

# Sexuality in Fishes



**T. J. Pandian**

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*Cover Illustration*

The picture illustrates the comparative distances between the vents among the mating partners during the event of pair spawning and group spawning that take place within a single species in some fishes. In both cases, about 95% fertilization success is achieved, though, at the cost of upto 50 times more sperm by the group spawning males. In pair spawning, sperm economy is achieved but group spawning enhances genetic diversity among the progenies.

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# Preface

Fishes constitute more than 50% of the living vertebrate species and are characterized by a level of sexual plasticity and flexibility that are unrivalled among other vertebrates. They display the most divergent expressions of sex, for instance, the normal male, primary and secondary males, hooknose and jack, parental and cuckolder, pairing and harem, stalker, sneaker, satellite and piracy males, a wide range of sexuality from gonochorism to unisexuality, and self-fertilizing to sequential and to serial hermaphroditism, and every conceivable form of mating system. Despite having to remain dependent on 'borrowed sperm' from males of sympatric bisexual species, gynogenesis and hybridogenesis have perpetuated the unisexuals such as, the *Poeciliopsis* over 100,000 generations.

There are many books on the sexuality in fishes. Some are 15–25 years old and require updating; others are concerned with a special group, like, coral reef fishes only and yet others are limited to one or another aspect of sexuality. Hence there is a need not only to update but also to look at the entire spectrum of sexuality of fishes from a holistic point of view. More than 52% of the references cited in the book are dated after 1991. This book is a synthesis of relevant and recent information on sexuality of fishes and points towards new directions of desired research. An array of male genotypes of fishes may indeed pose a challenge to molecular biologists searching for the sex determining gene(s) in the vertebrate system. The unprecedented level of diversity recorded among the clones of self-fertilizing gynogenetic unisexuals and self-fertilizing simultaneous hermaphrodites challenges current ideas on the predominant role of recombination in promoting evolution of biological diversity. Though limited only to a few species, the existence of self-fertilizing and cyclical sex changing hermaphrodites is a puzzle to many but provocative to geneticists and endocrinologists. Within a few days following the removal of a harem master or mistress, a successor changes sex and takes over the harem; the causes for the sex changing mechanism pose many challenges to socio-ecologists and behaviour specialists.

Japanese scientists should be complimented for the devotion and patience of their worthy and seminal contributions to this area of fish biology. Some of them remained underwater longer than 500 hours to collect the desired information. About 560 references cited in this book from widely scattered sources of 175 journals, books, proceedings and theses, 20% of the publications are by the Japanese.

Wanting to accomplish this book, the first of its series, was aching me for the last few years and is now delivered to the users, thanks to Science Publishers.

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**T.J. Pandian**

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## Introduction

Fishes constitute more than 50% of the living vertebrate species and are characterized by a level of sexual plasticity that is unrivalled among other vertebrates. They inhabit every conceivable aquatic habitat and are found in the world's highest lake, Titicaca (3,812 m above mean sea level) and the lowest lake, Baikal (7,000 m below sea level). Some are reported from almost distilled waters (0.01 g/kg salinity), while others live in very salty lakes (100 g/kg s). The African tilapias inhabit hot soda lakes (44°C), while *Trematomus* are found under the Antarctic ice sheet at -2°C. Some of them are capable of breathing atmospheric air and survive in stagnant tropical swamps (Pandian, 1987).

Interestingly, freshwater, which covers only about 1% of the earth's surface and accounts for a little less than 0.01% of its water, has about 10,000 species of fishes, while sea water, which covers about 70% of the globe with 97% of its water, has only 15,000 species (Bone et al., 1995). Most freshwater species are found in Southeast Asia and in the Amazon Basin; the Indo-Pacific is the richest region for marine species and is followed by the Caribbean. Species richness is known to decrease with increasing latitude; for example, Donaldson et al. (1994) recorded as many as 950 species (112 families) at 13° N around the South Mariana Islands in the Pacific but only 400 species belonging to 70 families at 34° N around the Izu islands. In the Palau Islands (13° 11'E-13° 19'E), known for the highest level of diversity, the increase in species richness is more acute; with a decrease in latitude from 5° 21'N to 2° 59'N, the richness and diversity are increased by about 2.5 and 2.0 times, respectively (Table 1). Besides, the ocean slope also accelerates the acuteness in the observed increases in diversity and richness (Donaldson, 1996).

Notably the tendency to small body size is more apparent among fishes than any other group. The smallest vertebrate known is the planktonic pedomorphic fish *Schindleria* sp measuring 9 mm (El-Regal and Kon, 2008). In terms of taxa present, small species are particularly abundant on coral reefs. For example, small species constitute 68% and 72% of species

## 2 Sexuality in Fishes

**Table 1** Physiography, species richness and diversity of fishes of the Palau Islands (from: Donaldson, 1996).

Locality	Longitude	Reef perimeter (ocean slope km <sup>2</sup> )	Species richness (no)	Shannon diversity (H)
Fanna	5° 21' N	4.3	195	1.29
Sonsorol	5° 9' N	7.0	250	1.66
Pulo Anna	4° 40' N	4.7	198	1.27
Merir	4° 19' N	9.5	294	1.68
Tobi	3° 1' N	7.0	317	1.55
Helen Reef	2° 59' N	61.7	488	2.00

of the Red Sea and Gulf of California, respectively. To avoid predation, smaller species occupy more restricted and sheltered habitat, and micro-habitat than larger species. The vertical rugosity of the reef measured in millimeters may be important in describing the distribution and abundance of small fishes like the blennies but for much larger fishes, an order of magnitude greater rugosity may be important. Not surprisingly, most published studies exclude the small and cryptic ichthyofauna because of the difficulties encountered in collecting, maintaining and identifying them. In fact, the small ichthyofaunas are more speciose. For instance, the family Gobiidae, whose mean body length is about 3 cm, is composed of as many as 1,500 species, whereas Epinephelinae with mean body length of about 50 cm has only 100 species (Munday and Jones, 1998).

