CHAPTER

12

Biology of Mullets - Revisited

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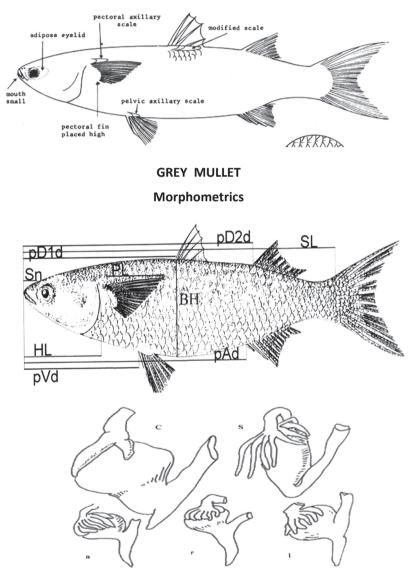
Members of the family mugilidae commonly known as mullets are one of the commercially important teleosts found in the coastal waters of the World. They have a Worldwide distribution including tropical, subtropical and temperate seas. Apart from inhabiting coastal and offshore waters, many mullets inhabit part or whole of their lifetime in coastal lagoons, lakes and even rivers. Mullets are moderate to large sized fishes reaching a maximum size of 120 cms. SL, but commonly reaching 30 cms. These fishes have a sub-cylindrical body, head often broad and flat dorsally. They have two widely separated dorsal fins. The first dorsal has 4 spines and the second one is with an unbranched ray and 6 to 10 branched rays. The pelvic fins are sub-abdominal with one spine and five branched rays. The anal fin has 2-3 spines and 8-12 branched rays. Lateral line is absent. Adults have ctenoid scales. The mouth is of moderate size with small labial or missing teeth. Their gill arches are long and they have a muscular stomach with a long intestine.

Taxonomic studies

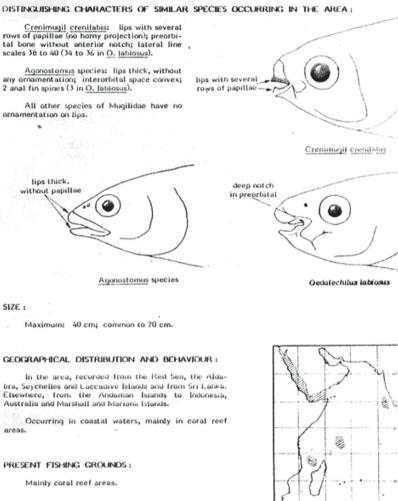
Thomson (1997), Eschmeyer & Fong, (2015), and Eschmeyer (2015) have done exhaustive work on systematics of mullets. Vide Eschmeyer (2015), there are 20 genera and 71 species. As of 2017, there are 75 species within the order Mugiliformes in the World (Thomson ,1977, Eschmayer & Fong, 2017). Currently there are conflicts on taxonomy of mullets at the generic level as well as the species level.

The taxonomy of mullets is mainly based on external morphology, meristics, morphometrics and the structure of some internal organs. Dentition, number of pyloric caeca, alimentary canal, otolith, morphology of the cephalic lateral line canals, pharyngo-branchial organs, pigmentation and melanophore pattern, are the characteristics used by many in taxonomy and identification of adults and fry. The adipose eyelid which is a fatty deposition on the head around the eyes, generally transparent and mostly well developed in adults is mentioned in most of the taxonomic keys and descriptions. The pyloric caeca can be of some taxonomic importance especially among different genera. Their number varies from 2 to 46 (generally between 5 and 8). The teeth are equally important in taxonomy. The teeth may be a single row or many rows and the shapes also vary from ciliform, setiform, caniniform, bicuspid, tricuspid etc. The head is equally important. The position and relationships of the different anatomical elements such as jaws, nostrils, lips and eyes, operculum and preorbital bones, jugular spines etc. aid in taxonomy. The preorbitals, a pair of triangular bones situated obliquely in front of the eyes are very useful in taxonomy. So also, the position of nostrils is equally useful in taxonomy. The presence or absence of axillary scales also aid in taxonomy. Among the meristic characters, the number of scales in the lateral series, the transverse scale count, the number of spines and rays in the fins are used in taxonomic study of these fishes. Morphometric differentiation of mullets such as the linear measurements are equally important along with molecular genetics or geometric morphometrics. The mullet taxonomy which is in a crisis can be

