cephalus* includes egg incubation for approximately 30 h from first cleavage to the eyed-egg stage, followed by larval rearing at densities of 100–200 ind./L for about 40 days until weaning and transformation into juveniles. Juveniles are then maintained in indoor circular tanks or nursery ponds until 100 DPH. At this stage the fish are graded and transferred to outdoor semi-intensive or intensive grow-out ponds; a second grading is carried out at 200 DPH, when individuals exceeding 5 g BW may be sold as juveniles for stocking or moved to grow-out systems for rearing to market size (0.8–1.0 kg BW) (Figure 5.1).

FIGURE 5.1
Productive cycle of *Mugil cephalus*
(a) Egg incubation to eyed stage; (b) larval rearing to weaning and juvenile stage; (c) nursery phase to 100 DPH; (d-e) grading and transfer to grow-out ponds, with further selection at 200 DPH and rearing to market size (0.8–1.0 kg) including selection of new breeders.



6. Egg and early larvae management

This chapter describes the technical procedures for the management of fertilized eggs and early larval stages of *Mugil cephalus*, from spawning and egg collection to incubation, transport, quality assessment, disinfection and transfer to larval rearing systems. These early developmental stages are particularly sensitive to mechanical stress, water quality and temperature fluctuations; therefore, strict adherence to handling protocols and environmental control is essential to ensure high hatching rates and optimal larval survival.

6.1 EGG DEVELOPMENT

Accurate identification of embryonic development stages is critical for determining appropriate handling, transport and incubation timing. The embryological sequence described below refers to eggs spawned and incubated at 23 ± 1 °C. Developmental timing is temperature-dependent; lower temperatures delay development, whereas higher temperatures accelerate it, potentially increasing the risk of abnormalities if not properly controlled.

Ripe unfertilized egg (immediately after spawning) – Figure 6.1 and Figure 6.2 (1)

Freshly spawned, unfertilized eggs of *M. cephalus* are spherical, transparent to slightly yellowish, pelagic and non-adhesive. Eggs have a diameter of 702 ± 9 µm (mean SD) and contain a single oil globule (OG) that provides buoyancy and an initial energy reserve.

Fertilized egg – first cleavage (2-cell stage) – Figure 6.2 (2)

Following fertilization, eggs remain pelagic and display a smooth, intact egg membrane (chorion). The first meroblastic cleavage occurs at the animal pole approximately 30–40 minutes post-fertilization, forming two blastomeres (B). The yolk mass (Y) remains concentrated at the vegetal pole. Egg diameter increases to 785 ± 12 µm (mean SD) due to hydration.

Four-cell stage – Figure 6.2 (3–4)

The second cleavage produces four equally sized blastomeres arranged in a single plane, forming a well-defined blastodisc (BD). This stage is reached approximately 40 minutes after fertilization. With each successive division, blastomeres decrease in size while the blastodisc becomes more clearly differentiated from the yolk.

From eight-cell stage to morula – Figure 6.2 (5–8)

The third cleavage (8-cell stage) occurs around 50 minutes post-fertilization, followed by the fourth cleavage (16-cell stage). Subsequent divisions result in a multicellular blastoderm (BL), and the embryo reaches the morula stage approximately 2 h after fertilization. Mean egg diameter is 786 ± 15 µm (mean SD).

Blastula and gastrula stages – Figure 6.2 (9–12)

Beyond the 32-cell stage, blastomere division becomes asynchronous and individual cells are difficult to distinguish under binocular microscope. The blastula stage begins as the blastoderm flattens and spreads over the yolk via epiboly, forming the germ ring and blastocoel. Gastrulation occurs approximately 4 h post-fertilization, during which the blastoderm thickens and invaginates to form the embryonic shield. At this stage, the embryo becomes multilayered. Egg diameter averages 786 ± 31 µm.

Blastopore closure and neurulation – Figure 6.2 (13–16)

At approximately 10 h post-fertilization, blastopore closure is completed and the embryonic axis becomes visible. Neurulation (neurula stage) begins around 11 h post-fertilization, marking the differentiation of neural tissues. Egg diameter is 787 ± 12 µm (mean SD).

Advanced embryo development – Figure 6.2 (17–20)

By 14–15 h post-fertilization, the embryo is clearly visible within the egg. Pigmentation initiates, the head region (HR) differentiates, and somites (S), the start of vertebrae, become distinguishable. The primordial fin forms, and by 26 h, the embryo occupies most of the egg volume. Eye capsules (E) and lenses are visible between 27 and 29 h post-fertilization. Egg diameter increases to 802 ± 4 µm (mean SD).

Hatching – Figure 6.2 (21–22)

Hatching occurs approximately 36 h post-fertilization. Larvae rupture the egg membrane (EM) through vigorous tail movements, with the head emerging first. Newly hatched larvae measure 2.1 ± 0.4 mm total length (TL) and remain poorly developed, relying on endogenous reserves in the yolk sac (YS).

FIGURE 6.1
Unfertilized eggs of *Mugil cephalus* seen under microscope

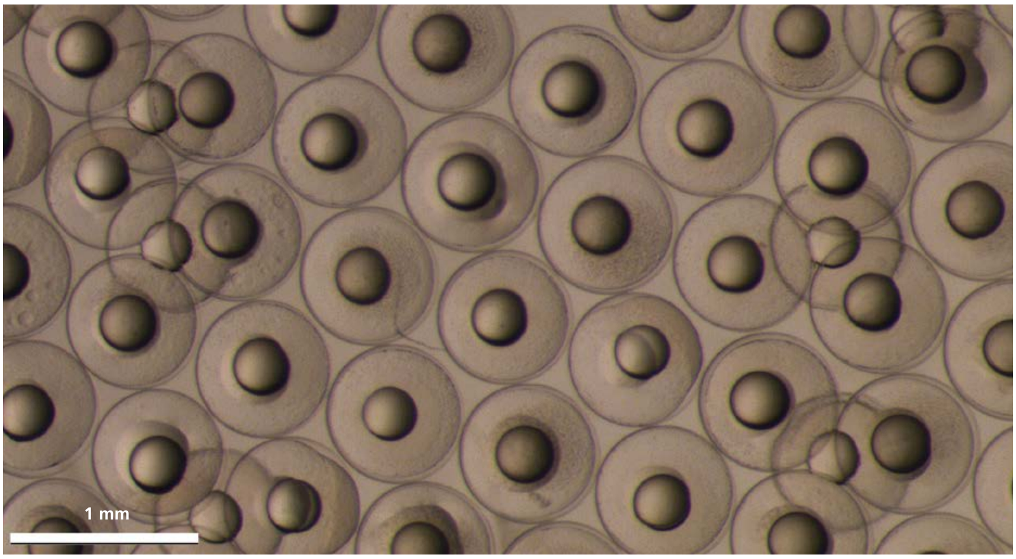
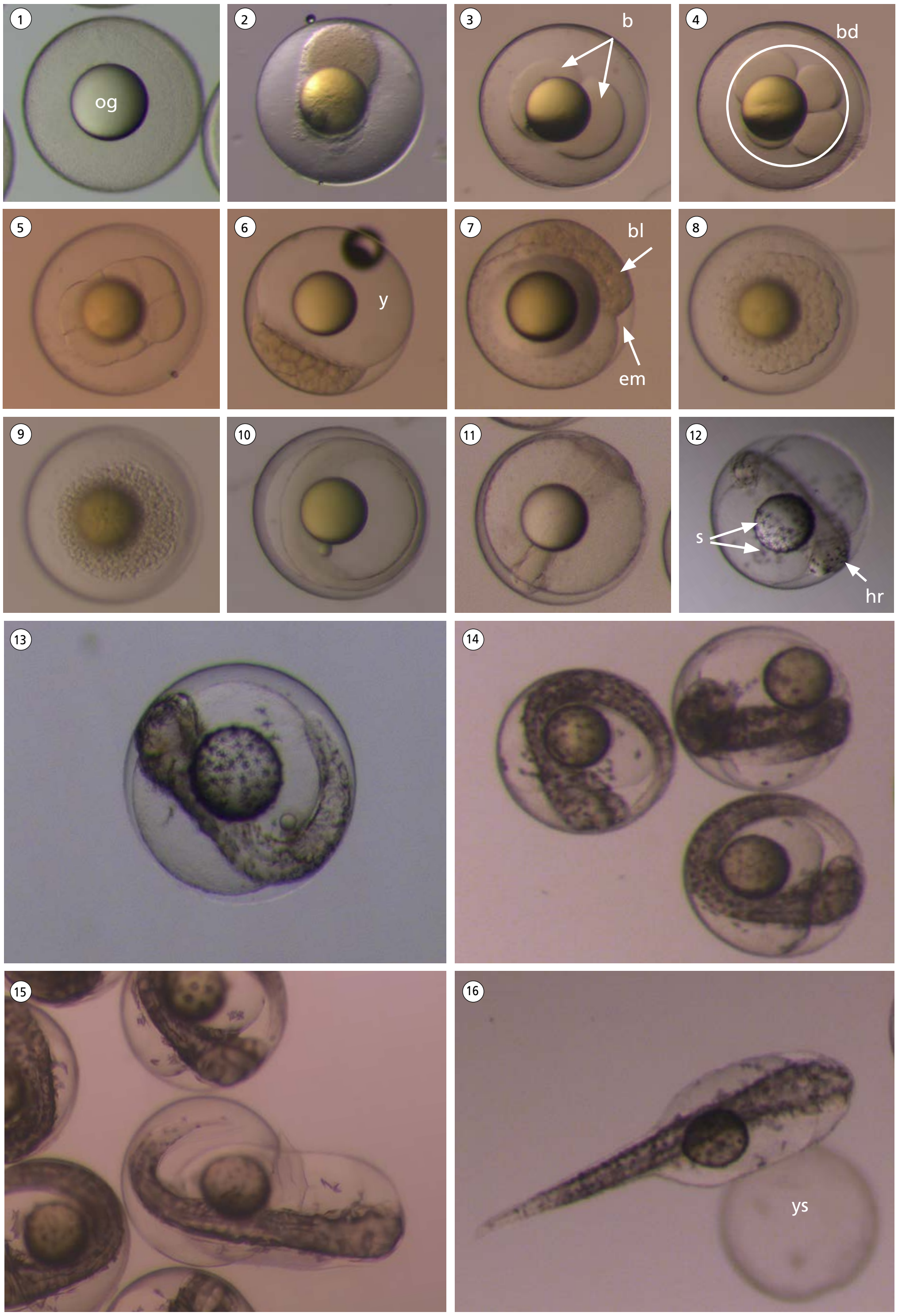


FIGURE 6.2
Embryonic development of *Mugil cephalus* from egg fertilization to hatching
(og) oil globule; (b) blastomers; (bd) blastodisc; (y) yolk material; (bl) blastoderm; (em) egg membrane; (hr) head region; (e) eye; (s) somites; and (ys) yolk sac



6.2 EGG PRODUCTION

Prior to spawning, water flow within the broodstock tank must be adjusted to ensure a gentle and continuous transfer of eggs toward the surface egg collector, minimizing turbulence and mechanical shock. *Mugil cephalus* spawns synchronously, releasing large numbers of eggs over a short time interval.

Eggs are positively buoyant in natural seawater and accumulate at the surface, where they are collected using an overflow egg collector (see Figure 4.8). Collectors must be inspected frequently, and eggs should be harvested promptly. A floating egg layer thicker than 1 cm should be avoided, as excessive accumulation rapidly leads to hypoxia, reduced fertilization success and increased embryo mortality.

6.3 EGG HARVEST

Egg viability and fertilization rate should be assessed approximately 40 minutes after the onset of spawning using a light microscope (6.5–50× magnification). Two standardized methods can be applied to estimate egg numbers.

Option 1 – Estimation by weight

Use this option when eggs are already concentrated, partially drained, or need to be harvested rapidly for transport or incubation. This method is faster and more practical for large egg volumes.

- Collect eggs from the overflow egg collector using a 500 µm mesh net;
- Gently blot the eggs with absorbent paper to remove excess surface water;
- Weigh 1 g of eggs and divide it into three subsamples of 0.33 g each;
- Count all viable eggs in each subsample under a light microscope;
- Calculate the mean number of eggs/g based on the three subsamples;
- Use this value to estimate the total number of eggs and harvest the desired quantity by weight;
- To avoid damaging the eggs by keeping them out of water, fill a 2-L cylinder with water from the spawning system and weigh its contents to establish the tare weight. Add the harvested eggs to the cylinder and weigh it again. Calculate the net weight of the eggs by subtracting the tare weight (cylinder with water only) from the total weight (cylinder with water and eggs). This value represents the exact weight of the harvested eggs.

Option 2 – Estimation by volume

Use estimation by volume when eggs are highly diluted, when precise volumetric control is required, or when working with smaller egg batches where higher counting accuracy is needed.

- Collect all eggs from the overflow egg collector using a 500 µm mesh net;
- Transfer the eggs into a 10-L bucket filled with clean seawater;
- Apply gentle aeration to ensure a homogeneous distribution of eggs in the water column;
- Collect 3–5 aliquots of 10 ml each using a pipette with a cut-tip, samples can be diluted to have a manageable number of eggs to count in the sample;
- Count all eggs present in each 10 ml aliquot under a light microscope;
- Calculate the total number of eggs based on the total volume of the bucket;
- Repeat the procedure at least three times and calculate the average number of eggs.

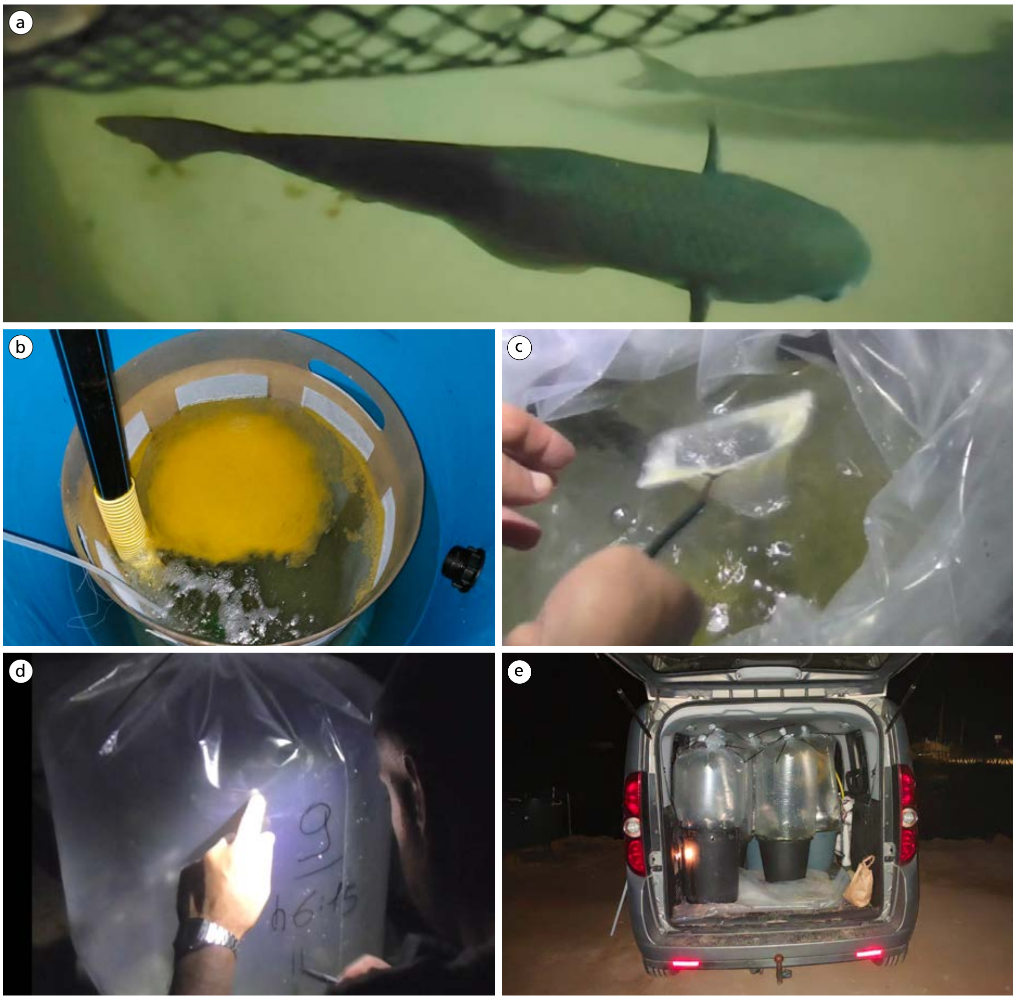
> *While counting eggs under the light microscope it is possible to determine concurrently the percentage of viable and fertilized eggs.*

6.4 EGG TRANSPORT

Eggs are transported at a maximum density of 4 000 eggs/L in 50-L transport bags (120 × 50 cm), filled 1/3 with seawater and 2/3 with pure oxygen. Bags must be kept in complete darkness and transported at a constant temperature of 22 °C. Transport duration should not exceed 28 h (Figure 6.3).

Egg manipulation should be avoided during sensitive developmental stages (8–11 h after fertilization), particularly during blastopore closure [see Figure 6.2 (16)] and

FIGURE 6.3
Eggs collection and preparation for transport
(a) *Mugil cephalus* female spawning; (b) eggs drained in the collector; (c) eggs collected using a hand net; (d) eggs are packed into transport bags; and (e) the egg bags are placed inside rigid containers and transported to the hatchery for incubation



early segmentation [see Figure 6.2 (17)]. Counting and handling at the eye stage (~28 h post-fertilization) generally results in high hatching rates and good larval survival (see Figure 6.5).

6.5 QUALITY CONTROL

Egg quality is evaluated by monitoring embryonic development and hatching success using a representative subsample of floating, fertilized eggs (minimum 2-cell stage) incubated in 96-well EIA plates, as follows (Figure 6.4):

- Collect a representative subsample of floating, fertilized eggs at the early cleavage stage (≥2-cell stage);
- Add 200 µL of autoclaved seawater (same temperature and salinity as the spawning tank) to each well of a 96-well EIA plate;
- Transfer one egg per well using a tip-cut, single-use Pasteur pipette to avoid mechanical damage;
- Prepare the plates preferably in triplicate to improve statistical reliability;
- Incubate the plates in complete darkness at a constant temperature identical to that of the spawning tank; plates may be wrapped in aluminium foil to prevent light exposure;

- Inspect the plates daily under a stereomicroscope to record embryo development, hatching success, and mortality;
- Continue observations until all viable larvae have hatched and no further survival is observed.

Calculate the survival percentage as:

$$\%\ \text{survival} = \frac{\text{Number of larvae alive}}{\text{Total number of hatched larvae}} \times 100$$

FIGURE 6.4
Assessment of *Mugil cephalus* egg quality using and monitoring embryonic development, hatching success and larval survival in 96-well EIA plates

6.6 EGG INCUBATION IN DEDICATED FACILITIES

Fertilized eggs are incubated in truncated-cone incubators equipped with gentle aeration to maintain eggs in suspension and prevent sedimentation. Water inflow may be supplied either from the surface or from the bottom of the tank, provided that a uniform and low-turbulence circulation is ensured. The system should operate with a recirculation rate of 40–50 percent/h and a water renewal rate of 20–30 percent/hour. Temperature and salinity must be kept identical to those of the spawning tank to prevent thermal or osmotic shock, which can negatively affect embryo development and hatching success.

Eggs are incubated in darkness at densities of 400–1 000 eggs/L. Dissolved oxygen should remain near saturation (~8 mg/L), ammonia at 0 mg/L, and nitrite concentrations below 0.25 mg/L. At 23 °C, hatching occurs after approximately 36 h. Sinking or dead eggs must be removed regularly by siphoning to prevent water quality deterioration. Dead eggs can be collected using a 500 µm net mesh size filter and counted volumetrically or by weight.

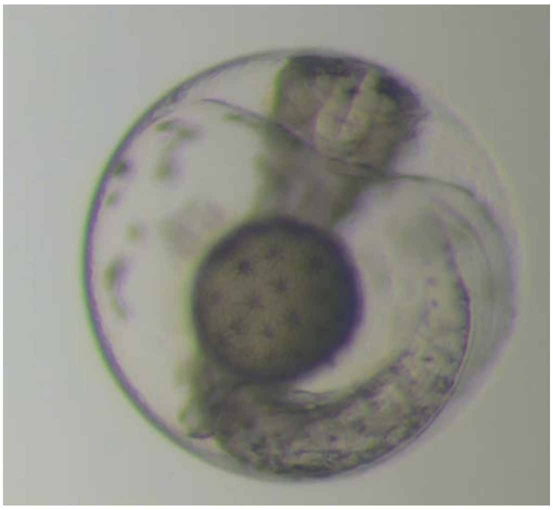
6.7 EGG INCUBATION IN THE LARVAL REARING TANKS

Eggs may be incubated directly within the larval rearing tanks, provided that the same hydraulic and aeration conditions described for dedicated incubation units are applied. Under these conditions, stocking density should be limited to 100–200 eggs/L. The total number of eggs introduced must be adjusted in advance by accounting for expected egg mortality, in order to achieve the target larval density at hatching.

However, the stocking with eggs that have embryos at the eye stage (Figure 6.5) is strongly recommended as the preferred practice for egg incubation in larval rearing tanks. At this developmental stage, embryos exhibit high tolerance to handling, counting, and transfer, with no measurable adverse effects on hatching rate or early larval survival. This approach allows more accurate control of stocking density and improves standardization among rearing batches.

During the hatching period, aeration should be temporarily increased to maintain dissolved oxygen near saturation and to promote uniform hatching. Once hatching is complete, both aeration and water inflow should be suspended. Unhatched eggs, dead embryos, and egg shells should then be gently removed from the tank bottom by siphoning, collected using a 50 µm mesh filter, and quantified by volume or weight to assess hatching success and incubation efficiency.

FIGURE 6.5
Fertilized egg of *Mugil cephalus* at the embryonic eye stage



6.8 EGG DISINFECTION

To reduce the risk of bacterial, fungal and protozoan contamination, fertilized eggs should be disinfected prior to stocking in larval rearing systems. Recommended procedures are as follows:

- *Hydrogen peroxide* (H_2O_2): Treat eggs at a concentration of 350 µl/L for 15 minutes; or
- *Active iodine*: Treat eggs at a concentration of 50 µl/L for 30 minutes.

Disinfection should be performed at the early embryonic stage, and eggs must be thoroughly rinsed with clean seawater afterward to remove any residual chemicals and minimize potential toxicity.

During treatment, eggs should be handled gently to avoid mechanical damage, and gentle aeration should be applied to ensure uniform exposure and prevent eggs from settling or clumping. Protective gloves and eyewear are recommended when handling disinfectants. Following disinfection, eggs should be immediately transferred to larval rearing tanks with water conditions (temperature, salinity and oxygen) matching those of the incubation system. For detailed procedures, see Appendix IV.