Off from the coral reefs, the course of evolution seems to have taken some fishes to what may be called miniaturization. Many records on the occurrence of smallest fishes belonging to different taxonomic groups from both freshwater and marine habitats have been published. The clupeomorph fish *Amazonsprattus scintilla* (Roberts, 1984), the Burmish cyprinid *Danionella translucida* (Roberts, 1986), *Paedogobius kimurai* (generation time: 42–67 days, Kon and Yoshino, 2002), a diandric goby with planktonic female and demersal secondary male phases from the Ryukyu Island, Thailand (Iwata et al., 2001), *Panderka pygmaea* (12 mm, see Pandian, 1987), the syngnathid trunk-brooding Denise's pigmy seahorse *Hippocampus denise* (13 mm, Lourie and Randall, 2003) and *Schindleria praematura* from the Red Sea (9 mm, El-Regal and Kon, 2008) are some examples. Compressing the vertebrate body into about 1 cm in some of these miniaturized fishes may be of inherent interest to cytologists, anatomists, chronobiologists and evolutionary biologists (Martin and Palumbi, 1993; Hanken and Wake (1993). Some of these fishes may serve as tools for genetic models, (see Table 32) as their generation time, for instance, is only 37 days as in the oviparous *Schindleria* (Kon and Yoshino, 2002) and 48 days, as in the viviparous guppy *Poecilia reticulata* which are shorter than that (50 days) known for the viviparous pygmy mice (Nowak, 1991).

## 1.1 Life span and sexual maturity

The life span of fishes ranges from a few days in some tropical paedomorphs (e.g. 60–90 days, *Schindleria praematura*; Kon and Yoshino, 2002) to about 200 years. The bathybenthic, benthic sea-mount and cave fishes are estimated to live longer than 100 years (Poulson, 2001). Otolith studies have shown that the life span of the bathymetric (1,400–1,650 m depth) fishes like the orange roughy *Hoplostethus atlanticus* is longer than 175 years; the fish matures at the age of 27.5 years and breeds up to the age of 175 years (Minto and Nolan, 2006). In the temperate zone, sturgeons live for 60 years and attain sexual maturity at the age of 14–21 years; some of them like *Huso dauricus* grow to a body length of 4 m and weight of 500 kg, and spawn once every 4 years and have a sex ratio of 1 male: 2–3 females (Bernstein et al. 1997; Wei et al., 1997). In the tropics, the females and males of the yellowmouth grouper *Mycteroperca interstitialis* attain 5.7 and 6.1 kg body weight at the age of 16 and 28 years, respectively (Bullock and Murphy, 1994). In the Gulf of Mexico, the jewfish *Epinephelus itajara* grows to a maximum body size of 2 m during its life span of 26–37 years; its female attains sexual maturity at the age of 4–6 years, when it grows to a body length of 1.3 m, whereas the male matures at the age of 6–7 years and size of 1.2 m (Bullock et al., 1992). Unlike the sturgeons, tunas have a male biased sex ratio of 3 males: 1female; the largest yellowfin tuna *Thunnus albacares* captured from the Bay of Bengal weighed 140 kg (Rohit and Rammohan, 2009). The 13 year-old bluefin tuna *Thunnus thynnus* attains the largest body weight of 330 kg in the Pacific (Wu et al., 2005). Quite contrastingly, among the three species of marine paedomorphic fishes recorded, *Schindleria praematura* matures at the age of 37 days and has a life span of no more than 60–90 days (Kon and Yoshino, 2002).

The onset of sexual maturity represents a critical transition in the life of an individual, as allocation of time and resources has to be provided for reproduction besides survival and growth. Temperature is a master factor significantly affecting the life span and age of maturity. For instance, the European cod *Gadus morhua* matures at the age of 3 years and lives for about 8 years in the relatively warmer British waters but in the colder Barent Sea within the Arctic Circle, it begins to mature at the age of 7 years and may live longer than 20 years (see Wootton, 1998).