tackled by an integration of all the above. Concerning phylogeny of the family Mugilidae, it appears exceptionally obscure at both the intra and inter-specific levels, it is extremely challenging to distinguish among species (Papasotiropoulos et al., 2002). The effectiveness of the CO1 gene in Mitochondrial DNA has been promising in distinguishing morphologically cryptic organisms including fishes. Chew et al., (2018), successfully conducted a study on identification of mullet species of Setiu Wetland, Malaysia using the approaches of morphological assessment using CO1 gene analysis.



Pyloric caeca



CATCHES, FISHING GEAR AND FORMS OF UTILIZATION :

Separate statistics are not reported for this species.

Caught with gillnets, liftnets and seines.



Morphological characters helping in taxonomy

Distribution

The mullets are distributed in coastal regions of tropical, subtropical and temperate areas. Mullets are distributed approximately from 42R"-degree N to 42R" S. They are found in marine, inshore waters, lakes, estuaries and fresh water systems. Since mullets are euryhaline, they can tolerate salinities from 0 to 80 ppt., inhabiting hypersaline to brackish water lagoons, estuaries and fresh water systems (Gonzalez-Castro,2007). The biogeography and distribution of mullets remain unclear mainly because of the difficulty in separating species based on morphological characters. However biogeographic characterization is essential for developing ecological and conservation planning. Recent advances in molecular, phylogenetic and phylogeographic analysis provide new tools for better understanding of the distribution and the biogeographic pattern of mullets of the World.

Food and feeding habits

The Grey mullets have always been described as mud eaters, iliophagous, detritus feeders, deposit feeders and interface feeders (Brustle,1981). This is mainly because the diet of majority of mullet species is based on the organic matter present in the bottom sediment. They can also exploit benthic invertebrates, green filamentous microalgae, as well as plankton. While feeding, the mullets angle their heads downward, protract the premaxillaries and scrape the surface of the sediment. They take a mouthful of sediment and associated food items. (Odum, 1970, King, 1988). After working on the material between the pharyngeal bones, they reject some particles through their gills and mouth. The toothed lips help in scrapping microbial films, the pharyngeal teeth help in removing large sediment particles from the oral cavity and the densely packed gill rakers help in retaining finer particles. A two chambered stomach with a powerful gizzard helps in breaking cell walls and the long intestine facilitates digestion of plant materials. The organic content in the upper layer of the sediment ranges from 10 to 250 mg./gm. dry weight.

The stomach contents of mullets are a fine compact paste without microscopic details. When the contents are observed under low power, only the largest prey such as the red larvae of chironomid midges and green filamentous algae are observed amidst sand grains, mud and detritus. When observed under high power, we can observe highly diversified and abundance of single celled algae and a few copepods. A standard method of analyzing the stomach contents is as follows. The stomach contents are first mixed with 70% ethanol. (1:3, sample - ethanol rates) and stirred. A subsample is taken and observed under high power for identification of microscopic algae. The process is repeated several times and the number of species counted. At this point, the sediment sample is stained with Bengal Rose to make evident transparent invertebrates and count them under a dissecting microscope. The presence of filamentous algae, sand and detritus are recorded. A small subsample of the stomach content in ethanol may also be preserved without staining for future work. However, such examination does not give a full picture since the heterotrophic microbes are seldom detected or quantified accurately. Complex methods using stable isotope ratios and fatty acid markers are modern tools in dietary studies.