6.9 LARVAL TRANSFER TO THE REARING FACILITIES

Larvae can be safely transferred from incubators to larval rearing systems while maintaining high survival rates. To ensure optimal handling, the incubation density should not exceed 200 eggs/L. The transfer procedure should be carried out as follows:

- At the onset of hatching, turn off both aeration and water flow in the incubators to allow larvae to stabilize.
- Maintain larvae under static and dark conditions and plan the transfer for 2–3 DPH when larvae are more robust and resilient.
- Prepare sterile transfer containers, such as bowls with handles, and adjust the temperature and salinity of the larval rearing system to match those of the incubators exactly, minimizing thermal or osmotic stress (see Figure 7.10).
- Turn off aeration and water flow in the larval rearing tanks to avoid turbulence during larval introduction.
- Take advantage of the phototactic behaviour of newly hatched larvae by directing a light source onto the incubator surface to gently aggregate them near the water surface.
- Carefully collect the larvae from the incubator surface using the prepared containers, minimizing disturbance to both water and larvae to prevent injury or stress.
- Introduce the larvae into the larval rearing system by slowly submerging the container, allowing the larvae to disperse gently into the tank while avoiding rapid water movement that could stress the larvae.

7. Larval rearing

Despite the wide geographic distribution and high ecological plasticity of the flathead grey mullet, *Mugil cephalus*, full-scale commercial production remains limited. The species is euryhaline and widely distributed in temperate and tropical regions and has traditionally been farmed under extensive conditions in ponds and coastal enclosures. Such practices are well established in the Mediterranean region, Southeast Asia, the Republic of Korea, Taiwan Province of China, China, Japan, and Hawaii (United States of America). Owing to its fast growth, omnivorous feeding habits, tolerance to a wide range of environmental conditions, and high market value, *M. cephalus* is considered a strong candidate for the development of more intensive aquaculture systems.

Experimental and semi-commercial induced spawning and fry production were first achieved in the 1970s in the United States of America and Taiwan Province of China. More recently, commercial-scale fry production from hormonally induced broodstock has been achieved in Israel, while limited fry production mainly for research and pilot-scale aquaculture has been reported in Italy, Spain and Egypt. These advances have renewed interest in the optimization of larval rearing protocols, which remain one of the principal bottlenecks to the expansion of grey mullet aquaculture.

Larval rearing of *M. cephalus* is conducted under intensive hatchery conditions, requiring strict control of environmental parameters such as light intensity and photoperiod, temperature, dissolved oxygen, salinity and water exchange rates. When reared at 23 °C, early development follows a predictable sequence of embryonic and larval stages (Table 7.1). This developmental timeline provides an essential reference for scheduling feeding regimes, adjusting environmental conditions, and managing tank operations throughout the larval phase.

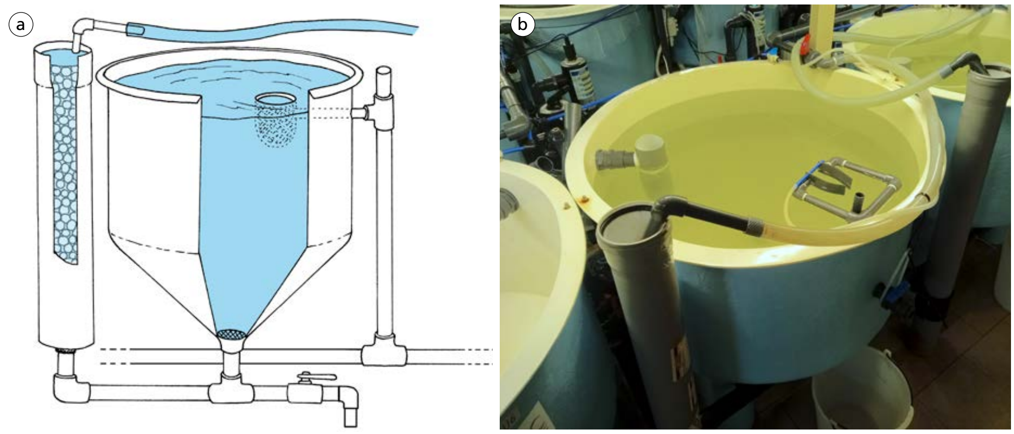
Larvae are typically reared in circular fiberglass tanks with volumes ranging from 500–5 000 L, depending on hatchery capacity and production scale. Tanks may be white or black; however, black-coloured tanks are often preferred, as they reduce light reflection and localized zones of excessive light intensity, conditions that have been associated with improved larval survival. Tanks are equipped with either a central bottom drain fitted with a vertical standpipe or a side-mounted overflow elbow fitted with a fine-mesh filter (Figure 7.1). The tank bottom should have a slight

TABLE 7.1
Description and timeline for the development of early life stages of *Mugil cephalus* reared at 23 °C

Developmental stage	Predicted timing post-fertilization
First cell division	40 minutes
Embryonated egg	32 hours
Newly hatched larva	36 hours
Pre-flexion larva	3 days
Flexion larva	13 days
Post-flexion larva	25 days
Juvenile	40 days

FIGURE 7.1
Larval rearing systems with lateral overflow elbow filter

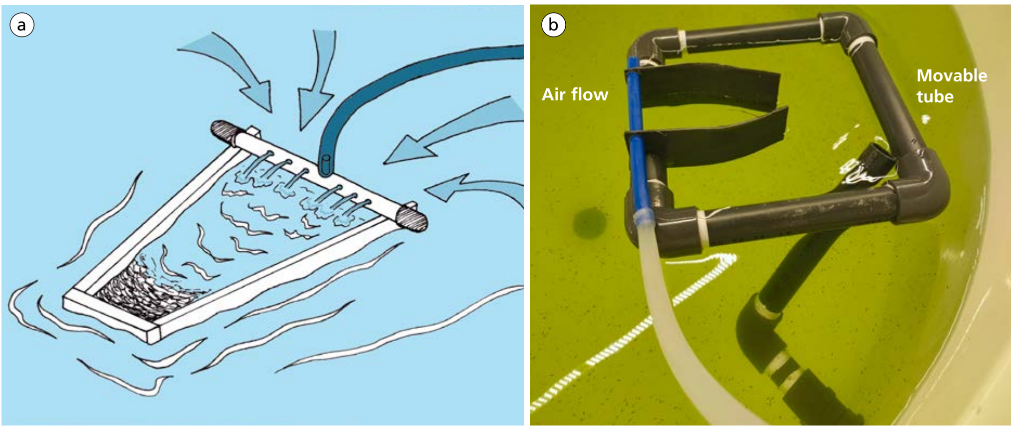
FIGURE 7.2
Schematic setup and photograph of a larval rearing tank
(a) The tank inlet passes through a vertical tube that produces a gentle, turbulence-free flow delivered from the bottom centre of the tank. The water returns to the recirculation system by passing through the basket filter placed on the lateral overflow and (b) photo of the larval rearing tank showing the surface skimmer used to retain surface oil and debris



slope toward the centre to facilitate waste removal and prevent debris accumulation (Figure 7.2). To avoid accumulation of prey and debris in the surface a skimmer is placed on the surface of the tank and connected to the air supply to produce a current of water that brings the oil to a small floating PVC structure.

Seawater supply may be provided under flow-through conditions or within a RAS incorporating appropriate mechanical and biological filtration. Each rearing tank is equipped with an independent lighting system, allowing precise regulation of light intensity and photoperiod, which is critical to support visual prey detection and optimize feeding success during early larval development. The number and size of larval rearing tanks are determined by available infrastructure and spatial constraints but are ultimately limited by the hatchery's live feed production capacity – particularly phytoplankton and rotifer cultures – which must be carefully synchronized with larval stocking densities and developmental timelines.

FIGURE 7.3
Schematic setup and photograph of a larval tank surface skimmer
(a) illustration that schematizes the functioning of the surface skimmer; and (b) photo of the surface skimmer and the movable elbow tube for removing biofilm from the surface of the rearing tank. The airflow pushes the surface biofilm into the surface skimmer, and a movable tube allows for the removal of the biofilm



7.1 PREPARING THE LARVAL REARING SYSTEM

Prior to larval transfer, all rearing units and associated equipment – including tanks, filtration systems, aeration lines, air stones, siphons and mesh screens – must be thoroughly cleaned using a suitable detergent and subsequently disinfected with a sodium hypochlorite (NaOCl) solution at a concentration of 500 ppm. After disinfection, all components should be carefully rinsed with clean freshwater or seawater to remove residual disinfectant before use.

When larval rearing is conducted under RAS conditions, the system must be operated at least 30 days before the start of larval rearing to ensure adequate colonization and stabilization of the biological filters by nitrifying bacteria. During this conditioning period, water quality parameters should be regularly monitored to confirm full biofilter functionality and system stability.

All consumables and operational materials required for larval rearing must be prepared in advance and readily available. These include live feeds (e.g. *Artemia* cysts), formulated microdiets for weaning, and all equipment necessary for larval handling, feeding, grading and routine husbandry. Proper advance preparation is essential to ensure uninterrupted operations and to minimize stress and mortality during the critical early larval stages.

7.2 ENVIRONMENTAL PARAMETERS FOR LARVAL REARING

Because information on the optimal photoperiod for *M. cephalus* larvae is limited, rearing units should operate under a natural autumn light regime, typically 12L:12D or 14L:10D, with a controlled light intensity of 600–1 000 lux. These conditions provide sufficient illumination for prey detection while preventing light-induced stress. One study reported successful larval rearing under continuous illumination (24L:0D) during the first 19 DPH, after which the photoperiod was reduced to 12L:12D. This approach may enhance prey detection during early feeding but should only be implemented in hatcheries with prior experience using continuous light, as it increases the risk of stress and behavioural disruption if not carefully managed. Grey mullet larvae exhibit strong light sensitivity: phototaxis develops by 4 DPH, and diel vertical migration patterns appear by 6 DPH. Feeding occurs exclusively during daylight hours. Larvae avoid intense light but are attracted to dim illumination in the range of 600–1 400 lux. Excessive or uneven lighting within rearing tanks should be avoided.

Natural seawater with a salinity between 32–41 ppt is recommended, as this range supports normal buoyancy regulation and vertical migration. Temperature control is essential during all larval stages. Newly hatched larvae through metamorphosis tolerate 19–29 °C, although a narrower range of 20–24 °C is recommended for optimal growth and survival. During live-prey feeding, when water exchange rates are reduced, temperature stability becomes critical, and fluctuations greater than 0.5 °C within 24 hours must be avoided.

Gentle aeration should be applied to each tank to maintain adequate oxygenation and promote the homogeneous distribution of live prey throughout the water column. Excessive aeration or strong currents must be avoided, as these can damage larvae or hinder feeding behaviour. Water recirculation rates must be adjusted according to larval age and feeding phase. During the first six DPH, recirculation rates of 2–5 percent/day are adequate. From approximately 22 DPH, recirculation should be increased to 15–20 percent/day, rising to 25 percent during weaning. Water recirculation should be suspended during each live-prey administration and reactivated in the evening after the final feeding to remove uneaten prey and stabilize water quality.

Water renewal rates must also be adapted to the type of live prey used. A nightly renewal of approximately 15 percent is sufficient during the rotifer and *Artemia* nauplii feeding phases. When enriched *Artemia* metanauplii are introduced, renewal should be increased to 40–60 percent to maintain stable water quality and remove uneaten prey. Outlet filters must be matched to the prey type: mesh sizes of 200–250 µm for rotifers and 500 µm for *Artemia* metanauplii. Each tank should be equipped with a complete set of mesh filters of different sizes. Filters must be rinsed after use, disinfected, and stored in a dry location to avoid cross-contamination and ensure proper water flow during subsequent operations.

7.3 FEEDING LARVAE

Newly hatched *M. cephalus* larvae are initially carnivorous and rely exclusively on zooplankton during early development. As they progress toward metamorphosis, they become omnivorous and gradually acquire the capacity to digest plant material. During the first ten days post-hatching, the highest rearing performance is obtained using a mixed feeding regime combining zooplankton (rotifers, copepods, *Artemia*) with phytoplankton species such as *Chlorella*, *Tetraselmis* or *Isochrysis*.

The application of the green-water technique is recommended, primarily to maintain the nutritional quality of zooplankton – particularly their omega-3 PUFA content – and to lower light intensity within the tank, improving prey visibility and capture efficiency. Additional documented benefits include the provision of essential micronutrients, the release of appetite-enhancing metabolites, and stabilization of the microbial community within the rearing water and larval gastrointestinal tract.

Feeding capability in *M. cephalus* larvae is closely linked to visual development. At hatching, larvae possess relatively large eyes that become pigmented within the first three days. Early visual acuity is limited, as the retina contains only a single photoreceptor type and is capable of detecting only coarse prey movements. Larvae are unable to rapidly adapt to abrupt light-dark transitions during this period. For this reason, dark-coloured tank surfaces are recommended to increase contrast between prey and background, thereby facilitating capture and improving early feeding success.

7.4 FIRST FEEDING

Initiation of exogenous feeding in *M. cephalus* requires the provision of prey that larvae can detect, capture and ingest efficiently. First-feeding larvae possess relatively large mouths but limited capture success; therefore, initial prey must fall within the 50–120 µm size range. As larvae grow, acceptable prey sizes increase rapidly. Maintaining an appropriate ratio of prey to larval density is critical, as both insufficient and excessive prey densities can compromise survival and growth. A high initial density of rotifers (>10 ind./ml) is recommended because newly feeding larvae show low hunting efficiency. Although natural zooplankton and copepods offer superior nutritional and behavioural characteristics, their mass culture remains operationally demanding; thus, rotifers (*Brachionus* spp.) constitute the standard first prey.

Rotifers provide several advantages: a suitable size range (100–250 µm), slow swimming behaviour that facilitates capture, rapid parthenogenetic reproduction, and tolerance to high-density culture conditions. A mixture of *B. plicatilis* (L-type) and *B. rotundiformis* (S-type) in a 1:1 ratio, supplied at 8–12 ind./ml, supports early-stage larvae during the period when mouth opening, visual acuity, and feeding coordination are developing. Rotifers must be enriched with omega-3 fatty acids using commercial enrichment products (e.g. Multigain®, Red Pepper®) or microalgae such as *Isochrysis galbana*. Enriched rotifers are introduced at 2–3 DPH, once the mouth and anus have opened, eye pigmentation has become funcional, and yolk sac resorption is underway. Rotifers remain the primary live prey until approximately 25 DPH (Figure 7.4).

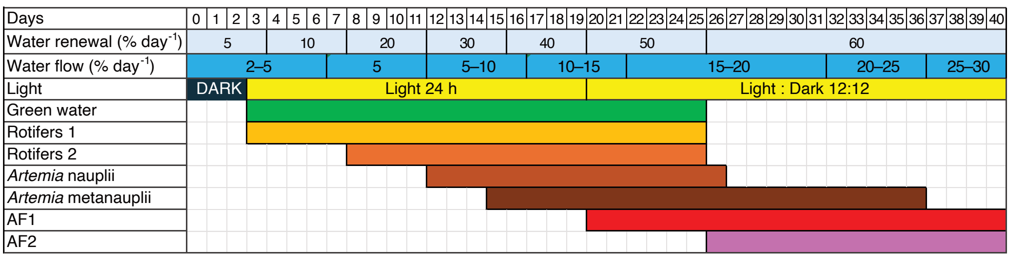
Throughout the early feeding phase, green-water conditions must be maintained to enhance prey visibility, stabilise rotifer nutritional quality, and support a favourable microbial environment. Phytoplankton species such as *Nannochloropsis* spp., *Isochrysis* spp. or *Tetraselmis* spp. should be provided at concentrations of 700 000, 350 000 and 200 000 cells/ml, respectively. When live microalgae are unavailable, concentrated commercial phytoplankton preparations (e.g. NannoStar Green, AlgaSpring, Netherlands) can be applied following manufacturer instructions usually at volumes ranging from 30–70 ml/m³ and continued until the end of rotifer feeding. In addition to enhancing visual contrast, phytoplankton contributes essential micronutrients and helps limit high-intensity light peaks within rearing tanks.

Newly hatched *Artemia* nauplii are introduced at approximately 12 DPH at an initial density of 0.3 ind./ml and co-fed with rotifers until around 26 DPH. From 16 to 36 DPH, enriched *Artemia* metanauplii are added, with density gradually increased to a maximum of 0.5 ind./ml as mouth gape and digestive capacity expand. To retain their fatty acid profile, enriched *Artemia* used for multiple daily feedings (2–6 times daily) must either be held in chilled seawater at approximately 4 °C or enriched and harvested twice daily to ensure that freshly enriched metanauplii are always supplied.

Weaning onto artificial microdiets begins at approximately 20 DPH, with feed particle size and quantity increased progressively in line with larval development (Figure 7.4; Table 7.3). Rations should be delivered in at least six daily feedings to maintain continuous feeding activity. Prey size remains a major determinant of feeding selectivity: larvae <10 DPH prefer rotifers, whereas larvae >15 DPH show increasing preference for *Artemia*. Early-stage larvae are unable to ingest *Artemia* nauplii (500–800 µm) efficiently due to their large size and rapid escape response, while rotifers eventually become too small to meet the dietary requirements of older larvae. Thus, prey size progression must track larval morphological development.

FIGURE 7.4

***Mugil cephalus* larval feeding protocol: timeline of live prey and microdiet administration**



Note: Rotifers 1: enriched with microalgae; Rotifers 2: enriched with commercial enrichment products.

FIGURE 7.5
(a) Digestive tract of *M. cephalus* larva (4 DPH) presenting rotifer mastax in the intestine (highlighted by red circles); and (b) digestive tract of a larva (4 DPH) fed with microalgae enriched with rotifers (arrows indicate undigested rotifers)

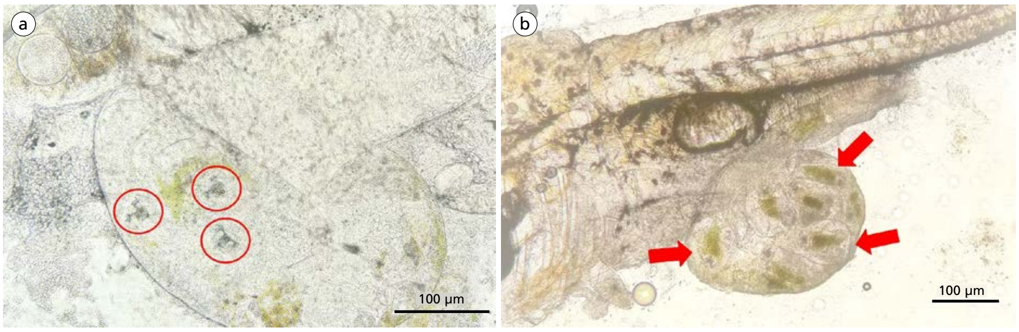
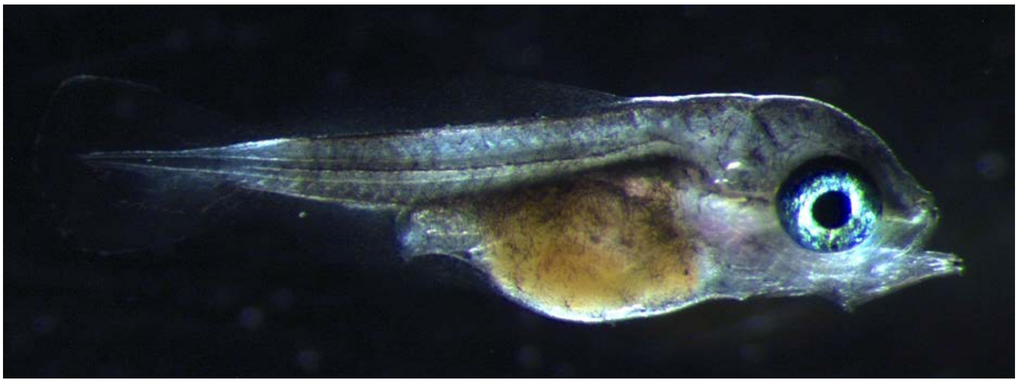


FIGURE 7.6
***Mugil cephalus* larva at 16 DPH fed with *Artemia* nauplii**
See orange-coloured material in the digestive tract



The ratio of larvae to prey and initial larval stocking density significantly influence growth and survival. Historical studies recommended 10–20 larvae/L, whereas recent trials suggest 100–200 larvae/L can achieve survival rates >20 percent at 40 DPH.

Environmental parameters, including temperature and dissolved oxygen, must be monitored daily. From 12 to 16 DPH, tanks should be cleaned every 2–3 days by siphoning dead larvae, uneaten prey and residual feed. Nitrite, nitrate and ammonia levels, as well as DO, should be measured daily. Recommended parameters are: ammonia 0 mg/L, nitrite <0.1 mg/L, nitrate <50 mg/L and DO ≥6 mg/L. Water renewal and recirculation rates should be adjusted accordingly.

Feeding performance should be monitored periodically, particularly during transitions between prey types. Larvae can be sampled to assess gut fullness. For rotifers, mastax counts (Figure 7.5a) provide an estimate of predation efficiency, while *Artemia* presence in the gut (Figure 7.5b and Figure 7.6) can be used to adjust prey densities and feeding strategies.

7.5 LARVAL WEANING

The introduction of artificial diets begins at approximately 20 DPH (see Figure 7.4; Table 7.3), in parallel with the continued provision of enriched *Artemia* metanauplii. At this stage, only small quantities of microdiet should be offered initially, increasing gradually once larvae are observed actively ingesting the feed. Hand-feeding with a plastic spoon is recommended during the first days of weaning, preferably during the early morning after the overnight fasting period and before metanauplii are introduced.

Delaying the distribution of *Artemia* encourages larvae to sample and accept the artificial diet.

Although mullet are detritivores and herbivores in the wild, hatchery production requires conditioning larvae to consume high-protein artificial feeds. No species-specific larval diet for *M. cephalus* is currently available; therefore, high-quality commercial weaning diets formulated for marine species such as European seabass (*Dicentrarchus labrax*) or gilthead seabream (*Sparus aurata*) – particularly those rich in omega-3 fatty acids – are recommended. Microdiets should be water-stable and semi-floating to align with larval feeding behaviour and minimise nutrient leaching.

Feed particle composition is described in Table 7.2. Size and total ration should be increased progressively according to larval growth and feeding activity ensuring that larvae have continuous access to appropriately sized particles throughout the weaning period. Weaning feeding protocol is described in Table 7.3 including the time for changes in live feed (*Artemia* nauplii and metanauplii) and weaning microdiets.

End-of-day assessment of feed residues is essential for adjusting ration size during weaning. Visible accumulation of uneaten microdiet indicates overfeeding and requires an immediate reduction in feed delivery, whereas consistently clean tank bottoms suggest the need for a gradual increase in ration. Once most larvae reliably ingest the artificial diet, automatic feeders may be introduced. During this transition, enriched *Artemia* metanauplii can be released near the feeder outlet to stimulate feeding and help larvae associate the automatic feeder with food.