As a major resource of nutrients and energy, food supply in natural habitats (e.g. Mann et al., 1984) and provision of higher rations in the laboratory (e.g. Donelson et al., 2008) are known to result in earlier sexual maturity and larger reproductive output. In the high productivity streams of southern Europe, the gudgeon *Gobio gobio* matures early, spawns several times and has a shorter life span. But in the northern and low productivity streams, it postpones maturity, spawns only once and has a longer life

span. The reciprocal transplantation of the fish between the northern and southern sites indicated that these differences in the life history traits are related to phenotypic plasticity of the fish. Consequent to the differences in food supply, the *size* of a fish may vary at a given *age* or the *age* of the fish may vary at a given *size* (length and/or weight). For instance, the sea bream *Pagrus pagrus* in its habitat attains sexual maturity at the age of 2 years, when the body length of the female ( $22 \pm 1.9$  cm) varies by 10% (Matsuyama et al., 1988). Even with equal provision of food in the laboratory the zebrafish *Danio rerio* male shows more than 5% variation in its body length ( $20.5 \pm 1.4$  mm) at the age of 120 days (Balasubramani and Pandian, 2008a).

Among shoaling fishes, isolation and assemblage may considerably widen the variation in size at which a fish sexually matures. Yamagishi (1969) developed a model that synthesized the potential effect of social/shoaling behaviour on the relative variation of size at which some marine fishes mature. Size variations at sexual maturity of non-shoaling fishes are more than two times wider than that observed for shoaling fishes.

Using adequate information on growth, fecundity and mortality of 19 populations of fish, Stearns and Crandall (1984) developed a model to predict whether age or size determines sexual maturity in fish. The model accounts for 82% of the variants in the age of maturity. Accordingly, when growth is either rapid or slow, changes in growth may produce large variations in *age* at maturity but less variation in *size* at maturity; consequently, the fishes may have a fixed *size*, at which they mature. Conversely, when growth rate is intermediate, changes in growth produce large variations in *size* at maturity but less variation in *age* at maturity and the fishes may have a fixed *age* at which they mature.

Two different life history patterns of reproduction are known. In the semelparous pattern, adults attaining sexual maturity die either while spawning or soon after. The Pacific salmon *Oncorhynchus* is a good example for the semelparous life history. A group of cyprinodontids provides unexpected but very interesting examples for the semelparous pattern of life history (Table 2). They all grow very rapidly and one of them *Notobranchius rachovii* matures within a period of 6–8 weeks after hatching. However, all of them die after spawning, when drought sets in. Buried in the substratum, their hard-shelled eggs survive dessication over a period of 6 months to 3 years.

Species in which adults in a population, survive to breed again, are called iteroparous. Majority of teleosts are iteroparous. However, there are interesting examples to suggest that the semelparity-iteroparity stands in a kind of continuum in fishes. In the Atlantic salmon *Salmo salar*, the repeat spawners comprise 2–22% of the Norwegian populations (Jonsson et al., 1997). In populations of the American shad *Alosa sapidissima*, the repeat

**Table 2** Semelparous cyprinodontid fishes and ability of their eggs to survive desiccation (from many sources).

Species	Remarks
<i>Aphyosemion australe</i> (Lyretail)	Survive annually through eggs, which remain alive in the dry beds of pools until the next rainy season
<i>Epiplatys dageti</i> (Firemouth epiplatys)	Annuals inhabiting shallow forest pools, that dry up each year. Adults die after depositing the eggs, as the waters dry up. Deposited hard shelled eggs survive in substratum and are stimulated to hatch by the next rain
<i>Notobranchius rachovii</i> (Toothcarp)	Annuals inhabiting pools and streams that dry up annually. Mature in 6 weeks after hatching. Males flick fertilized eggs into the substratum. Eggs survive desiccation for 6 months
<i>Terratonatus dolichopterus</i> (Sabrefin)	Annuals inhabiting muddy pools, which dry up between December and May. The eggs survive desiccation and hatch in 7 days after the rains fill the pools
<i>Cynolabias nigripinnis</i> (Pearlfish)	Life spans for 8 months. Courtship involves burrowing into the mud, where the eggs are deposited. Adults die after depositing the eggs. Left in the mud, eggs remain very resilient to drought and are viable for upto 3 years submerged in the anaerobic soil of pool bottom

spawners progressively increase from almost 0% from the Florida coast (32° N) to 75% at the coast of New Brunswick (46° N). Hence, the shad are semelparous in Florida, whereas the population is strongly iteroparous in New Brunswick (Leggett and Carscadden, 1978).

## 1.2 Spawning process

To ensure fertilization and to avoid egg predation and facilitate dispersal, fishes adopt an array of strategies by temporally and spatially scattering the actual process of releasing the eggs. Pelagic spawners ascend to a height of a few meters and the female releases her entire batch of eggs in a single burst, the exact process lasting from a few seconds (e.g. 2.6 seconds, *Aulichthys japonicus*, Akagawa et al., 2008) to a few minutes (1.7 to 7.0 minutes, *Pseudobagrus ichikawai*, Watanabe, 1994). To avoid predators, for instance, the mating partners of the angelfish *Centropyge ferrugatus* (Sakai and Kohda, 1995), who are ready to spawn, may delay the 'spawning rush', select an alternate site or may even avoid spawning on that day.