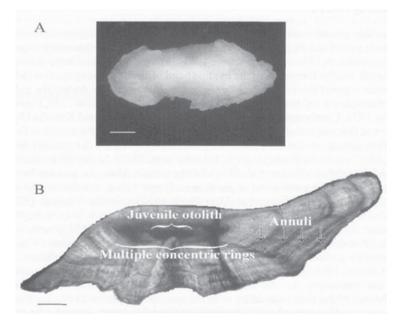
The food and feeding of the larvae are entirely different compared to the adults. In the larvae, the mouth opens three days after hatching. Even though very little is known about the food of larvae in nature the diet of hatchery reared larvae is known. Post larvae measuring 10-30 mm. TL usually referred to as fry are primarily zooplankton feeders in all species of mullets studied. Harpacticoid and cyclopoid copepods form the main prey of mullet fry in brackish water environments, whereas cyclopoid copepods, cladocerans and rotifers dominate the food of fry living in oligohaline and freshwater environments. The prey in general range in length between 700 –

1000 µm.

After recruitment to estuaries, juveniles of 20-55 mm length undergo a gradual transition from planktonic carnivory to benthic diet (de Silva,1997). The amount of plant materials and detritus increase steadily and the fish larger than 40-50 mm total length have an adult diet. The stomach contents of mullet fingerlings larger than 8 cms. is dominated by sand, detritus, microalgae and polychaetes. There are also a few mullets that feed on surf diatoms such as *Liza richardsoni, Liza ramnada*. *M.cephalus* is capable of feeding on zooplankton and phytoplankton when these occur in dense blooms.

Age and Growth and methods of studying the same

Growth is understood as the increase in length or weight generated by physiological processes and it is directly related to age. Environmental factors like temperature, availability of food, salinity and photoperiod influence mullet growth. *M.cephalus* has the greatest growth rates compared to other species. The annual migrations of mullets to coastal water from estuaries for spawning induce large variations in the growth rate that can change year after year with age as also with the biotope. Added to this are also other variations due to the long spawning period and the sexual physiology. Populations that are denied access to the Ocean for breeding and therefore do not spawn may exhibit greater length and weight showing that the energy used for gonad maturation may be used for somatic growth. The most frequently used method is the interpretation and counting of growth zones or of "stop growth annuli" which appear in the scale. Other hard parts of mullets such as otoliths, operculum, fin rays, and other methods such as tagging and length frequency distribution of a population (Peterson method) have also been used simultaneously with scale reading to get



authentic data. Generally, the scales used for age determination are situated under the first dorsal fin or the middle part of the body or at the extremity of the pectoral fin. The annuli (winter rings, marks, breaks or checks) which are the boundaries between two successive growth zones) are formed annually. However, there are some practical problems in age determination using the scales in mullets compared to other fishes since the markings on the scales are not always visible. In general, the rate of growth is highest during the first and sometimes the second year of life and noticeably slows down after sexual maturity. A linear relationship exists between scale radius and body length and between otolith radius and body length (Thomson,1951). This makes it possible to construct individual growth histories from the widths of scales or otolith increments. Female mullets grow faster than the males.

Sexuality and Reproduction of mullets

Mullets are heterosexual or gonochoristic. Cytological evidence of sexual differentiation begins in *M.cephalus* at lengths over 15 cms. SL. Normally adult females are larger than males. Males mature earlier and are smaller in size compared to females. There is however no sexual dimorphism and it is impossible to distinguish males from females, except the bulge of the belly of the females having growing ovaries. It has been observed that the girth of adult females (mature females) tend to increase by 3 cms. during the spawning season contributing to their selection in the nets. Female mullets are thought to be isochromal spawners, i.e. producing a single clutch of eggs per year. However, an individual may not release all eggs at once. A soft pressure applied near the vent brings out a drop of milt in case the males are ripe. Mullets are oviparous wherein the individuals shed their gametes into the water and fertilization is external.