With the shift to automatic feeding, water renewal rates must be progressively increased to remove fine feed particles and faecal waste, maintain low ammonia and nitrite concentrations, and ensure dissolved oxygen remains near saturation. Tanks should also be cleaned more frequently to prevent organic build-up and associated microbial deterioration of water quality.

After larvae pass the early critical period, husbandry challenges diminish. Weaned larvae typically feed vigorously, grow rapidly and show improved resilience. By the third week post-hatching, scale formation begins and larvae display coordinated schooling behaviour – an indicator of normal development.

Handling must be avoided before 40 DPH, as larvae at this stage are highly susceptible to stress and mortality. For operational planning, the rearing cycle should be treated as a 50-day process, with any transfer, grading or handling deferred until juveniles are older than 40 DPH and exhibit consistent schooling and greater physical robustness.

TABLE 7.2
Larval feed composition

Fishmeal
Hydrolysed aquatic invertebrates
Lecithin
Wheat gluten
Algae
Fish oil
Maize starch

Analytical constituents	%
Crude protein	59.0
Crude fat	14.0
Crude fibre	0.20
Crude ash	13.0
Phosphorous	2.00
Calcium	1.50
Sodium	0.70

Additives
Vitamin A (23 000 IU/kg) and Vitamin B3 (2 800 IU/kg)

Trace elements	mg/kg
Iron (ferrous sulphate monohydrous)	100
Iodine (calcium iodate anhydrous)	5
Copper (cupric sulfate pentahydrate	10
Manganese (manganese sulfate monohydrate)	40
Zinc (zinc chelate of amino acids hydrate)	100

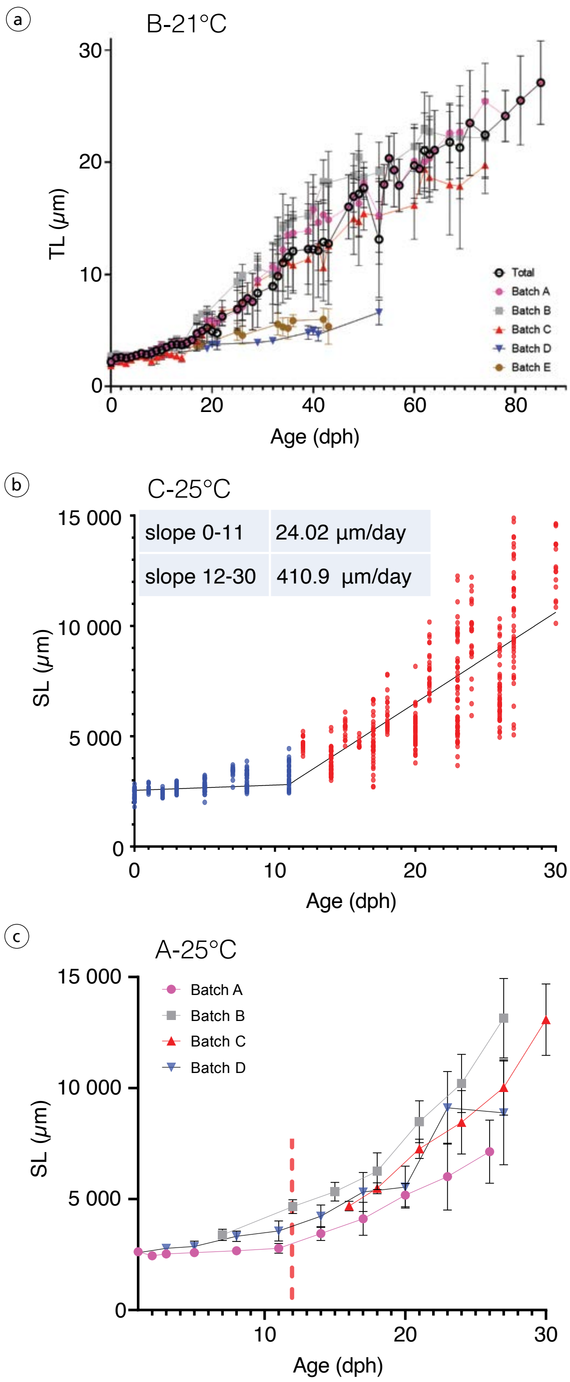
TABLE 7.3
The administration of Gemma micro 300 continues up to 100 DPH (juvenile rearing phase)

Timing	Feed type	Feed size (µm)	Commercial feed examples
12–22	*Artemia* nauplii	125–150	--
16–40	Enriched *Artemia* (metanauplii)	140–170	--
20–34	Dry feed	100–200	Gemma micro 150 (Skretting, France)
26–40	Dry feed	200–500	Gemma micro 300 (Skretting, France)

FIGURE 7.7

Growth pattern of grey mullet larvae

(a) Age-standard length (SL) relationship in for larval batches reared at 25 °C (the red dashed line indicates the approximate onset of metamorphosis); (b) Age–total length (TL) relationship for five batches reared at 21 °C (each batch is shown in a different colour); and (c) Linear regression of SL by age, illustrating the biphasic growth pattern: blue dots represent larvae 0–11 DPH (pre-metamorphosis) and red dots represent larvae 12–30 DPH in metamorphosis



Source: For a) and b) - Oz, I., Gajbhiye, D.S., Columbus-Shenkar, Y.Y., David, L. & Golan, M. 2022. Non-uniform metamorphosis underlies different development trajectories in hatchery-reared flathead grey mullet (*Mugil cephalus*). *Frontiers in Marine Science*, 9: 967984; for c) data provided by Dario Vallainc (IMC).

7.6 GROWTH PATTERNS

Newly hatched *M. cephalus* larvae display a highly uniform size distribution, with an average standard length (SL) of 2.36 ± 0.22 mm (Figure 7.7a). By the time the yolk sac and oil globule are fully absorbed, mean SL reaches approximately 3.2 ± 0.48 mm. Growth is strongly temperature-dependent: at 21 °C, larvae progress through early stages more slowly than at 23–25 °C (Figure 7.7b).

Two distinct growth phases characterize early ontogeny (Figure 7.7c). During the pre-metamorphic stage – extending to the onset of metamorphosis at approximately 12 DPH at 25 °C (Stage E) – growth is gradual, with an average increase of ~24 µm/day in standard length. Once metamorphosis begins, growth accelerates markedly: rates increase by roughly 17-fold, reaching approximately 411 µm/day.

The pre-metamorphic period is notable for its consistency, both within individual batches and across rearing cycles, with relatively low variance in larval length. In contrast, the metamorphic period exhibits a substantial increase in size heterogeneity (Figure 7.7a and Figure 7.7b). When larvae are reared at 25 °C, this divergence becomes evident from around 12 DPH onward, reflecting differences in feeding efficiency, metabolic capacity and developmental progression. Size variability peaks around 18 DPH – during metamorphic activity – where uniformity is four to five times lower compared to early development.

Pre-metamorphosis typically spans from first feeding (~3 DPH) to approximately 10–12 DPH and encompasses key developmental milestones such as swim-bladder inflation, fin fold differentiation, early pigmentation and gut maturation. This phase represents the most vulnerable period in hatchery rearing and demands strict management of live feed delivery, water quality and environmental stability.

Metamorphosis in mullet larvae begins at ~10–12 DPH, with completion of fin and scale formation, development of a functional stomach and progressive transition from live prey to artificial microdiets. By 35–40 DPH, juveniles have stable feeding behaviour, improved resilience to handling and are suitable for transfer to nursery systems.

7.7 DEVELOPMENT AND GROWTH CORRELATION

A clear understanding of how growth relates to developmental progression is essential for effective hatchery management, particularly when planning feeding transitions, adjusting water-exchange rates, and scheduling weaning. To support operational decision-making, the relationship between larval age, standard length (SL) and developmental progression was examined (Figure 7.8).

Both age and SL increase as *M. cephalus* larvae develop, but the two parameters differ markedly in their reliability as indicators of stage. Age shows wide variation within each stage (Figure 7.8a), demonstrating that larvae of the same chronological age may be at different developmental points. By contrast, SL shows comparatively low variability within stages (Figure 7.8b), indicating that developmental transitions are primarily size-dependent rather than age.

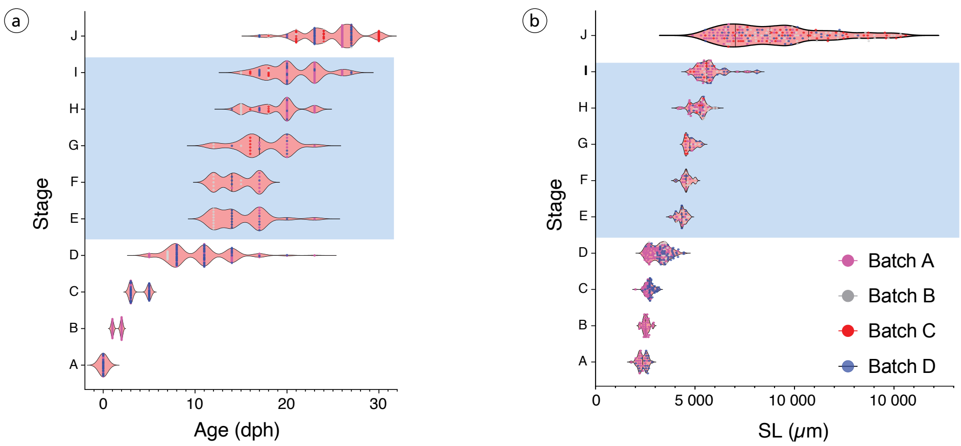
Analysis of multiple batches confirms that larvae must reach a critical size of approximately 4.3 mm SL before initiating metamorphosis. Some individuals remain in the post–oil globule absorption stage until they reach this threshold, regardless of how many days post-hatching they have accumulated. This explains the increasing size dispersion observed after metamorphosis (Section 7.6).

For hatchery operations, SL should therefore be used as the primary indicator of developmental readiness rather than age alone. Reliance on SL allows more accurate timing of prey transitions (e.g. rotifer to *Artemia*), introduction of microdiets, modification of water-exchange rates and system hygiene routines, and planning of handling and grading once larvae reach the juvenile stage. Integrating SL-based monitoring into routine assessments substantially improves the precision and consistency of rearing protocols.

FIGURE 7.8

Relationship between age, standard length (SL) and developmental stage in *Mugil cephalus* larvae

Graphs depict developmental stage as a function of age (a) and standard length (b). Each dot represents a single larva. Dots are color-coded according to the batch of individual larvae. Shaded area marks the metamorphosis period

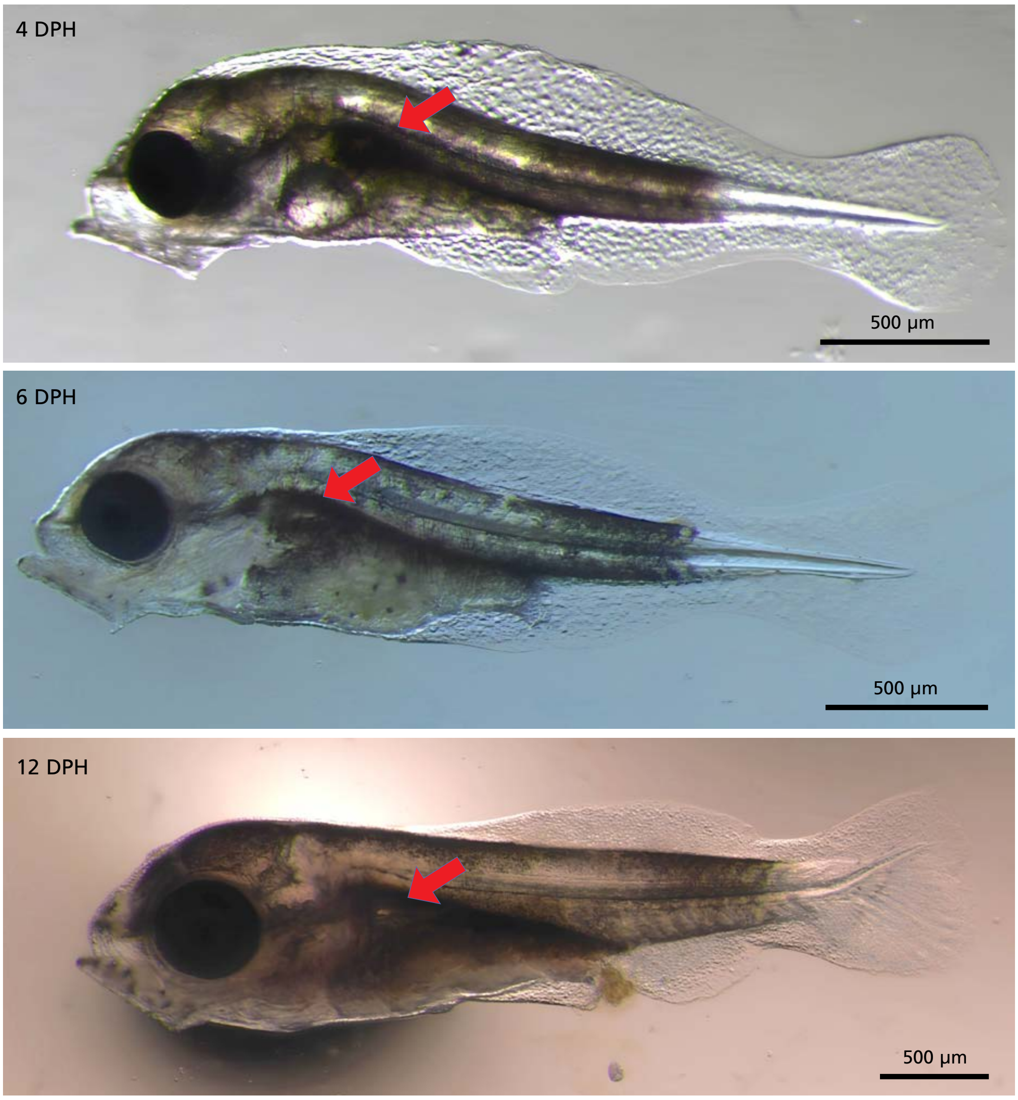


Source: Oz, I., Gajbhiye, D.S., Columbus-Shenkar, Y.Y., David, L. & Golan, M. 2022. Non-uniform metamorphosis underlies different development trajectories in hatchery-reared flathead grey mullet (*Mugil cephalus*). *Frontiers in Marine Science*, 9: 967984.

7.8 CONTROL OF SWIM BLADDER INFLATION

Successful swim bladder inflation is a critical developmental event in *Mugil cephalus* larvae and must be carefully managed during the early rearing period. The swim bladder forms as an invagination of the digestive tract (Figure 7.9) and enables larvae to achieve neutral buoyancy. Proper inflation reduces energy expenditure during swimming, improves stability in the water column, and enhances prey capture efficiency. Inadequate or failed inflation is a major cause of poor larval performance in many

FIGURE 7.9
Development of the swim bladder (red arrow) in grey mullet larvae at 4, 6 and 12 DPH



marine finfish species, including European seabass (*D. labrax*) and gilthead seabream (*S. aurata*), and is associated with reduced growth, erratic swimming behaviour, and a high incidence of skeletal deformities.

In grey mullet, initial inflation typically occurs between 3–5 DPH, shortly after mouth opening and the onset of exogenous feeding. During this period, larvae rise to the surface and ingest a small volume of air. Complete and functional inflation is generally observed by 5–7 DPH under optimal hatchery conditions. This process occurs within a narrow developmental window; therefore, the integrity of the water surface must be strictly controlled. A clean, stable air–water interface is essential for successful inflation. Any oil film, foam, or surface debris inhibits air ingestion and leads to high rates of non-inflation.

Swim bladder inflation can be assessed using a simple buoyancy-based sorting technique. Larvae are anaesthetised with MS 222 (80 ppm) or clove oil (0.08 %). Individuals with inflated swim bladders become positively buoyant and float, while non-inflated larvae sink. This method provides a rapid and reliable means of monitoring batch quality and, if required, selectively retaining larvae with proper inflation.

Timing of swim bladder inflation in grey mullet fry

Initial inflation

- Occurs at approximately 3–5 DPH, immediately after the mouth opens and larvae begin exogenous feeding.
- Inflation is achieved by gulping air at the water surface.
- It is essential that the water surface is calm, clean, and free from oil films or excessive bubbles to facilitate air ingestion.

Complete inflation

- Typically achieved by 5–7 DPH under optimal hatchery conditions.
- At this stage, the swim bladder is fully functional, allowing larvae to maintain neutral buoyancy and regulate vertical position in the water column.

Hatchery management recommendations

As mentioned above, a clean water surface is essential to ensure proper swim bladder inflation. Larval rearing tanks should be equipped with surface skimmers or floating devices to remove debris and grease. Skimming can be performed continuously or intermittently during the inflation window, using a low-pressure air stream directed tangentially across the water surface, or manually with a beaker, soft paper, or the mobile discharge elbow system (Figure 7.3). Skimmers and collection devices must be inspected and cleaned several times per day to maintain optimal surface conditions. Failure to keep the water surface clean can compromise swim bladder inflation, negatively affecting larval buoyancy, feeding efficiency, growth, and survival.

7.9 DAILY ROUTINES DURING LARVAL REARING AND WEANING

During larval rearing, strict daily monitoring and maintenance of water quality is essential to ensure optimal growth and survival. Water temperature, salinity, and dissolved oxygen (DO) must be measured daily. Other parameters, including pH, nitrites, and ammonia, should be monitored at least 3–4 times per week, and adjusted as necessary to maintain recommended levels.

Feeding routines must be carefully managed. Live prey, including rotifers and *Artemia* nauplii, should be distributed after assessing prey density in the tank, allowing adjustments to maintain the optimal prey-to-larva ratio. Depending on larval age and feeding behaviour, live prey may be offered in two to three daily feedings.

During the weaning phase, dry feed should initially be administered by hand while observing larval behaviour to ensure acceptance. Once larvae consistently consume dry feed, automatic feeders may be employed, delivering feed at regular intervals. Care must be taken to ensure even dispersion across the water surface to maximize accessibility for all larvae.

All observations and measurements – including water quality, feed quantities, larval behaviour, and aeration settings – must be recorded daily in a dedicated data sheet. Routine tank maintenance should include siphoning the bottom to remove uneaten feed, debris, and dead larvae. Tanks should be cleaned every 2–3 days, or more frequently if elevated mortality is observed. Dead larvae should be counted and recorded.

In the event of high mortality, a systematic review of feeding rates, prey density, and water quality parameters should be conducted to identify and correct potential causes. Consistent adherence to these daily routines ensures healthy larval development, successful weaning, and high overall survival.

7.10 CONTROL OF GROWTH AND DEFORMITY RATES

Monitoring larval growth and identifying deformities are critical components of larval rearing and quality control in hatchery operations. Standard length (SL) is the preferred parameter for assessing growth, as it is rapid, reliable and easy to measure. To determine SL, larvae should be sampled randomly from the rearing tank and anaesthetised using MS-222 (80 ppm) or clove oil (0.08%). Once immobilized, transfer the larvae onto a glass slide and measure SL under a dissecting microscope with a calibrated ocular micrometre. Alternatively, photographs can be taken and analysed using image analysis software (e.g. ImageJ or Analysis) to obtain accurate length measurements. Measurements should be recorded for at least 20 ind./batch to obtain representative growth data.

Dry weight can also be used as a complementary growth parameter. Collect larvae in a fine mesh, rinse briefly with sterile freshwater, and place them on a pre-weighed cover slide. Dry the samples in an oven for approximately 12 h (i.e. during the night). Weigh the dried larvae together with the cover slide using a precise balance. A correlation between larval SL and dry weight can then be established to monitor growth performance.

Furthermore, deformity assessment provides important information about larval health, nutrition and rearing conditions. Early juveniles can be examined using the double staining method to identify skeletal and soft-tissue deformities, including abnormalities in the jaw, head, opercula, vertebral column and fins. Deformities may indicate issues with nutrition (e.g. vitamin deficiencies), water quality or broodstock quality.

At commercial scale, deformities are typically addressed during size grading prior to transferring juveniles to on-growing systems. Juveniles are visually inspected, and those exhibiting deformities or abnormal swimming behaviour are removed. Such individuals are counted and discarded because they exhibit slower growth, higher susceptibility to disease and lower market value. Visual sorting can be combined with mechanical grading using fixed-bar or automatic roller graders to separate juveniles by size while identifying abnormal individuals efficiently.

Regular monitoring of SL, dry weight, and deformities ensures early detection of rearing issues, allowing hatchery staff to implement corrective actions to maintain high-quality fry production.

7.11 STAFF DAILY OPERATIONS

Daily operations in the larval culture unit are critical to ensure optimal growth, survival, and larval quality. Staff must follow a structured routine, including environmental monitoring, feeding management and larval health checks (Table 7.4).

Environmental monitoring and water management

- Measure key water quality parameters daily, including temperature, dissolved oxygen and water flow. Nitrite and ammonia concentrations should be measured 3–4 times/week.
- Adjust water renewal rates according to feeding schedules, recording the flow rate in the control log.
- Inspect and maintain tank equipment, including outflow meshes, and change mesh size according to the live prey being administered.

Feeding and prey management

- Sample the rearing water to assess live prey concentrations (rotifers and *Artemia* nauplii/metanauplii) and adjust feeding rates accordingly.
- Distribute live prey in 2–3 doses/day, depending on larval age and feeding behaviour.

TABLE 7.4
Daily data recording sheet for larval rearing

Tank:
Stock N°:
Incubation date:
Hatching date:
% Hatching:
Larvae initial N°:

Day/month	Age	T (°C)	S ‰	O_2 (mg/L)	pH	NO_2 (mg/L)	NH_2 (mg/L)	Flow rate (L/min)	Volume (L)	Phytoplankton (L)	Rotifers/ml (initial)	Rotifers/ml (final)	Rotifers added	Nauplii/ml (initial)	Nauplii/ml (final)	Naupli added	Dead larvae	SL (mm)	Observations/ cleaning

- For weaning larvae, record the amount, size and type of dry feed administered. Feed should initially be distributed by hand and progressively transitioned to automatic feeders once the larvae accept the diet.

Larval health and behavioural observations

- Monitor larval behaviour daily, noting swimming activity, buoyancy, schooling and prey capture efficiency.
- Periodically assess feeding performance, particularly during prey transitions, by sampling several larvae under a microscope. Examine gut contents for rotifers (mastax, lorica, eggs) and *Artemia* and verify swim bladder inflation. Record findings in the larval monitoring table.
- Count and record dead larvae, noting any signs of deformities, abnormal behaviour or other clinical indicators.

Record keeping

- Maintain detailed daily logs (Table 7.4) including tank identification, stock numbers, incubation and hatching dates, hatch percentage, larval counts, environmental parameters, feeding quantities and cleaning activities.
- For weaned larvae on dry feed, maintain a separate log (Table 7.5) recording feed type, quantity, pellet size, mortality, growth measurements and any grading or separation events.