Incidentally, there are also fishes, which adopt quite an ingenious tactic to safely disperse the eggs avoiding predation of not only their eggs/progenies but also their symbiotic partner. According to Hunnam et al. (1991), the European bitterling *Rhodeus sericeus* has a peculiar mode of safely dispersing eggs and progenies. The fish has symbiotic relationship

with the mussel *Unio*. When the female is ready to spawn, she maintains an oblique head-down position over the mussel, touches its exhalant siphon with an erect proximal part of her egg-laying tube. Then she inserts the stiffened tube into the siphon and releases her eggs, usually one to four in each pulse into the mussel's gill chamber. Once she has withdrawn her tube, the male releases his milt over the mussel's inhalant siphon, so that it is drawn through the mussel and fertilizes the eggs. This sequence is repeated from 10 to 25 pulses until the female lays 40 to 100 eggs. In the mussel, the eggs are safely incubated for 2–3 weeks. At the time of hatching, the mussel's glochidia larvae, which are also hatched out just then, attach themselves to the young bitterling and remain in this symbiotic state until developing into small mussel and dropped to the substratum. The bitterling is bitter in taste, which avoids predation of the fish larvae and glochidia. Thus, the mussel safely incubates the fish eggs and the mussel larvae are also safely dispersed and settled.

A male of the armoured catfish *Corydoras aeneus* courts a ready-to-spawn female, who drinks the semen directly from a male by attaching her mouth around his anus in a 'T-position'. Unaffected by digestive enzymes, the semen passes through intestine and is safely delivered into a pouch beside her anus formed by her two pelvic fins. Following the appearance of semen in the pouch, a clutch of ca 30 eggs is released into the pouch in which the eggs are inseminated. Fertilized eggs are then deposited on a substratum. After ca 5 minutes, the female repeats this act with the same or another courting male and the spawning bouts successively continue for 10–20 times in 1–2 hours (Kohda et al., 1995, 2002).

On the eve of spawning, the clutch may be released in 'pulses', 'bouts' or 'parcels'. For instance, the fightingfish *Betta splendens* releases her eggs in 19 pulses and during each interval of a few seconds between successive pulses, the male fertilizes the released eggs and draws them into his bubble nest (Kirankumar and Pandian, 2003). The number of bouts in the goby *Valenciennea longipinnis* ranges from 10 to 15, and during each bout, which lasts for a period of 5–25 seconds, the female deposits and glues her eggs on the ceiling of the burrow, over which the male milts (Takegaki and Nakazono, 1999a). In the serranid black hamlet *Hypoplectrus nigricans*, the eggs are released in 4–5 'parcels' and during the interval of 3.0 minutes between successive pulses, the female 'trades off' her eggs to be fertilized by her mating partner (Fischer, 1980). During the spawning period, the lizardfish *Synodus dermatogenys* spawns 6.5 times/day with an interval of 1 to 3 minutes between successive pulses and spawning is spread over 4 days; such multiple spawnings may avoid predation and ensure wider dispersal of eggs (Donaldson, 1990). The Indian glassfish *Chandra ranga* releases 4–6 eggs in a pulse and the process is repeated about 40 times until 200 sticky eggs are produced (Hunnam et al., 1991). Examples for the

number of such pulses within a single spawning act of selected cichlids are listed by Kuwamura (1986).

### 1.3 Fecundity

The number of eggs that a female spawns over a definite period depends on the number of eggs released per spawning. Batch fecundity or a clutch includes the number of eggs produced per spawning. Season fecundity is the batch fecundity multiplied by the number of spawning during a breeding season. Life time fecundity is the season fecundity multiplied by the number of spawning seasons, during which spawnings have occurred (Wooton, 1998).

Batch fecundity ( $F$ ) is shown to be a function of body size ( $L$ ).  $F$ , as indicated by ovary weight, also increases with  $L$  (Fig. 1). Because the  $F$  is related to the volume of body cavity available to accommodate the ripe ovaries ( $F = aL^b$ ), geometry suggests that length exponent 'b' would be 3.0. Surveying this relationship in 62 species, Wooton (1979) found that the exponent ranges from 1.0 to 5.0, and lies mostly between 3.25 and 3.75. The exponent ranges (2.57 to 7.12) even wider in *Lethrinus nebulosus* (Ebisawa, 1990). For a larger sample of 238 species, including both marine and freshwater species, the exponent for the relationship between the total