Male reproductive system

The testes are tubular, longitudinal and paired. They are suspended in the cavity by mesenteries and lie lateral to the gas bladders. Spermatogonia occur along the entire length of the tubular organ. The morphology of the testis along with the fact that spermatogenesis takes place in enclosed cysts precludes monitoring of the actual development of the sperm. The stage of gonad maturity according to the shape of the testis and the condition of milt inside can be categorized into six stages. 1 immature, 2 mature, 3 ripening, 4 nearly ripe, 5 ripe, and 6, spent. Mature sperms are stored in the ducts surrounding each testis (vas deferens) and connecting to the urogenital pore (sperm duct) in a fluid called milt. Milt can be extruded from the urogenital pore by applying pressure to each side of the abdomen in an anterior to posterior direction. This process is called "squeeze check". The actual procedure is as follows: after being anaesthetized, the male is turned upside down and the abdomen is gently squeezed three times between the thumb and the fore finger, from mid body to the urogenital pore. If no milt is extruded, the fish is recorded as negative. If milt is extruded the male is staged as a +1, +2 or +3 depending on whether the amount of milt which came out is scanty or copious.

Female reproductive system

Ovaries are paired and hollow consisting of two ovarian lobes separated by a septum. The lobes are joined near the urogenital pore. A number of oviferous folds project into the ovarian

cavity which are lined by the germinal epithelium and which contain nests of oogonia. In *M. cephalus* it was observed that more than 83% of the specimens above 20 cm had become sexually differentiated. Total length at 50% maturity is as low as 22/24 cms. (male /female) which correspond to an age of two years.

Stages of oocyte development in mullets

Oogonia are small cells with a large nucleolus in the center and are observed in immature specimens or in post spawning females. The process which involves the transformation of oogonia to oocytes is called oogenesis. The stages in oogenesis are briefly described below.

a. Previtellogenic stage (primary growth stage)

In this stage, in mullets oocytes reach a diameter which varies from 50 to 200 μ m, polyhedral in shape and rounded nucleoli. At the peri nucleolus stage concomitant with the growth of the oocytes, the nucleus increases in size to form the germinal vesicle.

b. Cortical alveolar stage (secondary growth stage)

The oocyte measures from 200 to $300 \ \mu$ m. In this stage the endogenous vitellogenesis starts. The zona radiata, an acellular vitelline envelope which develop around the oocyte, continues to differentiate and increase in complexity throughout the oocyte growth.

c. Vitellogenesis (3 stages)

In this stage, the extraovarian proteins are processed and packaged into oocyte yolk proteins and are accumulated in fluid filled yolk globules. These globules fuse centripetally forming a continuous mass of fluid yolk during post vitellogenic maturation. Yolked oocytes of mullets have a range of 350-800 μ m. diameter.

d. Maturation, Germinal vesicle migration.

At this stage the GV migrates towards the periphery of the oocyte and the nuclear envelope dissociates. (GV break down). Hydration of the oocytes occur just before the spawning. Hydration takes place when the mullets migrate to coastal area from the estuaries and the hydrated oocytes may vary from 900-1000 μ m. in diameter.

e. Post ovulatory follicle

Once the oocytes are ovulated or released, the follicular cells remain in the ovary and constitute the post-ovulatory follicle, which also shows clear evidence of recent spawning.

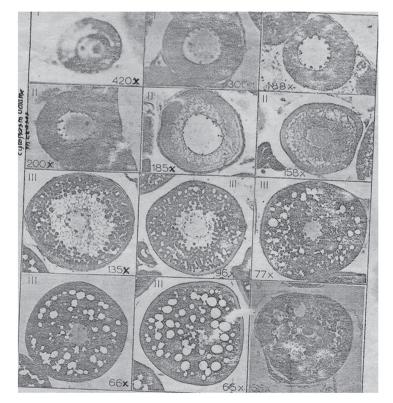
f. Atretic follicle

Atresia is oocyte degeneration that may occur at any stage of ovarian development. In this stage a series of vacuoles of various sizes will appear within the oocytes. The oocytes start breaking also.