TABLE 7.5
Daily data recording sheet for the weaning phase

Tank:
Date of Transfer:
Initial Number:
Origin:

Day/month	T (°C)	S (‰)	O_2 (mg/L)	Flow rate	Volume	Dry feed (mark and size)	Dry feed (g/day)	Dead fish	Number	Weight (mg)	Observations/ cleaning

Tank maintenance

- During the larval phase, tanks should be cleaned every 2–3 days, or more frequently if mortality is high.
- During weaning, increase cleaning frequency and replace outflow meshes with larger sizes (500–1 000 µm) once the larvae are fully adapted to dry feed.

7.12 TRANSFERRING FISH FROM LARVAL TO JUVENILE SECTION

Grey mullet juveniles become sufficiently robust for handling and transfer between 40–50 DPH, at a size of approximately 1 cm TL. At this stage, fry can be moved from the larval rearing system to larger juvenile tanks ranging from 2 000–20 000 L. Transfers must be performed carefully to minimise stress, avoid physical injury, and prevent abrupt environmental transitions.

Preparation of juvenile rearing tanks

Before transferring fish, prepare the receiving tanks as follows:

- Fill each tank with seawater adjusted to the same temperature and salinity as the larval tanks.
- Ensure proper water inflow, stable aeration and lighting conditions (600–1 000 lux, natural or artificial).
- Install outlet screens of 1 000 µm.
- Verify that water flow, dissolved oxygen, and tank hygiene meet the required operational standards.

Transfer procedure from larval tanks ≥2 m³ to juvenile tanks

1. *Deploy a soft seine net* – Place a knotless, soft nylon seine net (2 mm mesh) inside the larval tank and gently encircle a portion of the fry. Avoid capturing excessive numbers at once to prevent crowding and stress.
2. *Form a submerged transfer pouch* – Close the net and lift both ends to the surface, creating a submerged bag attached to the tank rim. Maintain the fish fully underwater at all times.
3. *Collect fry in a water-filled bucket* – Immerse a plastic bucket into the net pouch, gently allowing water and fry to enter without exposing fish to air.
4. *Transfer fish to the juvenile tanks* – If the receiving tank is adjacent, pour the bucket contents directly into the tank. If transport is required, pour the fry gently into a wheeled, aerated transport container, maintaining continuous oxygenation.
5. Repeat the procedure until the majority of fry have been removed.
6. *Collect remaining fish* – The last remaining fry – typically the strongest fish – can be collected through the bottom drain or by means of a dip net.
7. *Clean the larval tank* – Once emptied, thoroughly rinse and clean the tank before allowing it to dry.

Transfer procedure from larval tanks <2 m³ to juvenile tanks

When working with small-volume larval tanks, the following method is recommended:

1. *Prepare a 100-L transfer tank* – Fill with 30–50 L of seawater at the same salinity and temperature of the larval rearing system. Provide aeration via an air stone.
2. Reduce larval tank volume to approximately one-third to concentrate fish.
3. Collect small numbers of fish (10–15 fry) using soft hand nets and place them into the 100-L transfer tank until reaching 100–200 juveniles.
4. Position the transfer tank near the juvenile system.
5. Transfer to juvenile tanks – Using soft hand nets or a semi-spherical transfer bowl with handles release small groups of fish gently into the receiving tanks (Figure 7.10).

FIGURE 7.10
Hand-held bowl used for gentle transfer of early juveniles from small-volume tanks to the juvenile rearing system

8. Post-larval culture

8.1 FRY CULTURE

Following metamorphosis, grey mullet juveniles are transferred to the fry-rearing section of the hatchery, where they are maintained at a maximum initial density of approximately 20 fish/L. Fish entering this phase are typically 40–50 DPH, and they remain in this section until about 90–120 DPH, by which time they generally reach 0.3–0.7 g BW. The fry stage represents a critical developmental window in which uniform growth, skeletal development, and robust health must be consolidated before transfer to the on-growing sector.

The layout of the fry-rearing system is conceptually similar to that of the larval section but incorporates larger tanks to accommodate increasing biomass and provide improved hydrodynamic conditions. Tanks are preferably round, which ensures a uniform circular flow pattern, reduces the accumulation of solid wastes, and promotes consistent swimming activity. The operational capacity of fry tanks typically ranges between 2–10 m^3 (Figure 8.1), depending on the scale of production and the degree of water renewal available.

FIGURE 8.1
Mullet juvenile rearing system

The choice of water supply and treatment system depends strongly on environmental conditions. In areas where water temperature and quality remain stable and within the optimal range for juvenile grey mullet, a simple flow-through system equipped with mechanical filtration (50–100 µm) and UV sterilization is generally sufficient to maintain good rearing conditions. However, during periods of low environmental temperature, or when source water is of variable or insufficient quality, the fry sector should be operated as a RAS or as a semi-closed system with partial recirculation. These systems allow for precise control of temperature, improved water conservation, and efficient removal of metabolic wastes through a biofilter, thereby ensuring stable water parameters throughout the culture cycle.

The outlet screens of the fry tanks require progressive adjustment as the juveniles grow. Mesh sizes should typically be increased in stages to 1 mm, then 2 mm, and finally 3 mm, ensuring that the fish cannot escape while maintaining adequate water exchange. Because screens are prone to clogging from suspended solids, uneaten feed, and natural biofilm formation, they must be inspected throughout the day and routinely replaced. As a standard procedure, screens should be removed every evening or sooner if they show reduced flow. After removal, they need to be thoroughly rinsed with tap water, soaked overnight in a hypochlorite solution for disinfection, and allowed to dry completely before being returned to use. Each tank should have at least one full set of spare screens available to ensure uninterrupted operation.

From 80–90 DPH, juveniles begin to display pronounced jumping behaviour, particularly during periods of high activity or when startled. To prevent fish from escaping and becoming injured, all fry tanks must be equipped with securely fitted cover nets made of approximately 0.5 cm mesh. These nets must be positioned so that no gaps remain along the tank perimeter.

8.2 FINGERLING CULTURE

At approximately 90–120 DPH, grey mullet juveniles measuring 2–4 cm TL and weighing 0.3–0.7 g BW are transferred from the fry-rearing area to larger tanks or nursery ponds with capacities typically ranging from 10–25 m^3. Prior to transfer, a grading procedure should be performed to achieve size homogeneity. When the fingerling facility is located at a distance from the fry section, juveniles must be transported using fish transport tanks or trucks equipped with aeration to minimise stress and maintain high survival.

During the fingerling phase, juveniles may be reared intensively until approximately 200 DPH, at which point they are ready for on-growing. Grey mullet at this stage are highly euryhaline and can be successfully cultured across a broad salinity range (0–40 ppt) and water temperatures within 20–25 °C, which represent the optimal conditions for growth. Dissolved oxygen must be maintained above 5 mg/L, and aeration should be dimensioned to support biomass increases throughout the cycle.

The choice of rearing system depends largely on environmental conditions, available space, operational budget, climatic constraints, and the level of technical expertise within the farm. Flow-through systems, RAS, and semi-closed systems with partial recirculation are all suitable. In temperate regions or during cold seasons, RAS or partially recirculated systems allow for reliable temperature control and stable water quality. Where environmental conditions are favourable, flow-through tanks or nursery ponds can be used effectively.

Round fibreglass tanks, installed either indoors or outdoors, are recommended. When combined with an appropriately positioned water inlet, this configuration promotes continuous swimming, enhances muscle and skeletal development, reduces sediment accumulation, and contributes to high welfare standards. Nevertheless, rectangular tanks and earthen ponds remain viable alternatives, provided they are maintained to a high standard. Regardless of tank or pond shape, surfaces that come into contact with water are best coated with an inert waterproofing material (e.g. gelcoat) to prevent abrasion and reduce pathogen retention.

Stocking densities during the fingerling phase should not exceed 2 fish/L, although final densities should be adjusted based on water quality, feeding rate, and system configuration. In flow-through units, recommended water exchange rates range from 5 to 10 tank volumes/day, with higher renewal corresponding to higher biomass or feeding levels. In RAS, maintaining stable chemical–physical parameters is essential to avoid stress and growth suppression.

Feeding strategies differ slightly between system types. In RAS, juveniles should be offered formulated diets – either pelleted or extruded – with >30 percent crude protein and >10 percent lipid, at a daily ration of 2.5–4 percent of biomass (BW), adjusted according to appetite. In flow-through systems, moist diets can also be used at similar feeding rates. Routine monitoring of growth, condition factor, and behaviour is essential for adjusting feed allocation and ensuring uniform performance across the population.

8.3 JUVENILE GRADING AND COUNTING

Grading is essential at 90–120 DPH to maintain a uniform juvenile population. Even when originating from the same egg batch, grey mullet juveniles exhibit considerable size dispersion, often resulting in fish of very different sizes co-existing in the same tank (Figure 8.2). This heterogeneity reduces feeding efficiency, increases competition, and negatively affects overall performance.

A grading operation separates the fry into homogenous size classes, ensuring that each group has a narrower size range and a more balanced feeding hierarchy. Differences in weight between the smallest and largest individuals within a graded class should not exceed 10–20 percent.

The recommended grading device is a floating PVC tray (45 × 20 × 15 cm) fitted with a stainless-steel or plastic bar grid. The spacing between the bars (typically 3–6 mm) must be selected according to juvenile girth. When placed in the tank, the base of the grading tray remains submerged, allowing smaller fry to pass through while larger ones remain above (Figure 8.3).

FIGURE 8.2
***Mugil cephalus* juvenile at 100 days post-hatch, where size differences among individuals are evident**

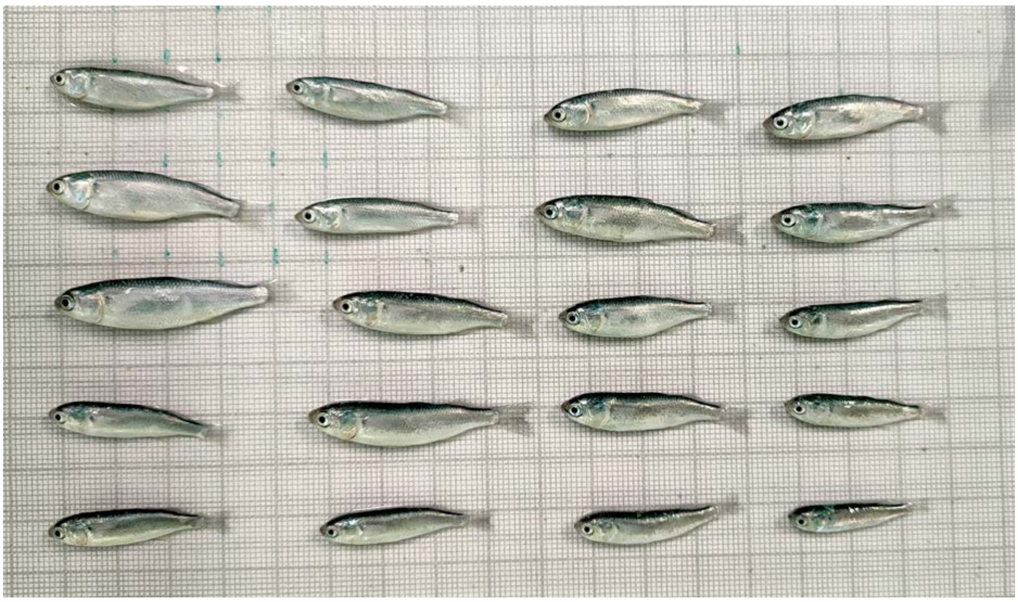
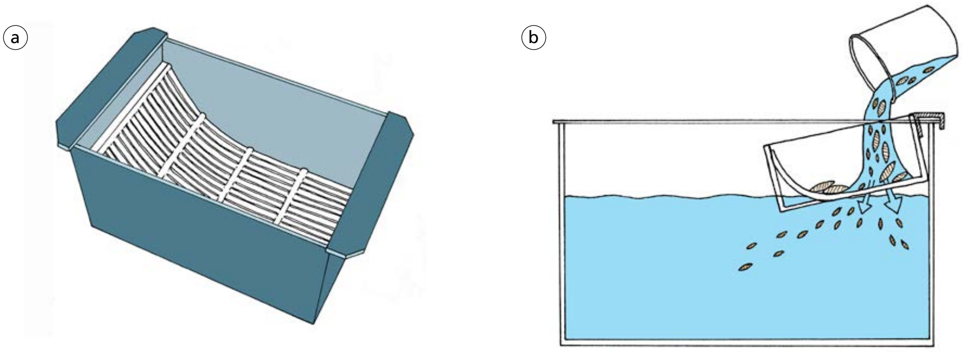


FIGURE 8.3
Juvenile *Mugil cephalus* grader used for size selection
Fish are gently poured into the grader (a), with smaller individuals passing through the bar spacing and moving into the tank below, while larger fish are retained for transfer (b)



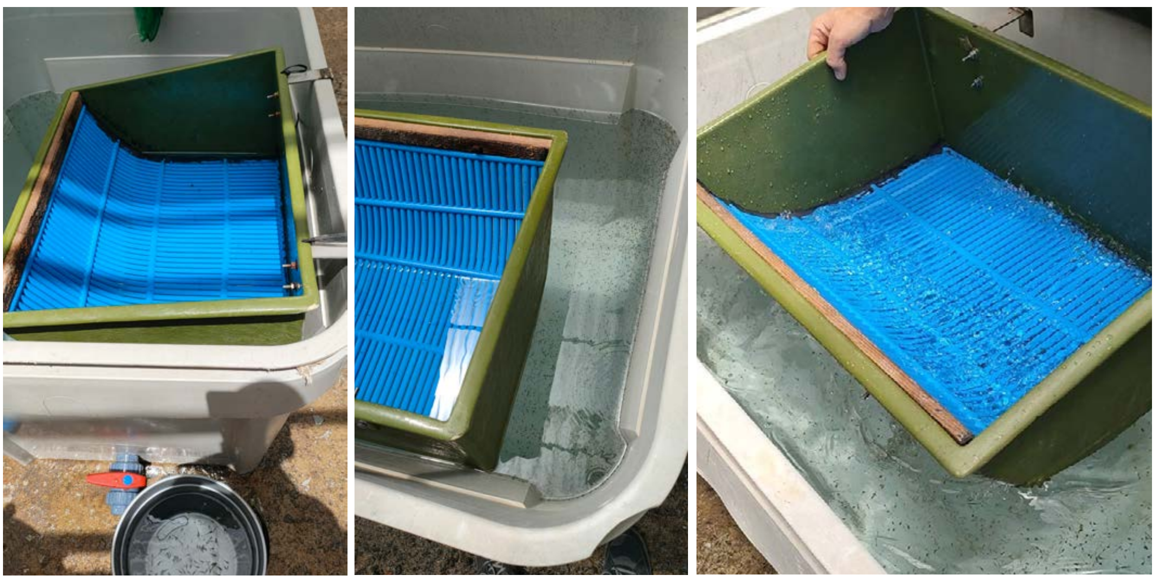
Before selecting a final grader size, a preliminary trial with a sample of at least 100 juvenile fish should be performed to confirm that the mesh spacing produces the desired size classes. Average weight for each class should be determined using the juvenile handling procedures described later in this chapter.

Grading operations require the following equipment: tanks prepared for receiving graded juveniles (or a transport truck if relocation is needed), two or more graders of appropriate bar spacing, one or more 100-L plastic tanks filled with system water, hand nets or bowls with handles, and a 100-L tank placed on an electronic scale with a capacity of approximately 100 kg and a measurement resolution of 5–10 g for counting fish by weight.

The grading procedure is carried out as follows (Figure 8.4):

1. Close the tank inlet and reduce water volume to approximately one third, ensuring continuous aeration.

FIGURE 8.4
Grey mullet juvenile grading operation
Juvenile fish of different sizes are transferred from a bucket into the grader positioned inside tank filled with water. Smaller fish pass through the calibrated bars and move directly into the tank below, while larger individuals are retained within the grader and subsequently transferred to a separate tank



2. Insert the graders into the tank.
3. Collect 10–20 fry at a time using hand nets and transfer them to a bucket or small tank.
4. When 100–200 fry have been collected, pour them gently into the grader.
5. Fish retained in the upper section of the grader are transferred into the tank positioned on a scale; their number is calculated by subtracting water tare weight.
6. Transfer counted juveniles to their designated tank or transport container.
7. Repeat the procedure for each size class using graders with different bar spacings.
8. For smaller fish, rearing should continue until they reach the appropriate size for grow-out.

This procedure standardizes size classes, improves feeding efficiency, and supports uniform growth during the early juvenile phase.

8.4 FEEDING

During the weaning stage (20–40 DPH), *M. cephalus* juveniles transition from a carnivorous to an omnivorous digestive physiology, reflecting changes in the ontogeny of digestive enzymes. Feeding regimes at this stage must accommodate this shift to ensure optimal growth and survival.

In the absence of species-specific commercial feeds, an ideal diet can be provided using a 1:1 wet weight mixture of a high-protein microencapsulated starter feed designed for carnivorous species and the dried macroalga *Ulva lactuca*. Alternative protein sources include soy flour or highly digestible vegetable flours. Dietary lipids should comprise 5–10 percent of the feed, sourced from fish oils and/or vegetable oils such as soybean or linseed oil. Carbohydrates should account for 30–40 percent of the diet, preferably from digestible sources such as corn starch, wheat, or barley, as mullet efficiently utilize complex carbohydrates.

Formulated feeds intended for European seabass (*D. labrax*) and gilthead seabream (*S. aurata*) can be used as a practical alternative from 0.1 g BW (approximately 2 cm TL). Recommended particle sizes and feeding periods include: Gemma Micro 150 (100–200 µm) from 20–34 DPH, Gemma Micro 300 (200–400 µm) from 26–60 DPH, Gemma Micro 500 (400–600 µm) from 50–100 DPH, followed by M 0.8 Gemma Silk (600–800 µm) from 80–200 DPH.

An alternative protein source is zooplankton biomass meal, which has been shown to support superior growth performance, feed conversion, gut health, and overall profitability compared to traditional fishmeal-based diets.

Feeding should be performed 4–6 times/day, providing 2.5–4 percent of the biomass per feeding, with adjustments based on observed appetite and growth. Frequent feeding promotes feed intake and reduces competition during this critical developmental stage.

8.5 MANAGEMENT OF THE JUVENILE SECTION

Management of the juvenile rearing unit follows principles similar to those applied during larval culture, but with increased emphasis on maintaining size uniformity, ensuring equitable access to feed, meeting higher oxygen demands, and preventing disease outbreaks associated with increased biomass and stocking density.

Adequate staffing is essential and should include a designated unit supervisor supported by a sufficient number of trained technicians to cover daylight operating hours. Personnel requirements depend on stocking densities, system configuration, and the degree of automation. All staff must be trained in standard operating procedures, biosecurity protocols and data recording (Appendix VI). Accurate and consistent record keeping is important for traceability and for identifying deviations from normal production performance at an early stage.

Daily operations must be structured and coordinated to ensure continuity of care. Consumables such as feeds, chemicals, nets, and filtration materials should be inventoried regularly, and strict hygiene standards must be maintained throughout the unit to minimize the risk of cross-contamination between tanks or production batches. Equipment should be cleaned and disinfected according to established schedules, and footbaths and hand-washing stations should be used where appropriate.

Continuous monitoring of environmental and biological parameters is fundamental to juvenile management. Water temperature, dissolved oxygen, salinity, flow rate, and basic water quality indicators must be measured daily. Biological observations should include swimming behaviour, feeding response, growth performance, size dispersion, deformity prevalence, swim bladder status and daily mortality. Any abnormal patterns must be recorded and investigated promptly, and corrective actions implemented without delay to safeguard stock health and production efficiency.

8.5.1 Cleaning of the juvenile section

Cleaning and sanitation procedures in the juvenile rearing unit are essential for maintaining fish health, minimizing disease risk, and sustaining high survival and growth rates. Juvenile grey mullet are particularly susceptible to bacterial, parasitic, and fungal infections under intensive culture conditions. Routine sanitation reduces pathogen loads, limits biofilm formation on tank surfaces and equipment, and prevents cross-contamination between production batches.

Cleaning protocols follow the same general principles applied in larval units but must be implemented more rigorously because of higher biomass, feeding rates, and organic loading, especially at elevated rearing temperatures. Mortalities must be recorded daily for each tank.

Daily cleaning tasks:

- Siphoning tank bottoms to remove faeces and uneaten feed.
- Purging settled solids through bottom drains.
- Wiping tank walls and the air–water interface when biofilms or grease films appear.
- Replacing outlet screens with clean, disinfected units.
- Removing dust and feed residues from automatic feeders.

Water treatment and filtration equipment:

- UV sterilizers: clean quartz sleeves monthly using calcium–lime–rust remover, vinegar, or another mild acid, rinse thoroughly, and replace lamps according to manufacturer specifications.
- Biofilters: wipe fouled internal walls with disposable tissues and siphon sump bottoms using disinfected equipment.
- Mechanical filters: verify backwashing at least twice daily and dismantle, clean, and disinfect filter components weekly or more frequently if clogging occurs.

Tank and system sanitation:

- Empty tanks must be drained, scrubbed with detergent and hot water, rinsed, disinfected with hypochlorite solution and air-dried before reuse.
- Used outlet screens should be washed with hot or high-pressure water, soaked in hypochlorite, rinsed thoroughly and dried prior to storage.
- Air hoses and diffusers should be cleaned weekly by washing with detergent and hot water, soaking in dilute hydrochloric acid to remove mineral deposits, and rinsing thoroughly before reinstallation.

Ancillary equipment and facilities:

- Automatic feeders: clean hoppers and dispensing mechanisms weekly with ethanol and disposable tissues.
- Disinfectant baths and containers: empty, clean and refill weekly.
- Floors: wash at least twice per week using high-pressure water jets followed by application of hypochlorite solution, ensuring adequate drainage and ventilation.

8.6 FISH BEHAVIOUR

Alterations in fish behaviour are often the first indication of sub-optimal environmental conditions and usually precede clear signs of stress such as elevated mortality. Daily visual inspections by trained staff are therefore essential in the juvenile section.

Routine observations allow early detection of problems related to water quality, oxygen supply, feeding management, stocking density, hydraulic malfunction, or emerging disease. Particular attention should be paid to swimming activity, distribution in the water column, schooling patterns, surface aggregation, feeding response, and buoyancy control.

Any abnormal behaviour should be recorded immediately and followed by verification of environmental parameters and system operation. Early intervention at this stage can prevent escalation into major health or production problems. Table 8.1 summarizes the main behavioural patterns observed in juvenile mullet, distinguishing normal from abnormal responses.