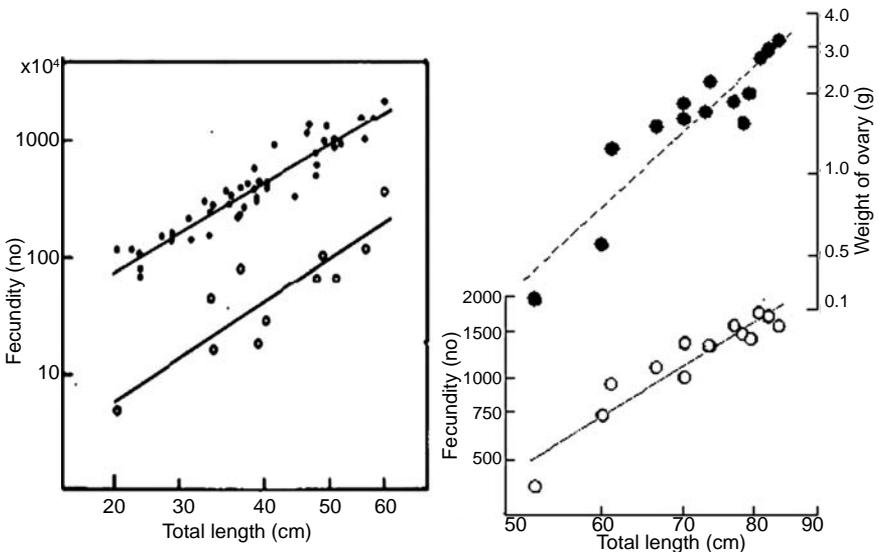
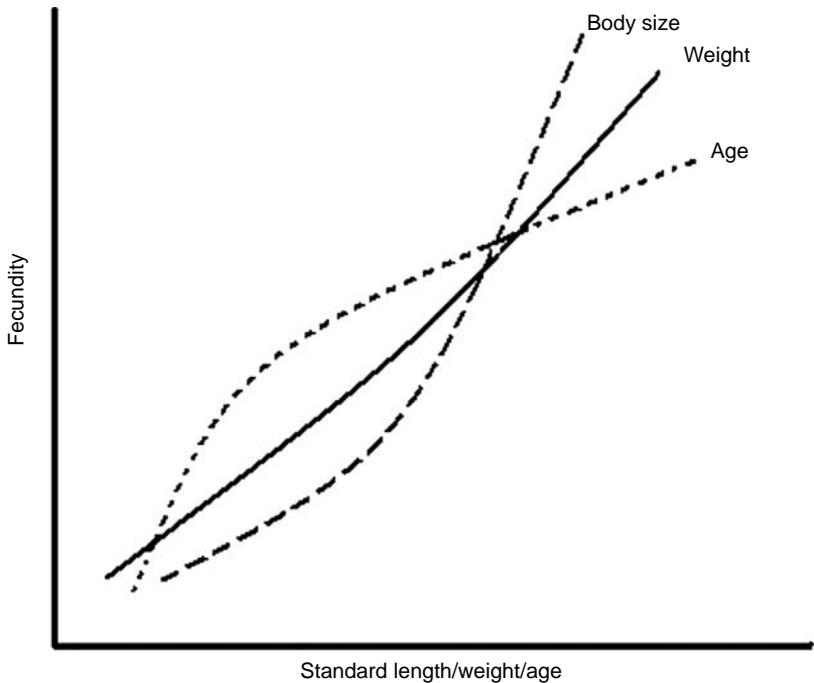


Fig. 1 Left panel: Relations of total body length to batch fecundity ( $\circ$ ) and season fecundity ( $\bullet$ ) in the tuskfish *Choerodon schoenleinii* (from Ebisawa et al., 1995). Right panel: Relations of body length to ovarian fecundity ( $\circ$ - $\circ$ ) and ovary weight ( $\bullet$ - $\bullet$ ) in *Pseudobagrus ichikawai* (from Watanabe, 1994).



**Fig. 2** Fecundity model, showing the relationship of fecundity to body size (-----), weight (—) and age (.....) of fishes.

volume (batch fecundity) and body length is found to have shrunken to 3.1 (Wootton, 1992).

In the black spot tuskfish *Choerodon schoenleinii* the trend between body size and batch fecundity is parallel to that between size-season fecundity (Fig. 1, Ebisawa et al., 1995). Likewise, the body size-ovary weight relation is also parallel to that of size *vs* ovarian fecundity in the bagrid fish *Pseudobagrus ichikawai* (Watanabe, 1994). Gomez-Marquez et al. (2003) showed that in the Nile tilapia *Oreochromis niloticus*, fecundity is better correlated with body weight rather than body length. In general fecundity is correlated with body size and is strongly correlated with body weight; however it is not correlated with age, as senescence seems to set in after a particular age (Fig. 2). For instance, in fishes with a large body size and long life span like the orange roughy *Hoplostethus atlanticus*, significant, yet highly variable relationships are found between total fecundity and body length and weight (Minto and Nolan, 2006). Weight is the single predictor of fecundity, explaining 40% of the variants (see also Macchi, 1998; Macchi and Acha, 2000). A significant relation is also present between fecundity and age from 27.5 to 122 years of the orange roughy

but beyond which the fecundity begins to decline (Minto and Nolan, 2006). Experimental studies with the amphidromous goby *Rhinogobius* sp have shown that almost all smaller (3–4 cm body length) and young (1+ to 2+ year old) females spawn twice but the larger (> 5 cm) and older (> 3+ year old) females do not spawn twice (Tamada and Iwata, 2005). Wide range of variations observed in the exponent may be a reflection of the differences in the belly diameter of the fishes; ideally, the values may be closer to 3.0 in slim fishes; the exponent may be more than 3.0 in fusiform fishes and less than 3.0 in fishes characterized by horizontally or laterally compressed body. As the body weight may neutralize the differences in body length and belly diameter of most fishes, a better formula to express the relation between fecundity and body size is  $F \times aW^b$ , in which  $W$  represents the body weight. Figure 2 is a generalized fecundity model, showing the relationship of fecundity to body size, weight and age of fishes.

As the season fecundity represents the batch fecundity multiplied by the number of eggs per spawning season, the trend obtained for the season fecundity-body length relationship (Fig. 1) runs parallel to that of the former (Ebisawa et al., 1995). The life time fecundity of the laboratory reared viviparous molly *Poecilia sphenops* is estimated as progenies. During her life spawn of 20 months, a female released 7 batches of progenies within the breeding period from the age of 7 to 16 months. The number of progenies increases from a few in the first batch to 44 in the seventh batch (George and Pandian, 1997). The trend obtained for the batch fecundity *vs* age relationship is also indicative of the same relationship between  $F = aL^b$ , as proposed by Wootton (1979). However the molly is also known not to produce progenies after 16 months of its age (see also Tamada and Iwata, 2005).