Ovarian maturity stages

Four to seven stages have been traditionally described in Mugilidae

- 1. Virginal. very small translucent. At the microscopic level only oogonia and incipient primary oocytes occur.
- 2. Immature, slightly smaller, ovary weight 1.3 gm and light pinkish coloured. Microscopically primary growth oocytes, with compact and organized lamellar structure.
- 3. Incipient maturity, ovarian weight 10-20 gms. Colour pale yellow to dark yellow two stages of oocytes, primary growth oocytes and cortical alveoli oocytes. The females are considered mature at this stage.
- 4. Advanced maturity. Ovaries occupy half to ¾ of abdominal cavity. 30 to 280 gm. weight. Dark yellow to orange. Oocytes appear visually
- 5. Spawning running stage: Ovaries occupy the entire abdominal cavity, translucent, hydrated oocytes. Can be seen with naked eye.
- 6. Spent: Flaccid ovaries, highly vascularized, notably shrinking- occupying 25% of abdominal cavity



7. Resting: flaccid, reddish or greyish in colour – 4-10 gm.

Stages in oocyte development of Mullets

Fecundity:

Fecundity estimates are based on ovaries at stage IV. Fecundity or potential fecundity is estimated by removing three pieces of ovary (0.1 - 0.2 gm) from anterior, middle and posterior portions. These are rehydrated and weighed and all oocytes counted. P.F is estimated by the mean number of yolked oocytes per gram weight that is YO/G and the ovary weight OW

 $PF = YO/G \times OV. W$

For M.cephalus the fecundity 5 lakhs to 15 lakhs

Gonado-somatic index

GSI is an efficient estimate of the physiological state of gonads. GSI can be defined as GSI = OW.100/BW here OW is the ovarian weight in gms. and BW is the body weight. The male gonads are very small compared to the ovaries. In *M.cephalus* from Japan the ovarian weight was 20% of body weight whereas the testicular weight was only 11%. Males mature earlier and are smaller in size compared to females

Neuroendocrine function of reproduction

The hypothalamus, pituitary and gonads are particularly influenced by environmental factors such as photoperiod and water temperature. The control of reproduction is with these organs. Fish adenohypophysis synthesizes at least eight different hormones of which two are directly related to reproduction in fishes, the FSH and the LH. Reproduction in fishes is controlled by the actions of the above hormones. They stimulate gonadal steroidogenesis and are involved in oocyte maturation, ovulation and spermiation.

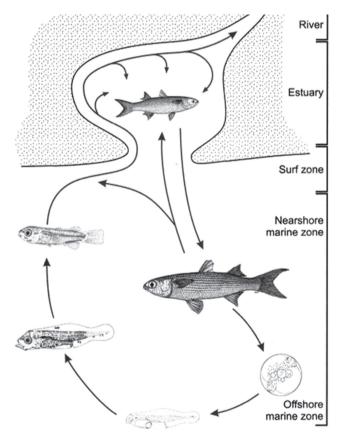
Spawning

Mullets spawn in different geographical areas at different times of the year. Spawning of each species takes place depending on an optimal temperature range.



A female mullet with swollen abdomen and ready to spawn

Migrations in fish happens on two reasons, the search and selection of suitable areas to migrate and the adoption of the appropriate environmental conditions that will mark the beginning of migration. All mullets are catadromous. They migrate to the sea to spawn. Before maturity the mullets stay predominantly in the coastal systems of rivers and lakes. As maturation begins they migrate to the sea where they complete maturation and spawning. About 2-3 months after the beginning of the reproduction period, there is an onshore migration of the post flexion larval stage followed by a temporary occupation of the surf zone as early juveniles. Once near the coast, they move in schools to coastal lagoons or lakes, estuarine waters or sometimes even reach fresh water, several kilometers away from the coast. These migrations are repeated every year. There are also records of mullets living permanently in sea water systems. Since mullets are euryhaline they have the ability to adopt to changes in salinity during migration. This is helped by many organs such as gills, kidney and the alimentary canal. The osmotic regulation performed during migration is also controlled by endocrine system and hormones.