Whereas observation of fish behaviour should be conducted daily, other assessments require sampling a representative subset of the population. To minimise stress and avoid sampling bias, these checks are typically carried out during routine harvests for grading and thinning of the weaning tanks. As fish are already handled for counting and weighing within each size batch, accuracy is improved. Periodical population checks are recommended at 80, 100, and 120 DPH. For populations up to 80 days of age, a sample of 100 fish is considered sufficient, while older populations require

TABLE 8.1
Behavioural indicators of marine fish fry health

Observations	Normal (healthy fry)	Abnormal (potential problem)
	BEHAVIOURAL	
Swimming pattern	Active and steady swimming with smooth, coordinated movements; fry remain upright and occasionally explore the tank.	Lethargic or slow movement, drifting with the current; erratic darting, circular swimming; loss of equilibrium, including tilting or swimming upside-down.
Schooling behaviour	Form loose schools with coordinated swimming behaviour.	Individuals isolated from the group, erratic or uncoordinated swimming, and aggregation at the water surface or in tank corners.
Feeding response	Rapid, coordinated movement toward feed; actively capturing and ingesting food.	Poor or no response to feed; food accumulates on the tank bottom; frequent rejection or spitting out of feed.
Position in water column	Evenly distributed in mid-water; perform vertical movements during feeding.	Gathering at the surface gulping air, resting on the tank bottom, or remaining motionless in one spot.
Reaction to disturbance	Rapid, coordinated startle response followed by a quick return to normal swimming.	No response to disturbance, or uncontrolled frantic swimming with collisions against tank walls.
Respiration rate	Regular, unlaboured gill movement; breathing appears normal.	Rapid or slow gill movement; gills constantly flared; fish gasping at the surface.
	PHYSIOLOGICAL	
Body condition and colour	Clear eyes, intact fins, normal pigmentation for age.	Pale, blotchy or darkened colour; clamped fins; damaged tails; visible lesions.
Interaction with the environment	Explore and interact normally with tank currents.	Avoids areas of the tank, becomes trapped against inlets or outlet screens, or cannot swim against even weak currents.

Source: Modified from Martins, C.I., Galhardo, L., Noble, C., Damsgård, B., Spedicato, M.T., Zupa, W., Beauchaud, M., Kulczykowska, E., Massabuau, J.-C., Carter, T., Rey Planellas, S. & Kristiansen, T. 2012. Behavioural indicators of welfare in farmed fish. *Fish Physiol. Biochem.*, 38: 17–41. https://doi.org/10.1007/s10695-011-9518-8

FIGURE 8.5
***Mugil cephalus* juvenile schooling in an intensive rearing tank**

at least 200 individuals to account for increased size variability. Juveniles must be anaesthetised (0.08% clove oil) before handling to prevent injury, mortality or stress-related pathologies.

The flathead grey mullet exhibits characteristic behavioural patterns under tank culture conditions. As a pelagic species, *M. cephalus* displays continuous swimming and utilises the entire water column (Figure 8.5). Individuals form cohesive shoals, reflecting natural schooling behaviour that serves social and anti-predator functions.

In captivity, *M. cephalus* shows high activity levels and a pronounced tendency for benthopelagic foraging. This behaviour is characterised by grazing or nipping movements along the substrate and tank surfaces as fish search for detritus, biofilm and other organic material. Activity and feeding responsiveness are generally diurnal, peaking during periods of light. Aggression is minimal, and intra-specific interactions are typically peaceful, although occasional nipping or competitive behaviour may occur during feeding.

Suboptimal environmental conditions, particularly poor water quality, induce stress behaviours, including erratic swimming, surface gasping, jumping and reduced feeding. Juvenile mullet require relatively large tank volumes to accommodate their active swimming and social structure. Moderate water flow promoting circular movement enhances normal movement patterns and reduces confinement stress.

Overall, the flathead grey mullet adapts well to captivity under suitable husbandry and environmental conditions, making it an appropriate species for aquaculture operations.

8.7 JUVENILE SKELETAL DEFORMITIES

Skeletal deformities in *M. cephalus* primarily originate during larval development. Severe anomalies, including jaw, opercular, and vertebral column deformities, can alter body shape and negatively impact growth, survival and overall performance. Such deformities are closely associated with hatchery practices, nutrition, and early-life environmental conditions.

Prevention is the most effective strategy to minimise skeletal anomalies. Key measures include:

- Maintaining optimal water quality and stable temperature and salinity in rearing tanks.

- Ensuring successful swim bladder inflation by keeping a calm, debris-free surface with appropriate aeration.
- Providing balanced larval diets enriched with vitamins, minerals, and essential fatty acids.
- Following gradual weaning protocols to avoid nutritional stress.
- Use of circular tanks with carefully adjusted flow rates to promote continuous swimming. Practising gentle handling and grading procedures to minimise mechanical stress.

The consequences of skeletal deformities extend beyond animal welfare to economic viability and market acceptance. Deformed juveniles exhibit reduced growth rates and feed efficiency, as abnormal morphology increases energy expenditure on compensatory swimming movements rather than biomass accumulation. Impaired buoyancy and reduced competitive ability for feed further compromise survival. High grading losses occur, as deformed juveniles are often discarded during sorting, resulting in wasted resources and elevated production costs. At market size, deformed fish are typically rejected by consumers, undermining the perceived quality and commercial value of farmed mullet. Addressing deformities is therefore critical both for animal welfare and for maintaining economic competitiveness.

Skeletal deformities in early juveniles (30–50 DPH) can be identified and quantified using a double-staining protocol. Fish are first fixed in 10 percent formalin and then stained to differentiate cartilage and bone. Cartilage is visualized using Alcian blue for 24 h, followed by rehydration through a graded alcohol series and maceration in an aqueous solution of potassium hydroxide (KOH) with hydrogen peroxide until skeletal structures are visible. Bone is then stained with Alizarin red for 24 h in a solution containing sodium borate and trypsin. Staining duration may vary depending on fish size. Excess dye is removed with distilled water and repeated KOH baths.

Once staining is complete, fish are positioned on their right side to examine meristic characters and skeletal structures, including the cranium, vertebral column and caudal fin complex. In adult specimens, it is recommended to use X-rays for the diagnosis of skeletal malformations, which is a quick and non-invasive method.

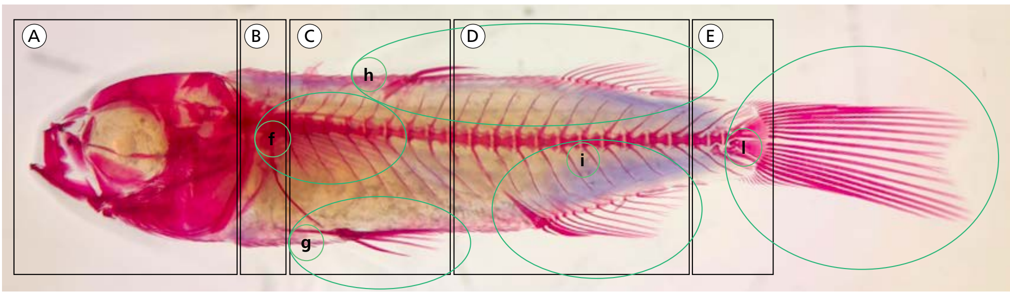
Figure 8.6 and Figure 8.7 illustrate an adult *M. cephalus* with pronounced skeletal deformities and a juvenile specimen with no observable skeletal abnormalities, respectively.

FIGURE 8.6
Skeletal deformity in an adult *Mugil cephalus* showing pronounced cervical vertebral *lordosis*

FIGURE 8.7
Juvenile *Mugil cephalus* (40 DPH) with completed ossification, exhibiting normal morphology and no visible deformities

Figure 8.8 shows a juvenile *M. cephalus* whose skeletal structures have been visualized using a double-staining protocol (Alcian blue–Alizarin red-S). Letters in the image indicate the cranium, the segmented vertebral column showing distinct vertebral regions and the fins, illustrating the skeletal supports and overall skeletal development in the juvenile fish. Table 8.2 describes the most commonly observed types of skeletal abnormalities observed in the flathead grey mullet. Figure 8.9 shows examples of skeletal anomalies in *M. cephalus*.

FIGURE 8.8
Juvenile *Mugil cephalus* treated with a double-staining protocol to highlight skeletal structures
Letters indicate cranium, anatomical segmentation of the vertebral column, highlighting distinct vertebral regions and fins



Vertebral regions	Vertebrae range	Key features
A	--	Cranium
B	1st–2nd	Cephalic vertebrae, carrying epipleural ribs.
C	3rd–10th	Pre-haemal vertebrae, carrying epipleural and pleural ribs; open haemal arch.
D	11th–21st	Haemal vertebrae; haemal arch closed by a haemal spine.
E	22nd–24th	Caudal vertebrae; haemal and neural arches closed by modified spines.

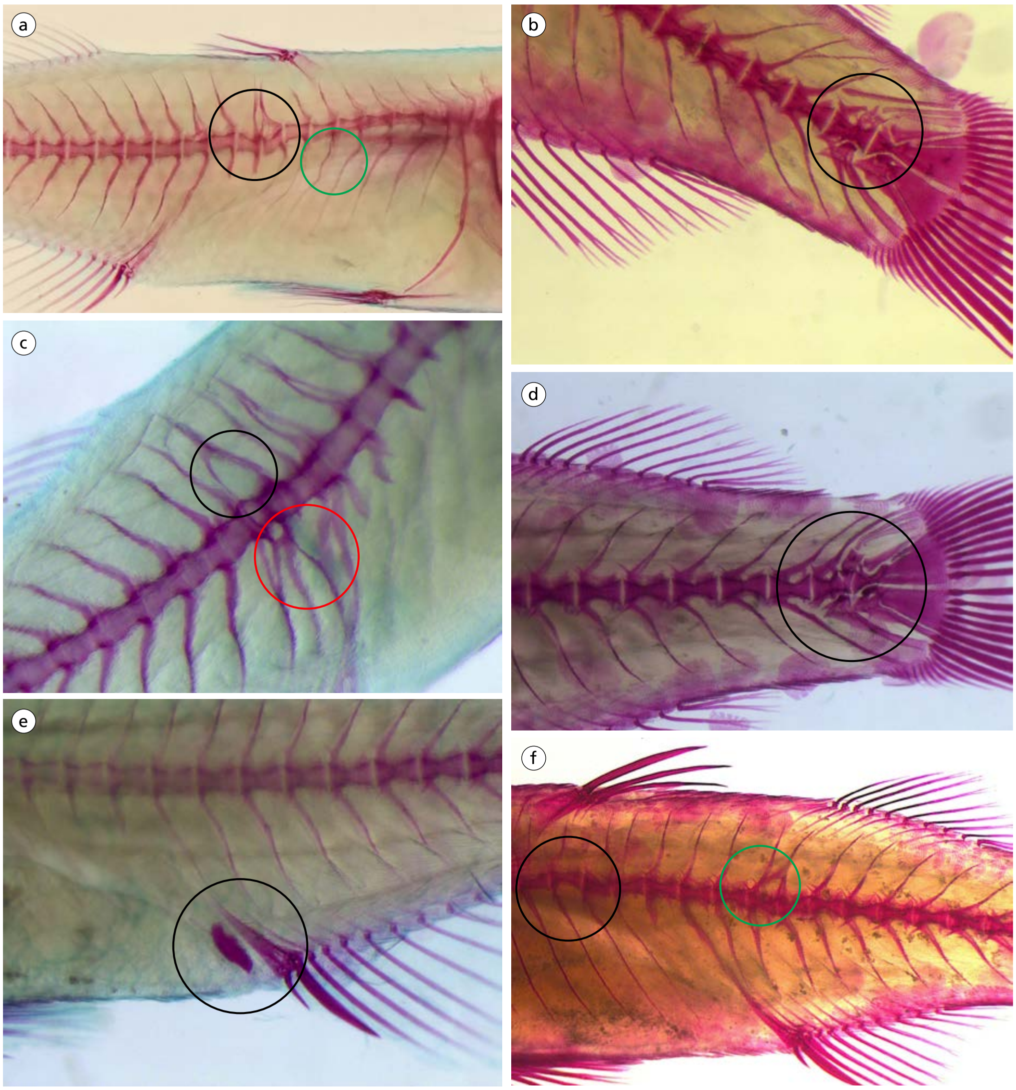
Note: Fins – **f**: pectoral; **g**: ventral; **h**: dorsal; **i**: anal; **l**: caudal.

TABLE 8.2
List of principal skeletal abnormalities in *Mugil cephalus*
Anomalies are categorized by type and location, with some affecting specific regions or structures (Bold text indicates severe anomalies)

Code	Abnormality	Description
1	**Kyphosis**	Exaggerated outward curvature of the spine (dorsal convexity).
2	**Lordosis**	Exaggerated inward curvature of the spine (ventral convexity).
3	**Partial vertebral fusion**	Incomplete fusion between adjacent vertebrae.
3*	**Total vertebral body fusion**	Complete fusion of vertebral bodies.
4	**Vertebral anomaly (shape, ossification ridges, length, intervertebral plates)**	Irregularities in vertebral morphology.
5	Anomalous neural arch and/or spine	Malformations in the neural arch or spine.
6	Anomalous haemal arch and/or spine	Malformations in the haemal arch or spine.
7	Anomalous rib	Deformities in epipleural, pleural, or dorsal ribs.
12	**Swim bladder anomaly**	Structural or functional issues in the swim bladder.
13	Presence of calculi in the urinary ducts	Mineral deposits obstructing urinary ducts.
14	**Anomalous maxillary and/or pre-maxillary**	Deformities in the upper jaw bones.
15	**Anomalous dentary**	Deformities in the lower jawbone.
16	Other cephalic anomalies (e.g. glossohyal, neurocranium)	Abnormalities in the skull or associated structures.
17L/R	**Anomalous left/right opercular plate**	Malformations in the operculum (gill cover).
17*L/R	Anomalous, absent, or fused branchiostegal ray	Issues with the branchiostegal rays (supporting gill arches).
21	Anomalous epipleural ribs	Deformities in the ribs attached to the cephalic vertebrae.
22	Anomalous dorsal ribs	Deformities in the ribs along the dorsal side.
23	Anomalous pleural ribs	Deformities in the ribs attached to the pre-haemal vertebrae.
26	**Supernumerary vertebra**	Presence of extra vertebrae.
29	Anomalous post cleithrum	Malformations in the post cleithrum (bone connecting pectoral fin to the skull).
S	**Scoliosis**	Lateral curvature of the spine.
Cl L/R	Anomalous left/right cleithrum	Deformities in the cleithrum (bone supporting the pectoral fin).
Cor L/R	Anomalous left/right coracoid	Deformities in the coracoid (part of the pectoral girdle).

Source: Boglione, C., Costa, C., Giganti, M., Cecchetti, M., Di Dato, P., Scardi, M. & Cataudella, S. 2006. Biological monitoring of wild thicklip grey mullet (*Chelon labrosus*), golden grey mullet (*Liza aurata*), thinlip mullet (*Liza ramada*) and flathead mullet (*Mugil cephalus*) (Pisces: Mugilidae) from different Adriatic sites: meristic counts and skeletal anomalies. *Ecological Indicators*, 6: 712–732. https://doi.org/10.1016/j.ecolind.2005.08.032

FIGURE 8.9
Examples of some skeletal anomalies
(a) Lordosis of the haemal region and fusion of neural arches, spines deformities of the pre-haemal; (b) Total fusion of vertebrae 22–23 in the caudal region; (c) Fusion of neural and haemal arches in the haemal region; (d) Partial fusion vertebrae caudal region; (e) Presence of calculi in the urinary ducts; and (f) Kyphosis of the pre-haemal region and fusion of neural arches in the haemal region



9. Juvenile grow-out

At approximately 200 DPH, *Mugil cephalus* juveniles (5–7 cm TL; 3–7 g BW) are transferred to larger tanks or ponds following a second grading procedure (Figure 9.1).

For grow-out, tanks or ponds exceeding 10 m³ in volume are recommended, particularly in flow-through systems. The Flathead grey mullet is a robust and adaptable species, characterised by low production risk and suitability for a wide range of farming scales, from small family enterprises to intensive commercial operations. The choice between extensive, semi-intensive, and intensive systems depends primarily on infrastructure availability, financial investment capacity, feed access and market demand.

FIGURE 9.1
***Mugil cephalus* juvenile (220 DPH)**

© Fondazione IMC, Italy

9.1 INTENSIVE REARING CONDITIONS

Under intensive conditions, juveniles are reared in a manner similar to that described for fingerlings in Chapter 8, at stocking densities not exceeding 1 fish/L or 10 kg/m³.

Fish can be cultured in lined ponds, flow-through raceways, semi-closed systems, or partial recirculating aquaculture systems (RAS) (Figure 9.2). Water exchange rates typically range from 5–10 tank volumes per day, depending on biomass loading and water quality parameters.

Feeding is carried out at 2–3 percent of standing biomass per day, distributed across five to six meals and delivered six days per week. Regular monthly biomass estimation through sampling and weighing is required to adjust feed rations, thereby optimising feed conversion and preventing water quality deterioration.

9.2 SEMI-INTENSIVE REARING EARTHEN PONDS

Following acclimation, fingerlings are stocked in earthen nursery ponds at densities of up to 125 fish/m², where production relies largely on natural productivity supplemented by formulated feeds.

Prior to stocking, ponds should be dried, ploughed and fertilised with 2.5–5.0 t/ha of cattle manure or equivalent organic fertiliser. Ponds are initially filled to a depth of 25–30 cm and maintained for 7–10 days to stimulate phytoplankton development. Subsequently, water depth is increased to 1.5–1.75 m before stocking. Pond productivity is maintained using chicken manure and/or inorganic fertilisers, guided by Secchi disk readings. Optimal dissolved oxygen concentrations must be supported

FIGURE 9.2
A nursery pond lined with food-grade PVC sheet, equipped with an on-demand feeder and a floating aerator

through mechanical aeration, especially after sunset and during early morning hours when oxygen minima typically occur.

Supplementary feeding is provided using moist or formulated feeds (pelleted or extruded), as well as agricultural by-products such as rice or wheat bran. In a 500 m² pond, water inflow should be maintained at approximately 1 L/s.

The fingerlings are kept in the nursery ponds for 4–6 months – preferably during spring – until they reach a body weight of about 10 g. Optimal temperatures range between 20–26 °C during both nursery and grow-out phases. Harvesting is achieved either by draining ponds into catch basins or by seine netting. Over-wintered fingerlings can be sold for further on-growing in other systems.

Part or all of the production may alternatively be retained in earthen ponds and grown to market size. When seawater salinity exceeds 25 ppt and dissolved oxygen is maintained near saturation, adult females older than two years can be reared to gonadal maturation (advanced vitellogenesis) for *bottarga* production, provided they are fed diets containing more than 35 percent crude protein and 6–10 percent lipid.

9.3 EXTENSIVE REARING CONDITIONS

In many regions, mullet fry and fingerlings are stocked in open natural systems such as lagoons, reservoirs and inland lakes – under freshwater, brackish or marine conditions – as part of fisheries enhancement programmes (culture-based fisheries). Flathead grey mullet is also frequently produced in polyculture within net enclosures in shallow coastal waters and can be successfully reared alongside species such as common carp, grass carp, silver carp, Nile tilapia, milkfish and gilthead seabream.

For restocking in confined environments, fingerlings of approximately 8 cm total length are recommended. Prior to release, fish must undergo gradual acclimation to the temperature and salinity of the receiving water body. This is achieved by holding fish for 10–14 days in pre-adaptation enclosures while progressively reducing feeding levels (Figure 9.3).

FIGURE 9.3
Lagoon acclimatization enclosures used for pre-release conditioning of mullet fingerlings
High-resistance nylon acclimatization enclosure (10 × 10 × 2 m; 7 × 7 mm mesh; 210/6 denier) with reinforced edges, corner grommets for installation, and a weighted perimeter bottom rope

Where supplementary feeding is prohibited, stocking densities within cages or enclosures should not exceed 50 fish/m³ to ensure adequate availability of natural food. Enclosures must be installed in areas characterised by adequate water circulation, stable thermal regimes, and high dissolved oxygen concentrations to minimise stress and mortality. It is also advisable to place the enclosures near the shore to facilitate inspection and routine management.

9.4 MARK AND RELEASE

The flathead grey mullet is a non-carnivorous and weakly competitive species and is therefore well suited for stock-enhancement and restocking programmes. For release operations, juveniles larger than 6–8 cm TL are recommended. Prior to release, fish can be marked or tagged to allow post-release monitoring and to evaluate the effectiveness of the stocking programme. Several marking techniques are commonly applied in mullet restocking initiatives.

Genetic tagging

In stock-enhancement programmes, parentage analysis can be used to determine the contribution of hatchery-reared fish to wild populations. This approach requires collecting a small fin clip from broodstock prior to spawning to obtain DNA samples for genetic profiling. When released fish are subsequently recaptured, tissue samples are taken and analysed, and their genetic markers are compared with those of the parental stock. This allows verification of kinship and estimation of survival, recruitment success, and reproductive contribution of released individuals. Genetic tagging provides robust information for evaluating the biological effectiveness of restocking programmes and their interaction with natural populations.

Elastomer tagging

Visible elastomer tagging is widely used to mark juvenile fish in enhancement programmes. The method involves injecting a small quantity of coloured elastomer – a biocompatible and inert polymer – beneath the skin, typically near the operculum or around the eye region, using a fine syringe. The elastomer cures in situ to form a flexible and permanent mark that can be observed under ambient or UV light. Different colours or injection patterns allow batch or group identification, usually for periods of up to six months. Elastomer tagging is minimally invasive and has negligible effects on growth or behaviour, making it suitable for short- to medium-term monitoring of survival, dispersal, and evaluating stock contribution following release.

Passive Integrated Transponder tagging

Passive Integrated Transponder (PIT) tagging is used for long-term individual identification in restocking programmes. PIT tags consist of small glass-encapsulated microchips containing unique alphanumeric codes and are implanted into the fish body cavity or musculature using sterile injection equipment (see Annex IV). Tag size must be appropriate for fish size to minimise handling stress and tagging-related mortality. Each tag is detected using a handheld reader, and the identification code is recorded together with biological data such as body size, tagging date and release location. After release, recaptured fish are scanned to assess survival, growth and movement patterns. PIT tagging provides high-resolution individual-based data and is particularly valuable for fisheries management, conservation planning, and evaluation of long-term stock-enhancement outcomes.

9.5 FEED SIZE IN RELATION TO GROWTH

At approximately 100 DPH, juveniles should undergo grading to separate the population into two size classes, typically ranging between 0.3 and 0.7 g BW. Following grading, fish are reared in open freshwater systems at a density of 2 ind./L and fed at a daily ration equivalent to 2.5 percent of total biomass, divided into six equal feedings.

Once juveniles exceed 200 DPH or reach approximately 15 g BW, a second grading is recommended. At this stage, rearing density should be reduced to 1 ind./L, corresponding to approximately 10–15 kg/m^3. This biomass volume should then be maintained throughout the remainder of the grow-out phase to ensure uniform growth, efficient feed utilisation and good welfare conditions.