With regard to the exponent of 3.0 for the relationship between batch or season fecundity on one hand and body length or age on the other (Wootton, 1992), it may be added that there are also reports for the batch and season fecundity of both marine and freshwater species, remaining independent of body size and age. From his field studies, Sakai (1996) showed that the batch fecundity of the angelfish *Centropyge ferrugatus* remained at around 1,400 eggs per spawning, independent of its body length varying from 7 to 10 cm. The laboratory reared zebrafish *Danio rerio* spawned 15 times, and its batch fecundity remained almost equal at 85 eggs/spawning, despite the fish growing from 24 mm to 41 mm body length during the period from 120 to 235 days of its age (Fig. 3, Balasubramani and Pandian, 2008a). In the fightingfish *Betta splendens* too, the batch fecundity remained equal at about 120 eggs/spawning, independent of its body size and age from 140 to 240 days (Balasubramani and Pandian, 2008b). Likewise, both fecundity and egg size of the Owens pupfish *Cyprinodon radiosus* remain independent of the mother's body size (Mire and Millett, 1994).

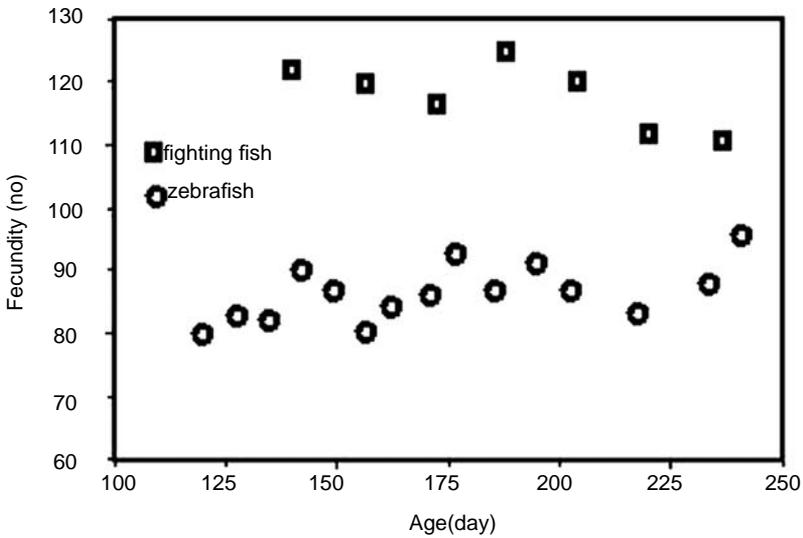
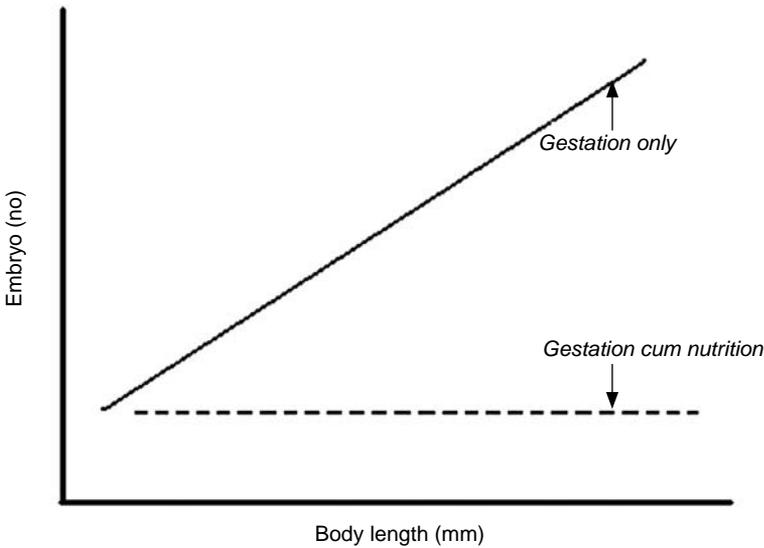


Fig. 3 Relationship between age and fecundity of laboratory reared zebrafish *Danio rerio* and fightingfish *Betta splendens* (from Balasubramani and Pandian, 2008a,b).

In general the body size-fecundity relationship among viviparous fishes depends on whether the developing embryos draw nutrients from the mothers or not. Using available data, two models namely the gestation and gestation cum nutrition are constructed. The gestation model includes those species, whose developing embryo do not draw much nutrient from their mothers, as in *Poecilia sphenops* (George and Pandian, 1997) and in them the expected linear size-fecundity relationship is apparent (Fig. 4). But in the gestation cum nutrition model, which includes *Poeciliopsis prolifera* (Thibault and Schultz, 1978) and *Heteroandria formosa* (Wourms, 1981), the developing embryos obligately depend on maternal nutrients and in them the size-fecundity relationship remains independent of the body size. Among the live-bearing poeciliids, the fecundity-size relationship is further complicated by superfoetation.