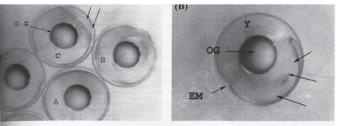
Migration of mullets

Observations on spawning behavior of Mullets in nature are few. After migrating to high saline coastal waters , large numbers of fish were observed schooling but scattered into small groups generally made up of one large female and a varying number of smaller but active males. Mullets are oviparous wherein the individuals shed their gametes into the water and fertilization is external. The spawning process as observed in hatchery conditions is as follows. Spawning is heralded by a violent quivering of the males which lie parallel to and touching the female. The first release of a few ripe eggs stimulates the males to liberate spermatozoa. The female then responds with an explosive and continuous release of eggs. Fertilization is external.

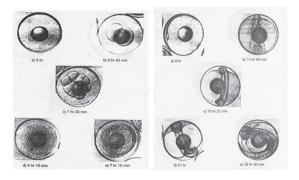
The embryonic phase:

This phase describes the development of the eggs from spawning till hatching

The morphology of the eggs before fertilization is described in many mullets studied. The unfertilized eggs are pelagic, spherical and transparent and the chorion is smooth. There is one large golden yellow oil globule that makes the egg extremely buoyant. The eggs are not adhesive (0.93 μ m. Dia.). Shortly after fertilization the cytoplasm becomes thickened at the animal pole of the egg where the nucleus occurs. The cells divide in a meroblastic fashion (cells form only at the animal pole). In about 50 minutes after fertilization, the first meroblastic division occurs. The developing embryos are visible after 15 hours post fertilization. The hatching of *M.cephalus* is evident in 36-38 hours after fertilization at 24R"C.



Eggs of Mugil cephalus (X31). (A) Unfertilized egg A), Fertilized egg B) Germinal disc stage C) Morula stage



Stages in development of eggs

Early larval development

The developmental stages of several mullet species have been described by many. This has been studied due to success in induced breeding techniques on many of the species. At hatching the fish becomes a larva. Most of the fish larvae have a yolk sac providing nourishment for the newly hatched larva (yolk sac or prelarva).

The newly hatched larva float upside down. Liao, (1978) gave a full description of the larval development of *M.cephalus*. Newly hatched larvae of *M.cephalus* vary in length between 2.2 - 3.5 mm length. Egg development and hatching are temperature dependent. At an age of 3-4 days, the mouth opens and the larva starts taking planktonic food. The larval period lasts till the fin rays reach the adult compliment when the juvenile stage begins. In about 25-28 days the scales and fin rays are well formed.

Artificial propagation

Eleven species of mullets have successfully been propagated artificially in many parts of the World. Mullets do not spawn spontaneously in captivity. Methods to build up brood stocks of mullets in captivity are also well developed. Induced breeding protocols are available in many publications. Hormones used vary from Pituitary homogenate, HCG, LH Rh a, Steroids, etc. A method of assessment of the state of maturity of egg in mullets is available called *live ovarian biopsy* (Shehadeh et al.,1973). The intra ovarian oocytes are removed in vivo from an unanesthetized female with the help of a polytene catheter and a sample of eggs from the middle portion of the ovary is sucked out. The oocytes collected are washed and preserved in a solution of 1% formalin in 0.6 % Nacl. They are then placed in a small Plexiglas plate and measured using an ocular micrometer. Average egg diameter is calculated and maturity determined. The spawning is induced in females at the tertiary yolk globule stage (600 μ m).