TABLE 9.1
Feed particle size in relation to fish body weight

Fish body weight (g)	Feed particle size (ø mm)
1–15	0.8
15–30	1
>30	1.3
>100	1.5
>300	2
>600	3

Table 9.1 presents the recommended feed particle sizes in relation to fish body weight.

9.6 JUVENILE GROWTH

The grow-out period for *M. cephalus* typically extends over 7–8 months. Under monoculture conditions in earthen ponds, fertilisation alone may be sufficient to sustain adequate natural food production. In many instances, mullets have been observed to ingest poultry manure directly, and satisfactory yields have been reported when pond productivity is properly managed. Growth performance should be monitored regularly through sampling. If growth rates fall below expectations, supplementary feeding with rice bran and/or wheat bran is recommended at 0.5–1 percent of total biomass per day to augment natural productivity.

In polyculture systems, mullet are commonly stocked together with Nile tilapia, common carp and silver carp. In such cases, fertilisation and feeding regimes are generally designed to meet the requirements of the primary species, while mullet exploit natural food resources, detritus and uneaten feed particles.

When acclimated to site salinity and stocked at 10–15 g BW, mullet reared in monoculture at densities of 6 200–7 400 ind./ha typically yield 4.3–5.6 t/ha by the end of the annual production cycle. In semi-intensive polyculture with tilapia and carp, mullet are stocked at lower densities (2 400–3 700 ind./ha), together with 1 850–2 450 common carp juveniles of approximately 100 g and 61 700–74 100 Nile tilapia fingerlings weighing 10–15 g. Under proper fertilization, feeding and water-quality management, total pond production can attain 20–30 t/ha, of which mullet typically contribute approximately 2–3 t/ha.

Following a single grow-out season of 7–8 months in subtropical environments, flathead grey mullet usually attain a body weight of 0.75–1.0 kg. After two consecutive production seasons, fish may reach 1.5–1.75 kg. Whether a second year of grow-out is implemented depends on market demand and economic considerations, as some markets favour mullet exceeding 1.5 kg. In two-season production cycles, fish remain in ponds during winter and continue growing through the subsequent spring and summer until harvest size is achieved. Selection of the production strategy should therefore be guided by market requirements, production costs and site-specific environmental conditions.

9.7 JUVENILE TRANSPORT AND ACCLIMATION

When juveniles reach 2–5 g BW, they can be transferred from the hatchery to on-growing facilities such as pre-growing tanks or net cages. Short-distance movements within the farm are relatively simple and generally do not require specialized transport systems. In contrast, long-distance transfers – particularly when fish are sold or relocated to external grow-out sites – are more demanding and require careful logistical planning and purpose-built transport equipment.

Successful transport depends on strict control of water quality, temperature and dissolved oxygen, together with appropriate loading densities and gentle handling procedures. Holding conditions must be monitored continuously throughout the journey to minimize stress, limit transport-related losses and safeguard juvenile welfare and subsequent growth potential.

9.7.1 Transport equipment

Fish transport success depends on the appropriate selection of containers, vehicles and tank design, combined with efficient oxygenation systems and continuous monitoring of water quality parameters. Equally important are correct stocking densities, careful handling during loading and unloading, and strict operational controls throughout the journey. This section describes the equipment requirements and technical procedures necessary to ensure safe and efficient transport of juveniles under commercial aquaculture conditions.

Containers:

- *Open containers* – Commonly used for transporting fry, these containers are installed on trucks and may include aeration-oxygenation systems, cooling devices, and automatic monitoring for dissolved oxygen and temperature.
- *Polyethylene bags* – Partly filled with seawater and inflated with oxygen, these bags are placed in insulated boxes to maintain stable temperatures. This method is less practical for large quantities of juvenile fish because of high costs and time-consuming packaging.

Vehicles and tanks:

Trucks are the primary means of transporting live fish. Light trucks fitted with round open tanks are used for short-distance transfers, whereas heavy truck–trailer units equipped with rectangular closed tanks are employed for long journeys and large consignments of fish.

- *Material* – Tanks are generally made of reinforced fibreglass, which is preferred for its strength, light weight and ease of cleaning.
- *Design* – Round, flat-bottom tanks with open tops are used for short-distance transport. For longer journeys, closed rectangular tanks with insulation and features such as trapdoors, sluice gates and vent holes are used.

Oxygenation systems:

Adequate supply of oxygen is critical because of the generally high stocking densities of fish and stress experienced during transport. Oxygenation can be provided through aeration and/or pure oxygen injection.

- *Aeration* – Uses air blowers and diffusers to supply filtered air. Less efficient but serves as an emergency backup.
- *Oxygenation* – Uses industrial-grade oxygen bottles or liquid oxygen containers with fine oxygen diffusers. Preferred for long-distance transport due to longer autonomy.

Monitoring and water quality

Oxygen monitoring:

- *Manual checks* – Traditionally done by observing bubbling and adjusting based on experience.
- *Automatic devices* – Portable oximeters and automatic DO monitoring devices with probes linked to a display in the driving cab. Some versions automatically adjust oxygen flow.

Water quality:

- *Dissolved oxygen (DO)* – Must be maintained between 150–200 percent saturation. Water should be hyper-oxygenated prior to transport and carefully regulated during the initial hours.
- *Salinity* – Should match the hatchery environment. Typically, full seawater at 35 ppt is used. Brackish water at 20–25 ppt can reduce stress and increase oxygen solubility.
- *pH* – Generally not problematic in seawater. The natural buffer capacity compensates for increased carbon dioxide levels.
- *Temperature* – Lower temperatures reduce metabolism rate and oxygen consumption. A gradual decrease to 18–20 °C is recommended for transport.
- *Ammonia* – Accumulates due to metabolic activity. Its concentration can be minimized by withholding feed 24–48 h before transport, partially renewing water, and maintaining lower temperatures.
- *Carbon dioxide* – Removed through aeration and tank ventilation. Sealed containers can lead to dangerous CO_2 build-up.
- *Turbidity and foam* – Clean, filtered water reduces the risk of gill clogging and bacterial proliferation. Foam should be removed as it interferes with gas exchange.

Stocking density and handling

Transport density must be adjusted based on fish size and the expected duration of transport to ensure optimal welfare and minimize stress-related physiological effects. The following table provides recommended stocking densities for fry and juveniles under different transport durations (Table 9.2):

TABLE 9.2
Recommended transport densities of mullet fry and juveniles

Transport duration (h)	Fry: 1–2 cm TL (0.2–0.5 g BW)	Juveniles: 5–8 cm TL (3–5 g BW)
Short (≤2 h)	8 000–10 000 fry/m³	1 000–1 500 fish/m³
Medium (2–6 h)	4 000–6 000 fry/m³	500–800 fish/m³
Long (6–12 h)	1 500–3 000 fry/m³	200–400 fish/m³

Handling:

- *Precautions* – Only healthy fish, free from disease and well-adapted to their rearing environment, should be selected for transport. Remove any injured or weak individuals prior to loading.
- *Starvation* – Fish should be starved for 24 hr before transport to reduce metabolic waste and faecal accumulation in the transport container.
- *Loading* – Water in transport containers should match the hatchery conditions in terms of temperature, salinity and quality. For long-distance transport, perform a complete water change before departure. External ice packs may be used to stabilize temperature but never place ice directly into the transport water.

Controls during transport

Monitoring:

- *Oxygen supply* – Regularly monitor for sufficient oxygen levels, paying attention to signs such as excessive foam or dead fish.
- *Water parameters* – Temperature, dissolved oxygen (DO), pH, and salinity should be checked whenever new water is added to the transport container.
- *Automatic devices* – Automated monitoring systems can save time and provide early warnings of oxygenation or water quality failures, ensuring timely corrective action.

Unloading:

- *Acclimatization* – Transport water should match the salinity and temperature of the receiving facility. Fish must be gradually acclimated to the new environment to minimize stress.
- *Discharge* – The method depends on the type of transport container used. Oxygen supply must be maintained throughout the discharge process to prevent critically low levels.
- *Post-transport care* – After arrival, the fish should be fed immediately if they have been long-starved. Remove any dead or moribund specimens and monitor mortality closely to ensure the health and welfare of the population.

10. Diseases and parasites

Diseases naturally occur in wild fish populations, but their effects are often subtle and go unnoticed. In aquaculture, however, diseases are more apparent and can spread rapidly due to high stocking densities and confined spaces. Outbreaks can cause significant economic losses and may have environmental impacts, including the transmission of pathogens from farmed to wild populations or the introduction of non-native pathogens during fish transport. Similarly, farmed stocks should be protected from pathogens carried by wild fish.

Even pathogens that exist at low levels in natural environments can cause significant disease in farmed fish under intensive rearing conditions. In hatcheries and nurseries, disease occurrence is primarily associated with:

1. High rearing densities, which increase physical contact and the risk of pathogen transmission;
2. Delayed removal of infected individuals, unlike natural predation in the wild;
3. Stress caused by suboptimal rearing conditions;
4. Accidental introduction of pathogens due to insufficient biosecurity;
5. Inappropriate feeding regimes that compromise fish health.

Flathead grey mullet, like other cultured fish species, are susceptible to a variety of diseases, particularly under conditions of environmental stress or poor water quality. Variations in salinity and temperature can further increase susceptibility. Major categories of pathogens include:

Viral infections:

Viral infections in *Mugil cephalus* may include diseases caused by Iridovirus and Betanodavirus. However, viral infections are generally less significant than bacterial or parasitic diseases. There are relatively few published reports documenting their impact.

Bacterial infections:

Bacterial pathogen, particularly those caused by species of *Vibrio*, can cause skin lesions, ulcers or internal infections. Infected fish often exhibit lethargy, abnormal swimming patterns, and visible changes in body appearance.

Parasitic infections:

Parasitic infections are common in *M. cephalus*. External parasites, such as monogenean worms or ectoparasitic copepods, primarily affect the skin and gills, causing irritation, lesions or respiratory difficulties. Internal parasites, including nematodes and cestodes, may infect the digestive system, potentially impairing nutrient absorption and growth.

Fungal infections:

Fungal infections typically develop in areas of injured skin. The fungi *Saprolegnia* and *Aphanomyces* are the most frequently reported causes of fungal disease in this species, often resulting in visible lesions and secondary infections.

As with other aquaculture species, disease outbreaks are frequently associated with stress and suboptimal rearing conditions. Environmental stressors, such as low dissolved oxygen, elevated ammonia concentrations, or rapid temperature fluctuations, can compromise the immune system of *M. cephalus*, increasing susceptibility to pathogens. Nutritional deficiencies, resulting from diets lacking essential vitamins or minerals, similarly weaken immunity and make the fish more prone to infections.

A comprehensive list of the most common diseases affecting *M. cephalus* is provided in Appendix VII. Effective prevention relies on maintaining optimal water quality, balanced nutrition, and strict biosecurity measures to reduce stress and minimize the risk of disease outbreaks.

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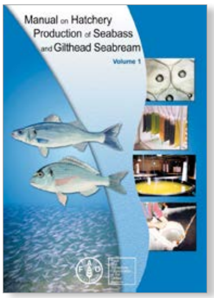

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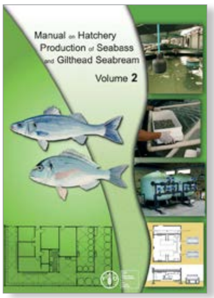

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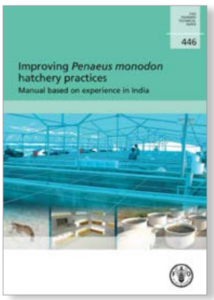

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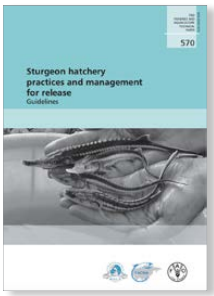

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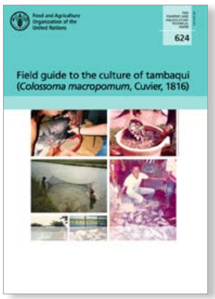

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Appendices

Appendix I – Mullet *bottarga* production

Bottarga is a traditional Mediterranean delicacy produced from the salted and dried roe (egg sacs) of grey mullet, most commonly *Mugil cephalus*, although similar products are also prepared in other regions of the world where mullet are harvested, such as Japan and Taiwan Province of China. The product is highly valued for its intense aroma, firm texture and distinctive savoury flavour, often described as rich, briny and umami. Depending on the degree of drying, *bottarga* ranges in colour from deep amber to dark orange. It is considered a premium product in several Mediterranean countries, where it is consumed thinly sliced or grated as a flavouring ingredient.

The preparation process requires careful handling to preserve the integrity of the roe sacs and to ensure proper salting and drying.

NECESSARY INGREDIENTS AND EQUIPMENT

Fresh mullet ovaries (preferably from *M. cephalus*), sea salt, a weighing scale, glass or food-grade plastic containers, a press or weights, fine mesh netting (e.g. mosquito net), a dehydrator or a dry and well-ventilated space, and a vacuum-sealing machine.

Procedure (see Figure A1.1 and Figure A1.2)

1. Extraction of ovaries

Before proceeding with ovary excision, the fish must be humanely euthanized to ensure immediate death and minimize suffering. The most effective method for euthanizing fish is cranial percussion, performed using a blunt instrument (officially recognized humane euthanasia technique for fish, compliant with animal welfare guidelines for laboratory and field settings). This method involves a single, rapid blow to the cranial region, ensuring instantaneous death.

The fish is first secured on a stable, sanitized surface. Scales are carefully removed using a blunt tool or the back of a knife, working from the tail toward the head to avoid damaging the skin (Figure A1.1a).

A ventral midline incision is then made along the abdominal cavity, starting just posterior to the pectoral fins and extending toward the anal fin, to expose the visceral mass (Figure A1.1b–c).

A vertical incision is then made between the urogenital papilla and the anal fin (Figure A1.0d). Additional cuts are performed to detach the muscle flap which delineates the base of the ovary and will remain attached to the extracted roe sac (Figure A1.0e). The ovaries are gently isolated to avoid rupturing the membrane. The operator detaches the ovaries from the surrounding mesentery and adipose tissue, supporting them at the base to prevent tearing (Figure A1.1f–g). The muscle flap is fully severed at its base to free the ovary (Figure A1.1h). The operator then continues to carefully detach the ovaries from the surrounding mesentery and adipose tissue, supporting them at the base to prevent tearing and severing the anterior blood vessels connecting the ovary to the fish's body to fully release the roe sac (Figure A1.1i–j). Once fully removed, the ovaries are placed on a clean surface and inspected for integrity. Any residual connective tissue or blood is removed with a damp cloth. Intact ovaries, free of lacerations are then ready for rinsing and subsequent salting (Figure A1.0k).

FIGURE A1.1
Photographic sequence illustrating the main stages of the extraction of the ovaries

Notes: Use a sharp, blunt-edged knife, disposable gloves, and a sanitized work surface. Perform the extraction at room temperature or in a cool environment to preserve product quality.

Retaining the muscle flap attached to the roe sac is optional and not strictly necessary; this practice is characteristic of the traditional Sardinian method of *bottarga* production.

2. Cleaning of the egg sacs

The ovaries are carefully removed from the fish, taking care not to rupture the membrane. They are gently rinsed under cold running water to remove blood residues and impurities.

3. Salting

After rinsing, the ovaries are dried with a clean cloth and weighed (Figure A1.2a). They are then placed in a food-grade container (Figure A1.2b). Sea salt is gently massaged over the entire surface to ensure complete and uniform coverage (Figure A1.2c–d). The ovaries are left to cure in salt for a period that depends on the weight of the ovary, as larger ovaries require a longer curing period. Each individual ovary is cured for approximately 30 minutes/100 g of fresh roe at room temperature. During this stage, osmotic dehydration begins and contributes to preservation and flavour development.

4. Pressing and drying

Following salting, the ovaries are rinsed briefly to remove excess surface salt and dried again with a clean cloth (Figure A1.2e–i). They may be lightly pressed to improve shape and texture. The roe sacs are then hung or placed on a flat surface in a dry, well-ventilated area and protected with a fine mesh net to prevent insect contact (Figure A1.2j–k). Alternatively, a dehydrator may be used at a low temperature (<30 °C). Drying typically requires 2–4 weeks, depending on environmental conditions and desired firmness. Slow drying is essential for proper flavour development and texture.

5. Conservation

When fully dried, the *bottarga* becomes firm and compact (Figure A1.2l). It should be stored in a cool and dry place. For extended preservation, the surface may be coated with melted beeswax or the product may be vacuum-packed to protect it from moisture and oxidation (Figure A1.2m–n).

FIGURE A1.2
Photographic sequence illustrating the main stages of *bottarga* production

FIGURE A1.3
A serving of sliced *bottarga* served with soft cheese, grilled fennel, bread, olive oil and lemon dressing

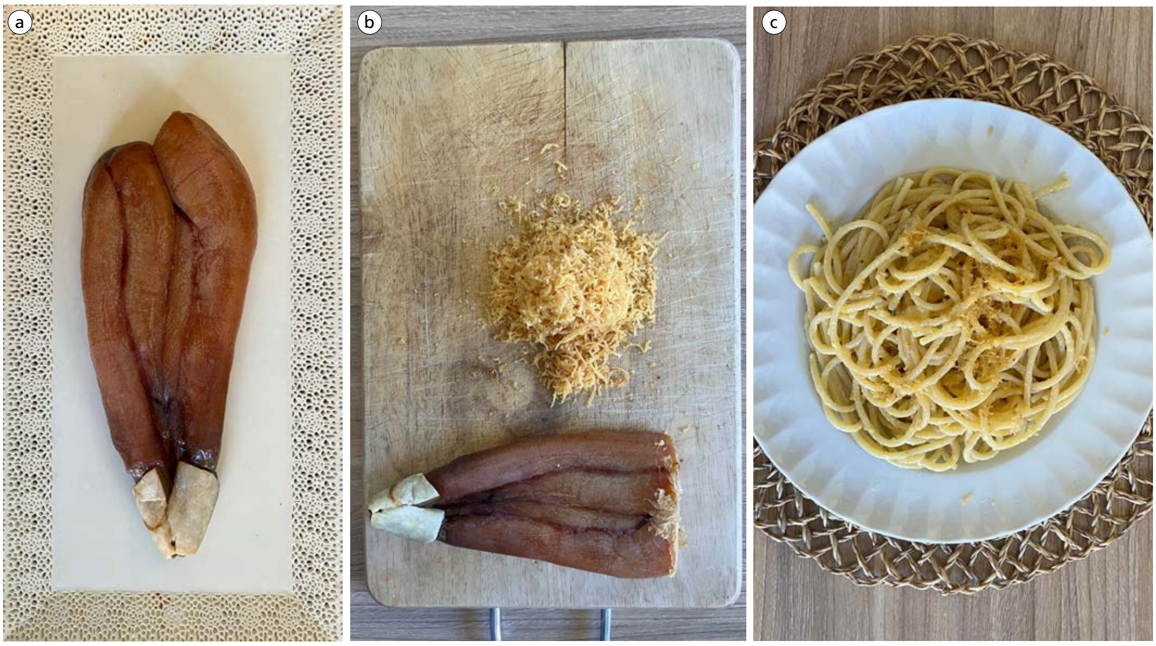
© N. Duncan

Consumption

Bottarga is typically grated over pasta or risotto or sliced thinly and served as an appetizer with olive oil. It may also be combined with salads, particularly with artichokes or celery (Figure 1.3 and Figure 1.4).

The procedure described above reflects traditional artisanal processing methods. Variations in salting time, pressing intensity, and drying conditions may occur depending on regional practices, climatic conditions, and desired product characteristics. In commercial production, strict hygiene standards, traceability, and food safety protocols should always be applied to ensure product quality and consumer safety.

FIGURE A1.4
Sardinian bottarga after removal from vacuum packaging (a); freshly grated bottarga (b); and spaghetti prepared with olive oil, garlic and grated bottarga (c)



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Appendix II – **Algal culture medium**

Reliable production of high-quality microalgae is essential for successful marine hatchery operations. Microalgae are used both directly in green-water systems and indirectly for the enrichment of live feeds such as rotifers and *Artemia*. Consistent culture performance depends on the use of an appropriate nutrient medium, strict hygiene procedures and careful monitoring of environmental conditions.

A widely used formulation in marine hatcheries is Guillard's F/2 medium, which supplies the macro-nutrients, trace elements and vitamins required for balanced algal growth. The standard composition is presented below. For more detailed information on microalgae production techniques, media preparation, scale-up procedures and culture management, reference can be made to the FAO manual by Helm, Bourne and Lovatelli (*Hatchery culture of bivalves: a practical manual*; see Other FAO publications), which provides comprehensive technical guidance applicable to finfish hatcheries.

F/2 GUILLARD'S MEDIUM

Macronutrients

Nutrient	Concentration
$NaNO_3$	8.82×10^{-4}
$NaH_2PO_4 \cdot H_2O$	3.62×10^{-5}
$Na_2SiO_3 \cdot 9H_2O$	1.06×10^{-4}
Trace metal solution	–
Vitamin solution	–

Trace metal solution

Component	Concentration
$FeCl_3 \cdot 6H_2O$	1.17×10^{-5}
$Na_2EDTA \cdot 2H_2O$	1.17×10^{-5}
$ZnSO_4 \cdot 7H_2O$	7.65×10^{-8}
$CoCl_2 \cdot 6H_2O$	4.20×10^{-8}
$CuSO_4 \cdot 5H_2O$	3.93×10^{-8}
$Na_2MoO_4 \cdot 2H_2O$	2.60×10^{-8}
$MnCl_2 \cdot 4H_2O$	9.10×10^{-7}

Vitamin solution

Component	Concentration
Biotin (Vitamin H)	2.05×10^{-9}
Thiamine · HCl (Vitamin B_1)	2.96×10^{-7}
Cyanocobalamin (Vitamin B_{12})	3.69×10^{-10}

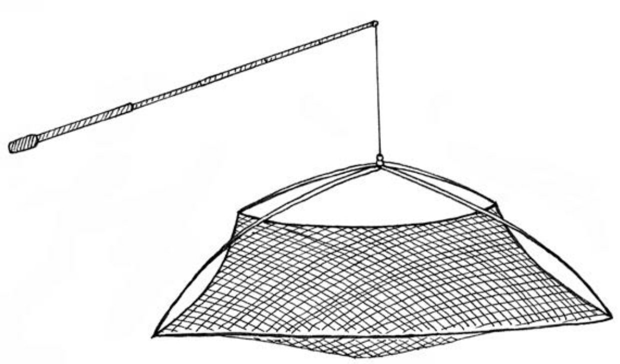
This formulation supports the growth of commonly used hatchery microalgae such as *Tetraselmis suecica*, *Isochrysis galbana* and *Nannochloropsis oculata*, ensuring consistent biomass production and appropriate nutritional quality for live-feed systems.