Incidentally, a distinction must be made between fish species with determinate and indeterminate fecundity (Hunter et al., 1992). Among the former, the potential fecundity is fixed prior to the commencement of a breeding season. As successive spawnings occur, the stock of oocytes remains to be spawned during that breeding season declines. Typically, *Lethrinus nebulosus*, a fish with determinate season fecundity, batch fecundity progressively decreases from 1,000,000 eggs during March, the first month in the breeding season to 70,000 eggs in May; the spawning



**Fig. 4** Generalized models of the size-fecundity relationships among the gestating poeciliids with and without maternal nutrition.

interval between successive spawnings during the breeding season is also prolonged from 2.2 days in March to 6.0 days in May (Ebisawa, 1990).

Due to one or other environmental factors like food supply, some yolked oocytes may also be resorbed through atresia. Consequently, the realized fecundity shall be lower than the potential fecundity. For instance, the realized fecundity decreases from 91% in well-fed herring *Clupea harengus* to 60% in herring group receiving low ration (Ma et al., 1998). Likewise, the realized fecundity of the Alaskan Atka mackerel *Pleurogrammus monopterygius* is 77% (McDernott et al., 2007). In species with indeterminate season fecundity, there is continuous recruitment to the stock of spawnable oocytes during a breeding season; hence the realized fecundity depends on the balance between the rate of oocyte recruitment, spawning and atresia.

A long breeding/spawning season is usually associated with multiple spawnings. In species characterized by indeterminate season fecundity, like *Cynoscion striatus* and *Brevoortia aurea*, spawning occurs at an interval of 8 days; the frequency is 22 times in the former (Macchi and Acha, 2000) but is limited to 15 times in the latter (Macchi, 1998). The blacktail comber *Serranus atricauda* is known to spawn 41 times/annum (Garcia-Diaz et al., 2006).

In commercially important fishes, relative fecundity, i.e. the number of oocytes/eggs per unit body weight, is reported. The number of eggs

spawned by demersal spawners is relatively small and can readily be counted. But the number is huge in pelagic spawners, especially commercially important fishes like the striped weakfish (Macchi, 1998). In such cases, the relative fecundity is calculated by counting the number of oocytes/eggs in small volumetric or gravimetric samples (the hydrated oocyte method, Hunter et al., 1985). For instance, the cod *Gadus morhua* reared under natural photoperiod in the laboratory, spawns 108.35 l containing 583,071 eggs/l; from these values, its batch fecundity ( $65.03 \times 6^{10}$  eggs) and relative fecundity ( $4.30 \times 10^5$  eggs/kg female) are estimated (Hansen et al., 2001). Unlike batch fecundity, the relative fecundity values provide a scope for comparative analysis of the reproductive potential (oocyte number)/reproductive effort (egg number) of different fish species. For instance, it ranges from 136 oocytes/g in *Brevoortia aurea* to 10,547/g in *Serranus atricauda*. Table 3 shows that the relative fecundity is higher for pelagic spawners than those for demersal spawners. Its values for the fishes with parental care are low (e.g. *Tilapia zilli*, Coward and Bromage, 1999) and those for live-bearers are the lowest. The trade off between egg size and number appears to result in the production of a large number of

**Table 3** Relative fecundity (RF) of selected fishes.

Species	RF (no/g bodyweight)	Reference
<b>Pelagic spawners</b>		
<i>Brevoortia aurea</i>	136	Macchi and Acha (2000)
<i>Lagocephalus spadiceus</i>	200	Sirisha and Rao (2007)
<i>Cynoscion striatus</i>	210	Macchi (1998)
<i>Hoplostethus atlanticus</i>	313	Minto and Nolan (2006)
<i>Gadus morhua</i>	430	Hansen et al. (2001)
<i>C. nebulosus</i>	451	Brown-Peterson et al. (1988)
<i>C. regalis</i>	475	Lowere-Barbieri et al. (1996)
<i>Engraulis anchoita</i>	600	Pajaro et al. (1997)
<i>Psenopsis cyanea</i>	700	Rajasree et al. (2004)
<i>Micromesistius australis</i>	706	Macchi and Pajaro (1999)
<i>Serranus atricauda</i>	10,547	Garcia-Diaz et al. (2006)
<b>Demersal spawners</b>		
<i>Bagre marinus</i>	3	Pinheiro et al. (2006)
<i>Pterygoplichthys disjunctivus</i>	8	Gibbs et al. (2008)
<i>Tilapia zilli</i>	32	Coward and Bromage (1999)
<i>Huso dauricus</i>	41	Wei et al. (1997)
<i>Acipenser schrenckii</i>	45	Wei et al. (1997)
<i>Pseudocorynopoma doriai</i>	50	Terriz et al. (2007)
<i>Huso dauricus</i>	92	Krykhtin and Svirskii (1997)
<i>Clupea harengus</i>	300	Ma et al. (1998)
<b>Live-bearers</b>		
<i>Poecilia formosa</i>	8	Monaco et al. (1983)
<i>Hippocampus kuda</i>	17	Thangaraj and Lipton (2009)

smaller eggs or smaller number of larger eggs. For instance, the herring spawn large number (300/g fish) of smaller (0.8 mm) eggs, whereas *Bagre marinus* produce only 3 eggs/g but large-sized (2 mm) eggs. The relative fecundity values of a fish species may also vary from river to river. For example, the values for the shovelnose sturgeon are 161, 174, 182 and 194 eggs/g in the rivers Red Cedar, Mississippi, Wabash, and Missouri, respectively (Kennedy et al., 2006).