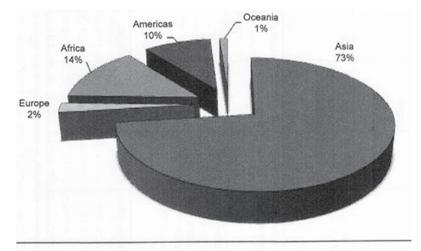
The females of *M. cephalus* are primed with commercial Carp Pituitary homogenate @ 20-40mg. /Kg. body weight followed by a resolving dose of LH RH a @ 100mg./kg. body weight administered 24 hrs. later. The males are sometimes administered with a dose of 17 Methyl testosterone for thinning of milt. Hormone treated female is left in a tank with 2-3 ripe males. Females spawn naturally in the tank and they are immediately fertilized by the males. The fertilized eggs are collected and left for hatching. The fecundity of *M. cephalus* is 8 lakhs - 2.7 million eggs per female. After hatching, the larvae are stocked in densities of 20-25 larvae / liters to start with and fed with rotifers (s-type) at densities of 10-20 rotifers/ml. Addition of cultured phytoplankton helps in growth of the larvae as well as keep the rotifers healthy. From day 12, *Artemia* nauplii are introduced as food for the larvae. Cultured copepods are also eagerly accepted by the larvae. Mortality of the larvae are high on the 2-3 day and 8-11 days. The use of hatchery produced larvae for culture is not viable for culture, since the cost of production is high.

Predation

Mullets are predated by piscivorous fish, crocodiles, sharks, birds and even by dolphins.

Mullet fisheries and culture:

Mullets are of great importance in fisheries and aquaculture. History tells that Egyptians captured mullets. Records of culture/capture of mullets by Romans, their culture in Italian 'vallis' the Egyptian "hoshas" and Hawaiian ponds, in Indonesian 'Tambaks', 'Bheris' of West Bengal are well documented. Mullets are grown/cultured in extensive, semi intensive and intensive systems. Vide FAO records mullet production was 698,293 tonnes in 2013, mainly 80.2% from capture fisheries (560150 tonnes) but with 138143 tonnes from aquaculture (FAO.2015). Capture fisheries for mullets are reported from 103 countries. China is the leading producer of mullets from Asia. Asia produces 73% of mullets and India is in the third place. 71 species of mullets are subjected to capture and culture. Mullet culture is practiced in the Mediterranean and Black sea region and Southeast Asia. Africa has the highest production among continents. Egypt, Indonesia, Republic of Korea, Taiwan and Israel are leaders in mullet culture. Other mullet producing countries are Tunisia in Africa, Guyana, Hongkong, Iraq, Saudi Arabia, Singapore, Greece, Italy, Spain, Ukraine, China and India. *M.cephalus* is the species used for culture in majority of countries since its growth rate is high. Capture methods of Mullets vary from seines, encircling nets, trammel nets, cast nets, dip nets and also pelagic trawling, spearing, rod and line, shooting arrows etc. Dolphins are found to help fishermen in catching Mullets by schooling them.



e 16.2. Mullet production from capture fisheries in 2013 by continent (%) (data from FAO

Fry collection for culture

Major sources of fry for culture are from wild since artificial propagation of mullets have not been commercially developed.

Fish traps, scoop nets, cast nets, seines and push seines also are used Worldwide. Fry are fragile and tend to get damaged during transport. Oxygen pack transport is safe. Acclimatization before release is a must and lessens the shock and increases survival.

Products from mullets

Mullets are sought after for their roes (ovaries). The Mullets roe is expensive and sold either fresh or salted. Mullet roe is known in different names in different countries. It is called Boutargue in Arab whereas in South east Asia it is called 'karasumi' and in Egypt as 'batarekh'. Ripe females are cultured in special ponds in Taiwan. The roe collected from them are vacuum packed, bee waxed or grated dry. The optimum weight of the roe collected from three-year-old females is around 300gms. In Taiwan male testes are sometime eaten raw.

Mullets are also filleted, salted or hot smoked and packed. Mullet gizzards are also eaten. Raw mullet fingerlings are eaten raw in Hawaii

Parasites and diseases

Mullets are fragile fishes and are easily prone for secondary infection with bacteria. Fungal infections are also common in culture systems. Parasite infection from microsporidians, myxosporidians, copepods, isopods, nematodes, cestodes are also common in natural as well as cultured mullets.



Raw mullet roe and testes

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