Appendix III – **Other ways to collect adults of *Mugil cephalus***

CAPTURING *MUGIL CEPHALUS* ADULTS WHEN LAVORIERI ARE UNAVAILABLE

When the capture technique described for traditional fixed traps (*lavorieri*) cannot be applied, adult *Mugil cephalus* can be collected using alternative fishing methods. These methods may be employed during the natural reproductive migration period as well as outside the spawning season, depending on broodstock requirements and local environmental conditions. The choice of method depends on site characteristics (e.g. lagoon, estuary, coastal area), available manpower and local regulations.

FIGURE A3.1
Illustration of a dip net



Collection using dip nets

A dip net consists of a long handle attached to a circular or rectangular frame supporting a net bag (Figure A3.1). The net is typically made of durable synthetic material (e.g. nylon) with a mesh size appropriate for the size of the target fish. The handle, constructed from wood, metal or composite materials, allows operators to reach into the water from the shore or from a boat. The frame must be sufficiently robust to withstand water resistance and the weight of captured fish.

Dip nets are most effective in shallow environments such as lagoons, estuarine margins and narrow channels where fish movements can be visually detected and anticipated. The operator submerges the net in the direction of fish movement and rapidly lifts it to capture passing specimens. Successful operation requires good timing, coordination and experience. This method is suitable for capturing limited numbers of broodfish with minimal equipment.

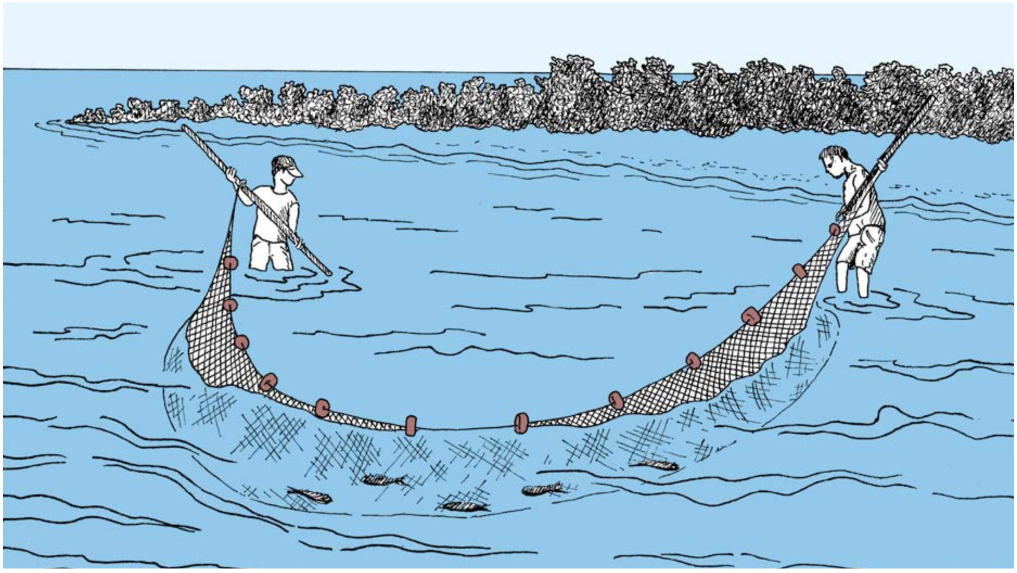
Compliance with local fisheries regulations is essential, as permits or seasonal restrictions may apply in certain regions.

Collection using beach seine

A beach seine is a type of seine net deployed from the shore, suitable for capturing larger numbers of adult mullet in shallow coastal waters, lagoons, and estuaries. The gear typically consists of two long wings and a central bunt (a bag or loose netting) where fish accumulate. The headrope is fitted with floats to keep the upper edge at the surface, while the footrope is weighted to maintain continuous contact with the seabed. The wings may be extended with ropes for towing the net to and along the beach. This arrangement forms a vertical barrier that prevents fish from escaping above, below or around the net (Figure A3.2).

Beach seines may be designed with or without a central bag. In bagless seines, the central section has smaller mesh and additional slack to retain fish. In bag seines, the bunt concentrates the catch and may be positioned centrally or asymmetrically, resulting in wings of unequal length. Operation generally requires several people to deploy and haul the gear to the shore. In some locations, a fixed wooden capstan may be installed onshore to assist with hauling.

FIGURE A3.2
Illustration of a beach seine



Deployment is typically carried out from a small boat. One towing line is secured onshore, after which the net is set in a wide semicircle: first wing, bunt (if present), second wing and the return towing line. The net is then hauled simultaneously from both ends, gradually reducing the enclosed area and concentrating the fish. Effective operation requires that the footrope maintains contact with the substrate and reaches the shore first, thereby preventing fish from escaping beneath the net.

Collection using bottle fishing

Bottle fishing is a simple trapping technique that can be applied in lagoons, estuaries or channels, particularly where fish densities are moderate and selective capture of individual broodfish is desired. The method uses modified plastic bottles to create small, baited traps (Figure A3.3).

Preparation involves removing the base of a plastic bottle and perforating it near the cut edge to attach a main retrieval line. Additional holes are made in the mid-section to secure a weight (approximately 30 g of lead) to ensure proper submersion and stability.

For baiting, one or two teaspoons of wheat or maize flour are placed inside the bottle before securely closing the cap. The bottle is then cast into the water while retaining the retrieval line. Fish entering the bottle to feed can be gently retrieved by pulling the line. This method generally captures one fish at a time and allows selection based on bottle size relative to target fish size.

Although simple and low-cost, this method is labour-intensive and suitable only for small-scale broodstock collection.

FIGURE A3.3
Illustration of a customized bottle adapted for the bottle-fishing technique

Appendix IV – **Chemical treatments for quarantine and egg disinfection**

Chemical treatments are commonly used during quarantine and in the disinfection of fish eggs to prevent the introduction or spread of pathogens in aquaculture facilities. The treatments target a range of pathogens, including external and internal parasites, bacteria, protozoa, and fungi. Depending on the target pathogen and life stage, chemicals are administered as baths for fish or eggs, orally via feed or cannula, or by injection. Proper selection of the chemical, dosage, and exposure duration is critical to ensure effective pathogen control while minimising stress or toxicity to the fish.

Pathogen to be controlled	Chemical	Species	Dosing	Administration
External parasites (including monogeneans and copepods), protozoans and epibiotic bacteria.	Formalin	Fish	100 µl/L	1 hour bath
External bacteria and protozoans (including *Amyloodinium* spp.)	H_2O_2	Fish	150 µl/L	1 hour bath
Endoparasites and ectoparasites	Praziquantel	Fish	5 mg/kg	In feed or orally with a canula
Systemic bacteria or internal bacterial pathogens	Enrofloxacin	Fish	10 mg/kg	Injection every other day for a minimum period of 10 days
Systemic bacteria or internal bacterial pathogens	Oxytetracycline	Fish	10 mg/kg	Injection every other day for a minimum period of 10 days
Ectoparasitic bacteria, fungi, and protozoans	H_2O_2	Fish eggs	500 µl/L	15 minute bath
Ectoparasitic bacteria and protozoans	Active iodine	Fish eggs	50 mg/L	10–60 minute bath

Source: U.S. Fish & Wildlife Service's Aquatic Animal Drug Approval Partnership Program. Quick Desk Reference Guide to Approved Drugs for Use in Aquaculture (2020). https://www.fws.gov/page/aquatic-animal-drug-approval-partnership/Resources-for-Selecting-Aquaculture-Medications

Appendix V – **Individual tagging of fish**

Individual tagging is an essential tool for tracking growth, behaviour, and health of fish in research or aquaculture operations. Proper handling, sterilization, and implantation techniques are critical to minimize stress, prevent infection, and ensure accurate identification over time. The following steps outline the recommended procedure for intramuscular tagging:

FIGURE A5.1
Application of a tag for fish identification

© A. Cannas, FLAG Sardegna Sud-Occidentale

1. Use a sterile tag implanter for the procedure. Prior to use, disinfect all components with 70 percent alcohol or another suitable disinfectant.
2. Record the tag identification code before insertion.
3. Capture the fish and place it in a small tank for anaesthesia according to standard protocols.
4. Disinfect the implantation site using an iodine-based solution. Consistently use the same site for all fish to facilitate future tag reading.
5. Load the tag into the implanter and insert it beneath the dorsal fin, within the dorsal epaxial muscles, parallel to the muscle fibres (Figure A5.1).
6. Slowly release the tag while withdrawing the implanter needle, ensuring the tag occupies the void left by the needle.
7. Reapply an iodine-based disinfectant to the implantation site.
8. Carefully release the fish into its holding tank.

Appendix VI – Standard operating procedures for the hatchery production of flathead grey mullet (*Mugil cephalus*)

These standard operating procedures (SOPs) are based largely on hatchery operational practices and regulatory conditions commonly applied in Italy and within the European Union. Where *Mugil cephalus* hatchery production is conducted outside Italy or the EU, additional national, regional, or local regulatory frameworks may apply, including veterinary authorizations, aquatic animal health surveillance requirements, environmental permits, biosecurity obligations and reporting systems. Hatchery operators should therefore verify and comply with the applicable legal and administrative requirements in the jurisdiction where the production facility operates and adapt these procedures accordingly.

PURPOSE AND REGULATORY FRAMEWORK

These standard operating procedures (SOPs) provide a risk-based, prevention-oriented operational framework for the intensive and sustainable hatchery production of *M. cephalus*. The objectives of these procedures are to:

- minimize the introduction and spread of listed and emerging pathogens, including *Nodavirus* and Viral Nervous Necrosis (VNN);
- preserve the biological integrity and wild-type traits of broodstock and progeny, maintaining genetic diversity and ecological fitness for potential restocking programmes; and
- standardize hatchery operations according to recognized best aquaculture practices (BAP) and aquatic animal biosecurity principles.

The overarching operational principle is that prevention is more effective than treatment, therefore emphasis is placed on proactive disease management, strict hygiene protocols, and full operational traceability.

REGULATORY CONTEXT

All hatchery operations should be conducted in compliance with relevant national and international regulatory frameworks governing aquatic animal health, biosecurity, environmental protection and traceability.

- Within the European Union this includes, in particular, Regulation (EU) 2016/429 (the Animal Health Law) and its delegated and implementing acts, including provisions related to biosecurity measures (Article 25), traceability and surveillance (Articles 64–68), and disease notification obligations (Articles 8–12).
- Internationally recognized technical standards may also apply, including the Aquatic Animal Health Code of the World Organisation for Animal Health (WOAH, formerly OIE), particularly with regard to health certification, disease reporting obligations and pathogen surveillance.
- Additional guidance may be drawn from the FAO Technical Guidelines for Responsible Aquaculture, including recommendations on genetic resource management, environmental sustainability and responsible hatchery practices.

DOCUMENTATION REQUIREMENTS

For biosecurity and traceability purposes, all hatchery activities should be documented and archived for a minimum period of three years, or longer if required by national legislation. Documentation should include records of broodstock origin and health status, quarantine and disinfection procedures, disease monitoring results, mortality records, movements of broodstock and eggs, and the use of chemicals or therapeutants. Maintaining comprehensive documentation supports disease surveillance, regulatory compliance and hatchery management transparency.

SOP-MUL-001: Selection, quarantine and genetic integration

Selection criteria

- **Source:** Prioritize wild-caught adults to maintain genetic diversity and rusticity.
- **Biometric standards:**
  → Females: 1.3–2.5 kg (optimal fecundity).
  → Males: 0.5–1.5 kg (optimal sperm quality).

- **Condition factor (K):**
  → Target K = 1.0–1.2 (calculated as: $K = \frac{\text{Weight (g)}}{\text{Lenght (cm)}^3} \times 100$)
  → Exclude individuals with K <0.9 or >1.3 (indicative of poor health or domestication effects).

Genetic integration

- **Objective:** Prevent domestication-induced loss of fitness (e.g. reduced disease resistance, behavioural changes).

- **Protocol:**
  → Annual integration of wild breeders (minimum 10–20% of broodstock pool).
  → Genetic screening (microsatellite DNA analysis) to confirm diversity maintenance.

Biosecurity entry and quarantine

- **Quarantine duration:** 21–28 days in isolated units with independent water supply.

- **Health monitoring:**
  → Daily observations for clinical signs (e.g. lethargy, lesions, abnormal swimming).
  → PCR testing for *Nodavirus*, *Photobacterium damselae* and *Vibrio* spp. (Day-7 and Day-14).

- **Release criteria:**
  → No clinical signs or positive PCR results.
  → Disinfection of quarantine tanks post-use (sodium hypochlorite 200 ppm, 1 h contact).

Transport hygiene

- **Vehicle disinfection:**
  → Pre-transport: High-pressure wash with detergent, followed by iodine (500 ppm) or chlorine (2 000 ppm).
  → Post-transport: Repeat disinfection; dry under UV light for 24 h.

- **Water quality during transport:**
  → DO >7 mg/L, temperature 18–22 °C, pH 7.5–8.2.
  → Aeration: Continuous with backup O_2 supply.

SOP-MUL-002: Gamete assessment and clinical hygiene

Sedation and biopsy

- **Anaesthesia protocol:**
  → Clove oil (0.08%) for 5–10 min (until operculum movement ceases).
  → Monitoring: Gill ventilation rate (target: 40–60 beats/min).

- **Gamete collection:**
  → Females: Abdominal massage or catheterization (sterile, single-use catheters).
  → Males: Strip sperm into sterile containers; avoid urine contamination.

Equipment sterilization

- **High-level disinfection:**
  → Heat-tolerant tools: Autoclave (121 °C, 20 min).
  → Heat-sensitive tools (e.g. catheters):
    - Glutaraldehyde (2%) for 3 h (sporicidal activity).
    - Final rinse: Sterile water (0.22 µm filtered) to remove residues.

- **Surface disinfection:**
  → Workbenches and tanks: Wipe with 70% ethanol followed by UV exposure (30 min).

SOP-MUL-003: Low sedation protocol for hormonal induction via intramuscular injection

To minimize stress and maximize hormonal uptake during GnRHa administration in *M. cephalus* broodstock, using a low-sedation protocol that maintains operculum movement and reflex responsiveness while ensuring immobility for precise injection.

Pre-injection acclimation and sedation

- **Environmental preparation:**
  → Timing: Conduct injections ≥1 h post-transfer to broodstock tanks to reduce acute stress.
  → Water level adjustment: Reduce water depth to 25–30 cm to facilitate handling. Stop water inflow to prevent hydraulic disturbance during sedation.

- **Low-sedation protocol:**
  → Agent: Clove oil (eugenol) at 0.01% (100 mg/L).
  → Rationale: Induces calm immobility without deep anaesthesia (operculum rate maintained at 60–80% of baseline).
  → Preparation: Dilute 100 µl clove oil in 1 L ethanol (95%), then add to 10 L tank water (final concentration: 0.01%). Mix thoroughly.
  → Sedation endpoint: Loss of equilibrium (fish remains upright but immobile). Retained reflex response (tail pinch elicits movement).
  → Duration: 5–10 min (monitor closely; prolong, if necessary).

  Note: Avoid oversedation (operculum rate <40 beats/min indicates excessive depth).

Hormonal dosage and preparation

Sex	GnRHa dose	Enantone® equivalent	Example calculation
Female	200 µg/kg BW	53.3 µl/kg	1.5 kg female → 80 µl (1.5 × 53.3)
Male	100 µg/kg BW	26.7 µl/kg	1.1 kg male → 29.4 µl (1.1 × 26.7)

- **Preparation steps:**
  → Vial handling: Warm Enantone® to room temperature (20–25 °C) before use.
  → Syringe priming: Use 1 ml insulin syringes (29G needle) for precise volume delivery.
  → Dose verification: Cross-check calculations with a second technician.

Injection administration

- **Restraint technique:**
  → Method: "Knee-and-towel" restraint to minimize stress and movement.
  → Cover the head with a damp towel to reduce visual stimuli.
  → Position the fish dorsally between the operator's knees, with the ventral side exposed.
  → Stabilize the body gently but firmly to prevent lateral movement.

- **Injection site and technique:**
  → Site: Epaxial musculature, 1–2 cm below the dorsal fin (avoid spinal column).
  → Needle insertion: Angle: 45° to the body surface. Depth: 5–7 mm (penetrate scales but avoid visceral organs).
  → Injection: Slow, steady deposition (3–5 s) to prevent leakage. Withdraw needle and apply gentle pressure with a sterile swab to the site.

- **Safety note:**
  → Single-use needles to prevent cross-contamination.
  → Disinfect injection site pre- and post-procedure with 70% ethanol.

Post-injection recovery and monitoring

- **Environmental restoration:**
  → Water flow resumption: Immediately reactivate pumps post-injection. Water exchange rate: 30%/h to flush metabolic waste and residual clove oil.
  → Oxygenation: Maintain dissolved oxygen (DO) at saturation (~8 mg/L) via aeration stones or pure O_2 diffusion.

- **Post-sedation monitoring:**
  → Recovery criteria: Operculum rate: return to ≥100 beats/min. Equilibrium: regain normal swimming posture within 10–15 min.
  → Stress Indicators: Erratic swimming or surface gasping → increase aeration. Lethargy >30 min → check for overdose (transfer to clean, highly oxygenated water).

- **Documentation:**
  → Record the following: Fish ID/tank number, sedation time (start/end), hormone dose administered, post-injection behaviour (normal/abnormal) and name of the operator.

Emergency protocols

- **Overdose response:**
  → Symptoms: Loss of reflexes, operculum rate <20 beats/min.
  → Action: Transfer to freshwater bath (0% salinity, 18–20 °C) for 5 min to accelerate clove oil elimination.

- **Injection site infection:**
  → Symptoms: Localized redness, swelling or ulceration.
  → Action: Isolate fish; treat with topical iodine (10 ppm, 5 min).

SOP-MUL-004: Egg collection and advanced disinfection

Harvesting and initial handling

- **Collection:**
  → Buoyancy-based separation: Collect positively buoyant eggs (viable).
  → Layer thickness: <1 cm in incubation trays to maximize oxygen diffusion.

- **Transport:**
  → Use sterile, aerated containers (DO >8 mg/L).

Hormonal dosage and preparation

Agent	Concentration	Contact time	Target pathogens
Active Iodine	50 ppm	30 min	Bacteria, fungi (*Saprolegnia*)
H_2O_2	350 µl/L	15 min	Viruses, bacterial biofilms

- **Transport:**
  → Rinse 3x with sterile seawater.
  → UV treatment: Expose eggs to UV-C (254 nm, 30 mJ/cm²) to inactivate residual pathogens.

Physical barriers and special techniques

- **Incubation water:**
  → Filtration: 5 µm mechanical filter + UV sterilization.
  → Flow rate: 0.5–1.0 L/min to prevent sedimentation.

- **Field-based repopulation:**
  → Vibert boxes: Bury fertilized eggs in gravel to mimic natural development (for restocking programmes).

SOP-MUL-005: Environmental and nutritional protocols for larval rearing

Water quality standards

Parameter	Target range	Critical limits	Monitoring frequency
Temperature	20–24 °C	<18 °C or >26 °C	Continuous
Salinity	32–41 ppt	<30 ppt or >45 ppt	Daily
Dissolved Oxygen	>7 mg/L	<5 mg/L	Hourly
pH	7.8–8.2	<7.5 or >8.5	2x daily
Ammonia (NH_3)	<0 mg/L	>0.1 mg/L	2x daily
Nitrite (NO_2^-)	<0.1 mg/L	>0.2 mg/L	2x daily

Flow dynamics and surface hygiene

- **Water circulation:**
  → Uniform flow (avoid "dead zones"); use air stones for gentle turbulence.

- **Surface skimming:**
  → Deploy protein skimmers (D3–D7) to remove oily films (critical for swim bladder inflation).

Nutritional sequence

Developmental stage	Day post hatch (DPH)	Feed type	Density	Enrichment
Yolk-sac larvae	D0–D2	None	–	–
First feeding	D2–D3	Rotifers (*Brachionus*)	8–12 ind./ml	DHA/Selco
Transition phase	D12–D25	*Artemia* nauplii	0.3–0.5 ind./ml	Algamac 3000
Weaning	D25–D40	Inert feed (50–10 µm)	Gradual increase	Phospholipids

Live feed hygiene

- ***Artemia* hatching containers:**
  → Disinfect post-use with chlorine (2 000 ppm, 1 h).
  → Rinse 3x with sterile seawater before reuse.

SOP-MUL-006: Cleaning cycles and the "sanitary break"

Mechanical and chemical cleaning

Agent	Concentration	Contact time	Application
NaOH (caustic soda)	2–5%	12 h	Cement basins (protein removal)
Sodium hypochlorite	2 000 ppm	1 h	All surfaces

Neutralization and sanitary break

- **Final rinses:**
  → 3x rinses with freshwater; test for residual chlorine (0 ppm).

- **Sanitary break:**
  → Dry tanks for 48–72 h (exposure to sunlight/UV disrupts pathogen life cycles).

SOP-MUL-007: Perimeter defence and pest control

Vector protection

- **Anti-bird netting:**
  → 100% coverage of rearing units to prevent heron/cormorant access (vectors for *Nodavirus*).

- **Footbaths:**
  → Iodine (500 ppm) at all entry points.

Rodent and pest control

- **Rodent control plan:**
  → Warfarin-based baits (placed in locked stations).
  → Monthly inspections; maintain pest sighting log.

- **Insect control:**
  → UV light traps for flying insects.
  → Larvicides (e.g. *Bacillus thuringiensis*) for mosquito control.

SOP-MUL-008: SURVEILLANCE, TRAINING AND RECORD-KEEPING

Mandatory documentation

All records must be maintained for ≥3 years and include:

- **Visitor log:**
  → Name, origin, date, and biosecurity compliance (footbath use, hand disinfection).

- **Movement register:**
  → Fish/egg/gamete transfers (source, destination, quantity, health status).

- **Mortality log:**
  → Daily counts; report "anomalous mortality" (>5%/day) to veterinarian immediately.

- **Disinfection log:**
  → Chemical used, concentration, contact time, and operator signature.

Training and compliance

- **Mandatory Training Topics:**
  → Biosecurity principles (zoning, disinfection, personal protective equipment).
  → Disease recognition (clinical signs of VNN, *Vibriosis*).
  → Prudent chemical use (safety data sheets for all disinfectants).

- **Antibiotic policy:**
  → Prohibited in restocking programs.
  → Veterinary oversight required for any therapeutic use (per EU 2019/6).

Appendix VII – *Mugil cephalus* common diseases and parasites

Disease	Agent	Type	Syndrome	Measures
		Viral disease		
Viral Erythrocytic Necrosis (VEN)	*Iridovirus*	Virus (dsDNA)	Anaemia: Fish may exhibit signs of anaemia due to the destruction of red blood cells. Pale gills: The gills may appear pale or anaemic because of the reduced number of circulating red blood cells. Weakness: Infected fish may show decreased activity, lethargy, and abnormal swimming behaviour. Haemorrhages: Internal bleeding may occur, leading to visible haemorrhages on the skin or internal organs.	Vaccination; environmental improvement.
Viral Nervous Necrosis Virus (VNNV)	*Betanodavirus*	Positive-strand RNA viruses	Abnormal swimming behaviour, loss of coordination, and high mortality in larvae and juvenile fish.	
		Bacterial disease		
Red pest of eels, red sore, red boil, saltwater furunculosis	*Vibrio anguillarum, Vibrio alginolyticus*	Bacterium Gram-negative	Systemic infection; acute haemorrhagic and septicaemic disease with mass mortality; anorexia; darkening; abdominal distension, dermal haemorrhages; skin ulcers; occurrence of exophthalmos.	Antibacterial drugs in feed; environmental improvement.
Streptococcosis	*Enterococcus faecalis*	Bacterium Gram-positive	Haemorrhagic areas on body surface. Abdominal swelling, lethargy, loss of appetite and changes in behaviour.	Antibacterial drugs in feed; environmental improvement.
Bacterial fin rot	*Aeromonas hydrophila*	Bacterium Gram-negative	Haemorrhagic Septicaemia, leading to internal bleeding and swelling; skin lesions, red patches (haemorrhaging), bloated abdomen, and lethargy; breakdown of tissues between fin rays (fin rot).	Antibacterial bath; environmental improvement.
Motile Aeromonad Septicemia (MAS)	*Aeromonas caviae, Aeromonas sobria*	Bacterium Gram-negative	Systemic infection; acute haemorrhagic and septicaemic disease; haemorrhagic spots on the skin and base of fins; ulcers and skin necrosis; exophthalmia and dropsy.	Antibacterial drugs in feed; environmental improvement.
Columnaris disease	*Flexibacter columnaris*	Bacterium Gram-negative	Skin lesions: White to greyish patches, often resembling cotton wool, on the skin, fins and gills. Gills: Fish may show signs of respiratory distress like rapid gill movement and gasping at the water's surface. Mouth ulcers. Affected fish may display abnormal swimming patterns or lethargy.	Antibiotics treatment; isolation; improved water quality.
		Parasitic disease		
Respiratory distress	*Myxobolus goensis*	Protozoan parasite, Cnidaria	Gills, kidneys, and sometimes the skin	Environmental improvement.
Respiratory distress	*Myxobolus ichkeulensis*	Protozoan parasite, Cnidaria	Damage to vital organs, including the gills and kidneys	Environmental improvement.
Skin and scales disease	*Myxobolus episquamalis*	Protozoan parasite, Cnidaria	Skin lesions, red or inflamed patches on the skin, often with a scabby or ulcerated appearance. Scale erosion or loss. A patchy appearance with missing or damaged scales along the fish's body.	Environmental improvement.

APPENDIX VII TABLE CONTINUED

Parasitic disease				
Caligidosis	*Caligus apodus, Caligus mugilis*	Copepod parasite	Infestation occurs commonly on the skin.	Medicated bath; environmental improvement; change environment to freshwater
Respiratory distress	*Nipergasilus bora*	Copepod parasite	External parasite that typically attach to the gills or other parts of the fish's body.	medicated bath; environmental improvement.
Ergasilidosis	*Ergasilus lizae*	Copepod parasite	Infestation occurs commonly in the gills.	Medicated bath; environmental improvement.
Epizootic Ulcerativesyndrome (EUS); Red Spot Disease (RSD); Mycotic Granulomatosis (MG)	*Aphanomyces invadans*	Fungus	Skin ulcers.	Environmental improvement.
Tissue lesions and inflammation	*Ascocotyle* (*Phagicola*) *longa, Haplosplanchnus pachysomus, Diplostomum spathaceum, Tylodelphys clavata, Heterophyes* sp., *Posthodiplostomum* sp.	Trematoda	Localized tissue damage at the site of infection, especially in muscle, eyes or organ tissues.	Environmental improvement.
Marine velvet disease	*Amyloodinium ocellatum*	Dinoflagellate	Inflict moderate-to-intense tissue reactions associated with serious gill hyperplasia, inflammation, haemorrhages and necrosis with subsequent death in less than 12 h in heavy infected specimens.	Freshwater baths are an effective way to detach trophonts from skin and gill epithelium due to the sudden osmotic shock experienced by the host. Peroxide H_2O_2 bath.
Respiratory distress	*Trichodina puytoraci, Trichodina lepsii*	Ciliated protozoan	Gill and skin damage, redness, lesions and thickening.	Water treatment; formalin or copper sulphate.
Thorny-headed worm infection	*Neoechinorhyncus agilis*	Acanhocephala (Nematoda)	Damage caused by the parasite's attachment to the intestinal wall.	Chemical treatments; broad-spectrum anthelmintics.

Appendix VIII – **Mullet aquaculture and capture production and value statistics (2015–2024)**

GENERAL NOTES

1) Production quantities of fish, crustaceans and molluscs are expressed in live weight, that is the nominal weight of the aquatic organisms at the time of capture.
2) Several countries still report their fisheries and aquaculture production at an aggregated level – due to deficiencies in their data collection and reporting systems – which can include production for many species. In these circumstances the production data presented at individual species items are therefore likely to be underestimated. Therefore, when examining the statistics for a particular species, it should be noted that an unknown proportion of the production for that species might have been reported by the national office under the generic, family or order name of the species, or even higher aggregated levels such as, for example, "Marine fishes nei" or "Freshwater fishes nei". Consequently, the production at species level may, in some cases, be underestimated and not reflecting the real production of the individual species.
3) Where necessary, any data published in previous releases of this dataset have been revised. Where the figures in the current release differ from those previously published, the amended data represent the most recent version. Some statistics provided to FAO by national offices, in particular those for the last year, are provisional and may be amended in future editions as better information becomes available and updates are made by national partners.

Symbols used

"E" = FAO estimate from available sources of information

List of tables

- Aquaculture production of flathead grey mullet (*Mugil cephalus*): top producing countries or territories
- Aquaculture production of mullets: top producing countries or territories
- Aquaculture production of flathead grey mullet (*Mugil cephalus*) and other mullets by continent
- Aquaculture production for flathead grey mullet (*Mugil cephalus*), all mullet species, total finfish production, and overall aquaculture output of aquatic animals
- Capture production of flathead grey mullet (*Mugil cephalus*): top producing countries or territories
- Capture production of flathead grey mullet (*Mugil cephalus*) and other mullets by continent
- Capture and aquaculture production of flathead grey mullet (*Mugil cephalus*) and other mullets
- Aquaculture production value of flathead grey mullet (*Mugil cephalus*) and other mullets by continent

Source: FAO. 2026. FishStat: Global aquaculture production 1950–2024. [Accessed on 28 March 2026]. In: FishStatJ. Available at www.fao.org/fishery/en/statistics/software/fishstatj.

Aquaculture production of Flathead grey mullet (*Mugil cephalus*): top producing countries or territories (2015–2024)

	2015	2016	2017	2018	2019	2020	2021	2022	2023	2024
Country/Territory	**Tonnes, live weight equivalent**									
Taiwan Province of China	2 145	1 739	2 244	2 588	2 182	1 520	1 738	1 962	1 760	2 838
Israel	2 250	1 792	1 900	1 900 E	1 900 E	2 100 E	2 000 E	1 950 E	2 050 E	2 050 E
China, Hong Kong SAR	738	1 003	846	928	984	1 088	1 405	993	327	416
Singapore	436	513	361	359	500	597	621	430	354	255
Guyana	27	5	0	0	0	0	0	121	200 E	200 E
Greece	251	315	198	341	251	308	386	270	150	124
Other countries	*1 100*	*967*	*1 216*	*301*	*247*	*268*	*5 631*	*246*	*212*	*52*
WORLD	**6 947**	**6 334**	**6 765**	**6 417**	**6 064**	**5 880**	**11 780**	**5 972**	**5 053**	**5 934**

"E" = estimated value.

Aquaculture production of mullets: top producing countries or territories (2015–2024)

		2015	2016	2017	2018	2019	2020	2021	2022	2023	2024
Country/Territory	**Mullet species**	**Tonnes, live weight equivalent**									
Egypt	Other mullets*	157 179 E	153 776 E	210 213 E	242 071 E	243 974 E	317 807 E	351 197 E	366 828 E	376 607	377 729
Indonesia	Other mullets*	7 176	7 430	323	7 220	7 789	7 865	1 569	8 295	9 181	9 643
Indonesia	Flathead grey mullet (*Mugil cephalus*)	0	0	0	0	0	0	5 366 E	0	0	0
Republic of Korea	Other mullets*	6 843	7 148	6 868	6 440 E	6 700	8 451	10 395	7 756	6 642	6 659
Taiwan Province of China	Flathead grey mullet (*Mugil cephalus*)	2 145	1 739	2 244	2 588	2 182	1 520	1 738	1 962	1 760	2 838
Israel	Flathead grey mullet (*Mugil cephalus*)	2 250	1 792	1 900	1 900 E	1 900 E	2 100 E	2 000 E	1 950 E	2 050 E	2 050 E
China, Hong Kong SAR	Flathead grey mullet (*Mugil cephalus*)	738	1 003	846	928	984	1 088	1 405	993	327	416
Guyana	Other mullets*	20	4	0	0	0	0	0	107	190 E	190 E
Guyana	Flathead grey mullet (*Mugil cephalus*)	27	5	0	0	0	0	0	121	200 E	200 E
Italy	Flathead grey mullet (*Mugil cephalus*)	690 E	700 E	700 E	0	0	0	0	0	0	0
Italy	Other mullets*	90 E	100 E	100 E	231	2 910 E	264	529	2 500	351	321
Singapore	Flathead grey mullet (*Mugil cephalus*)	436	513	361	359	500	597	621	430	354	255
Greece	Flathead grey mullet (*Mugil cephalus*)	251	315	198	341	251	308	386	270	150	124
Other countries		*1 839*	*354*	*659*	*363*	*365*	*354*	*391*	*365*	*336*	*181*
WORLD		**179 684**	**174 879**	**224 413**	**262 440**	**267 555**	**340 353**	**375 597**	**391 577**	**398 147**	**400 606**

"E" = estimated value.

Aquaculture production of Flathead grey mullet (*Mugil cephalus*) and other mullets by continent (2015–2024)

		2015	2016	2017	2018	2019	2020	2021	2022	2023	2024
Continent	**Mullet species**	**Tonnes, live weight equivalent**									
Africa		*157 545*	*153 997*	*210 716*	*242 335*	*244 264*	*318 115*	*351 529*	*367 141*	*376 886*	*377 835*
	Flathead grey mullet (*Mugil cephalus*)	363	217	466	251	247	268	265	246	212	39
	Other mullets*	157 182	153 780	210 250	242 084	244 017	317 847	351 264	366 895	376 674	377 796
Asia		*20 070*	*19 675*	*12 636*	*19 485*	*20 055*	*21 620*	*23 094*	*21 386*	*20 313*	*21 907*
	Flathead grey mullet (*Mugil cephalus*)	5 616	5 097	5 402	5 825	5 566	5 304	11 129	5 335	4 491	5 558
	Other mullets*	14 454	14 578	7 234	13 660	14 489	16 316	11 965	16 051	15 822	16 348
Europe		*2 022*	*1 197*	*1 061*	*621*	*3 236*	*618*	*973*	*2 820*	*557*	*473*
	Flathead grey mullet (*Mugil cephalus*)	941	1 015	898	341	251	308	386	270	150	137
	Other mullets*	1 081	183	164	280	2 985	311	587	2 551	407	336
Latin America and the Caribbean		*46*	*9*	*0*	*0*	*0*	*0*	*1*	*229*	*391*	*391*
	Flathead grey mullet (*Mugil cephalus*)	27	5	0	0	0	0	0	121	200	200
	Other mullets*	20	4	0	0	0	0	1	108	191	191

* "Other mullets" might include an unknow proportion of *Mugil cephalus* production, along with any other species of mugilidae.

Aquaculture production for Flathead grey mullet (*Mugil cephalus*), all mullet species, total finfish production, and overall aquaculture output of aquatic animals (2015–2024)

	2015	2016	2017	2018	2019	2020	2021	2022	2023	2024
Aquatic species	**Tonnes, live weight equivalent**									
Flathead grey mullet (*Mugil cephalus*)	6 947	6 334	6 765	6 417	6 064	5 880	11 780	5 972	5 053	5 934
All mullets	179 684	174 879	224 413	262 440	267 555	340 353	375 597	391 577	398 147	400 606
All finfish	49 082 657	51 083 987	52 741 967	54 598 686	56 330 991	57 693 416	59 738 443	61 784 799	63 947 170	66 257 339
All aquaculture production of aquatic animals	73 010 745	76 641 303	79 761 982	82 667 332	85 249 860	87 843 607	91 374 589	94 734 561	98 821 015	102 663 709
Share of flathead grey mullet (*Mugil cephalus*) in the production of all mullet species	3.87%	3.62%	3.01%	2.45%	2.27%	1.73%	3.14%	1.53%	1.27%	1.48%
Share of flathead grey mullet (*Mugil cephalus*) in the production of all finfish	0.01%	0.01%	0.01%	0.01%	0.01%	0.01%	0.02%	0.01%	0.01%	0.01%
Share of flathead grey mullet (*Mugil cephalus*) in aquaculture production of aquatic animals	0.01%	0.01%	0.01%	0.01%	0.01%	0.01%	0.01%	0.01%	0.01%	0.01%

Capture production of Flathead grey mullet (*Mugil cephalus*): top producing countries or territories (2015–2024)

	2015	2016	2017	2018	2019	2020	2021	2022	2023	2024
Country/Territory	**Tonnes, live weight equivalent**									
China *	126 733	103 894	102 102	90 800	83 344	73 365	77 324	69 534	69 611	69 028
Mauritania	0	0	0	5 723	3 650 E	3 430 E	12 895	9 690 E	9 880 E	10 982
Pakistan	0	0	0	0	0	0	0	0	0	10 641
Mexico	10 572	14 284	11 735	15 998	13 183	9 583	8 197	8 509	9 424	9 799
Republic of Korea	3 142	2 057	2 000	4 961	5 293	4 114	4 484	4 888	3 872	5 610
Oman	0	0	0	0	0	0	0	0	3 549	3 540 E
United States of America	6 044	6 089	5 111	4 721	4 189	3 331	3 911	4 837	3 001	3 198
Venezuela (Bolivarian Republic of)	0	2 814	2 368 E	1 671 E	1 580 E	1 853 E	1 700 E	1 685 E	1 740 E	1 855 E
Greece	1 143	2 315	2 164	2 016	2 042	1 895	1 445	1 506	1 346	1 357
Taiwan Province of China	1 306	1 838	1 445	1 143	1 624	1 044	641	569	610	836
Other countries	*1 228*	*1 342*	*2 013*	*2 060*	*2 506*	*1 996*	*7 653*	*4 467*	*4 148*	*2 390*
WORLD	**150 167**	**134 632**	**128 938**	**129 093**	**117 411**	**100 611**	**118 250**	**105 686**	**107 180**	**119 236**

* For statistical purposes, the data for China do not include Hong Kong Special Administrative Region (Hong Kong SAR), Macao SAR and Taiwan Province of China.
"E" = estimated value.

Capture production of Flathead grey mullet (*Mugil cephalus*) and other mullets by continent (2015–2024)

		2015	2016	2017	2018	2019	2020	2021	2022	2023	2024
Continent	**Mullet species**	**Tonnes, live weight equivalent**									
Africa		*92 904*	*80 442*	*94 441*	*123 433*	*114 799*	*118 243*	*197 342*	*193 461*	*216 265*	*260 859*
	Flathead grey mullet (*Mugil cephalus*)	736	612	686	6 528	4 412	4 194	13 820	10 624	10 661	11 743
	Other mullets*	92 168	79 830	93 755	116 905	110 387	114 049	183 522	182 838	205 604	249 116
Asia		*426 362*	*390 187*	*430 065*	*372 570*	*378 557*	*354 915*	*366 864*	*362 405*	*348 946*	*334 633*
	Flathead grey mullet (*Mugil cephalus*)	131 181	107 789	105 547	96 904	90 261	78 523	82 449	74 991	77 641	89 654
	Other mullets*	295 181	282 398	324 518	275 666	288 296	276 392	284 416	287 415	271 305	244 979
Europe		*8 392*	*12 390*	*10 837*	*12 942*	*12 620*	*11 256*	*11 235*	*11 369*	*11 288*	*10 986*
	Flathead grey mullet (*Mugil cephalus*)	1 634	3 044	3 491	3 272	3 328	2 697	2 307	2 434	2 133	2 058
	Other mullets*	6 757	9 346	7 346	9 671	9 292	8 560	8 928	8 934	9 155	8 928
Latin America and the Caribbean		*52 607*	*66 408*	*82 917*	*85 087*	*69 591*	*50 801*	*62 507*	*60 293*	*56 266*	*54 353*
	Flathead grey mullet (*Mugil cephalus*)	10 572	17 098	14 103	17 669	15 221	11 866	15 763	12 800	12 875	11 866
	Other mullets*	42 035	49 310	68 814	67 418	54 370	38 935	46 744	47 493	43 390	42 486
Northern America		6 213	6 227	5 252	4 938	4 382	3 519	4 154	5 030	3 216	3 457
	Flathead grey mullet (*Mugil cephalus*)	6 044	6 089	5 111	4 721	4 189	3 331	3 911	4 837	3 001	3 198
	Other mullets*	169	138	141	217	193	188	243	193	215	259
Oceania		6 805	6 422	6 245	6 087	6 147	6 781	6 550	5 081	5 683	5 183
	Flathead grey mullet (*Mugil cephalus*)	0	0	0	0	0	0	0	0	869	717
	Other mullets*	6 805	6 422	6 245	6 087	6 147	6 781	6 550	5 081	4 815	4 466
WORLD		**593 283**	**562 077**	**629 757**	**605 057**	**586 096**	**545 515**	**648 652**	**637 639**	**641 664**	**669 470**
	Flathead grey mullet (*Mugil cephalus*)	**150 167**	**134 632**	**128 938**	**129 093**	**117 411**	**100 611**	**118 250**	**105 686**	**107 180**	**119 236**
	Other mullets*	**443 116**	**427 444**	**500 819**	**475 963**	**468 685**	**444 904**	**530 402**	**531 953**	**534 484**	**550 234**

* "Other mullets" might include an unknow proportion of *Mugil cephalus* production, along with any other species of mugilidae.

Capture and aquaculture production of Flathead grey mullet (Mugil cephalus) and other mullets (2015–2024)

		2015	2016	2017	2018	2019	2020	2021	2022	2023	2024
Aquaculture/Capture	**Mullet species**	**Tonnes, live weight equivalent**									
Aquaculture production	Flathead grey mullet (*Mugil cephalus*)	6 947	6 334	6 765	6 417	6 064	5 880	11 780	5 972	5 053	5 934
Capture production	Flathead grey mullet (*Mugil cephalus*)	150 167	134 632	128 938	129 093	117 411	100 611	118 250	105 686	107 180	119 236
Share of aquaculture production		4.42%	4.49%	4.99%	4.74%	4.91%	5.52%	9.06%	5.35%	4.50%	4.74%
Aquaculture production	Other mullets*	172 737	168 546	217 647	256 024	261 491	334 473	363 816	385 604	393 094	394 672
Capture production	Other mullets*	443 116	427 444	500 819	475 963	468 685	444 904	530 402	531 953	534 484	550 234
Share of aquaculture production		*28.05%*	*28.28%*	*30.29%*	*34.98%*	*35.81%*	*42.92%*	*40.69%*	*42.03%*	*42.38%*	*41.77%*

* "Other mullets" might include an unknow proportion of *Mugil cephalus* production, along with any other species of mugilidae.

Aquaculture production value of Flathead grey mullet (*Mugil cephalus)* and other mullets by continent (2015–2024)

		2015	2016	2017	2018	2019	2020	2021	2022	2023	2024
Continents	**Mullet species**	**Value (USD 1 000)**									
Africa		*405 773*	*360 619*	*389 174*	*491 605*	*575 545*	*804 762*	*1 168 202*	*1 252 644*	*906 939*	*617 276*
	Flathead grey mullet (*Mugil cephalus*)	925	606	1 156	664	589	774	759	634	546	100
	Other mullets*	404 848	360 013	388 018	490 941	574 956	803 988	1 167 443	1 252 010	906 393	617 176
Asia		*72 156*	*71 188*	*71 963*	*77 805*	*78 042*	*84 238*	*116 075*	*109 530*	*92 492*	*92 523*
	Flathead grey mullet (*Mugil cephalus*)	20 364	20 985	23 156	23 381	25 032	24 832	35 132	26 097	22 582	24 716
	Other mullets*	51 792	50 203	48 807	54 424	53 010	59 406	80 942	83 433	69 909	67 807
Europe		*9 003*	*6 819*	*6 704*	*2 266*	*25 204*	*3 152*	*4 729*	*16 426*	*3 941*	*3 361*
	Flathead grey mullet (*Mugil cephalus*)	5 486	5 595	5 525	644	629	828	881	681	507	463
	Other mullets*	3 517	1 224	1 178	1 622	24 575	2 324	3 848	15 745	3 434	2 898
Latin America and the Caribbean		*104*	*4*	*0*	*0*	*0*	*0*	*1*	*330*	*563*	*562*
	Flathead grey mullet (*Mugil cephalus*)	59	2	0	0	0	0	0	175	288	288
	Other mullets*	44	2	0	0	0	0	1	155	275	275
WORLD		***487 036***	***438 630***	***467 840***	***571 676***	***678 791***	***892 152***	***1 289 007***	***1 378 931***	***1 003 934***	***713 722***
	Flathead grey mullet (*Mugil cephalus*)	**26 834**	**27 189**	**29 837**	**24 689**	**26 250**	**26 434**	**36 772**	**27 587**	**23 923**	**25 567**
	Other mullets*	**460 202**	**411 441**	**438 003**	**546 987**	**652 541**	**865 718**	**1 252 235**	**1 351 344**	**980 011**	**688 155**

* "Other mullets" might include an unknow proportion of *Mugil cephalus* production, along with any other species of mugilidae.

Notes and observations

This technical manual provides practical guidance on the hatchery production of the flathead grey mullet, *Mugil cephalus*, a high-value, low-trophic marine species with strong potential for sustainable aquaculture development. It compiles current knowledge and tested methodologies covering the entire production cycle, from broodstock management and captive reproduction to larval rearing, fry production and grow-out.

The manual presents the biological and developmental characteristics of the species, together with practical recommendations for hatchery site selection, design and operational management. It also describes live feed production, including microalgae, rotifers and Artemia, as well as health management and disease prevention in hatchery systems.

Based on experimental research validated at laboratory and pilot scales, the techniques described are suitable for application in commercial hatcheries and adaptable to both small- and large-scale operations. By supporting reliable seed production and improved culture practices, this publication contributes to the sustainable expansion of aquaculture in line with the FAO Blue Transformation Roadmap and the principles of the Guidelines for Sustainable Aquaculture.

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