Incidentally, Gonado Somatic Index (GSI), for which available reports are indeed plenty, is closer to the relative fecundity. But as it relates the ovary weight to the body weight of fish, it may not be as useful as the relative fecundity, especially for studies on egg hatching success, larval survival, recruitment and population dynamics, which are very important to commercial fisheries.

Besides body size, temperature and food supply are known to considerably influence the batch fecundity and spawning frequency. In England, the cottid *Cottus gobio* spawns once a year in the resource poor northern upland stream but several times in a year in the resource-rich southern chalk stream (Mann et al., 1984). In the tropics (~12° N), 6 species belonging to the anemonefish *Amphiprion* spawn 12.5 times/annum with a mean batch fecundity of 575 eggs/clutch, but the sub-tropical (~30° N) 3 species spawn only 7.1 times/annum (see also Madhu and Madhu, 2007). However, the batch (2,612 eggs/clutch) and season (=annual) (15,601 eggs/annum) fecundities of the sub-tropical anemonefishes are nearly 3–5 times higher than those of the tropical species (Richardson et al., 1997). Hence, the warmer tropics appear to increase the spawning frequency but decrease batch and season fecundities.

Experimental studies have shown that the effect of food supply on fecundity is mediated through somatic growth rather than directly on gonadal growth. Essentially, low ration is associated with reduced spawning frequency (e.g. three spined stickleback *Gasterosteus aculeatus*, Fletcher and Wootton, 1995), decreased batch fecundity (e.g. the coral reef fish *Acanthochromis polyacanthus*, Donelson et al., 2008) and fewer progenies (e.g. guppy *Lebistes reticulatus*, Hester, 1964). Feeding captive Pacific herring *Clupea harengus pallasii* in the late maturing stages advanced the spawning periodicity but starvation led to lower fecundity (Hay and Brett, 1988). A reason for the lower fecundity is traced to the atresia of early stage oocytes (Ma et al., 1998). However, the better fed *A. polyacanthus* produces more eggs in its 2 clutches itself within the first 2 months and the damselfish receiving low ration postpones sexual maturity, and produces fewer eggs in its 4 clutches spread over to 4 months (Donelson et al., 2008); the longer period is perhaps required to accumulate adequate reserves from the low ration supplied.

Data available for fecundity of fishes are amazing. The batch fecundity ranges from as few as 3 eggs for the bagrid *Bagre marinus* (Pinheiro et al., 2006) to 25,000,000 in *Molva molva* (Blaxter, 1969). The season fecundity of the pufferfish *Canthigaster valentine*, is 72,000 eggs and the fecundity is equivalent to 1.5 times of its body weight of 8.6 g (Robertson, 1991). In an intermittent lowland stream in Trinidad, West Indies *Corynopoma riisei* spawn about 5,000 eggs, which are equivalent to 2 times the body weight of a female (Alkins-Koo, 2000). Data reported for the life time fecundity of fishes are more amazing; the values range from about 200 for the small viviparous molly (George and Pandian, 1997) to 6 millions for the lethrinid fish *Lethrinus nebulosus* (Ebisawa, 1990). Likewise, information available for the breeding potential of a population of a fish species is even more amazing; for instance, the breeding potential of the Japanese sardine *Sardinops melanogaster* is indeed colossal; inhabiting 260,000 km<sup>2</sup> area of the Pacific Ocean, the sardine spawn 5,130 trillion eggs annually (Watanabe et al., 1996).

#### 1.4 Egg size

Within a species, changes in egg size are associated with latitude-dependent temperature and food supply. In the small cobitid *Barbatula barbatulus*, the eggs of the Finnish population have a volume of 2.5 times larger than that of a population of southern England (Mills and Eloranta, 1985). During the warmer years in a southern chalk stream in England, the annual variations in the egg volume (~1.6 mm<sup>3</sup>) of the dace *Leuciscus leuciscus* were usually associated with the expected inverse relation between egg size and fecundity, an expression of trade off between these factors by the fish (Mann and Mills, 1985). Among populations inhabiting the rivers of Cape Race, Newfoundland, the egg volume of the brook trout *Salvelinus fontinalis* varied by 35%. With better food supply, the females produced a large number of smaller eggs but a small number of larger eggs when receiving low food supply (Hutchings, 1991). These field observations confirm the trade off between egg size and fecundity by the fishes.

However, the results obtained from laboratory studies differ from these field observations. The tropical damselfish *Acanthochromis polyacanthus* produced a large number (~3,000) of large (5.76 mm<sup>3</sup>) eggs, when fed 3-times more food for 2 months prior to spawning but a smaller number (2,200 eggs) of smaller (4.38 mm<sup>3</sup>) eggs at lower ration (Donelson et al., 2008). From a controlled laboratory experiment, that lasted for 1.5 years and covered a complete maturation cycle of the Atlantic herring *Clupea harengus*, Ma et al. (1998) showed that ration had no effect on oocyte size and relative potential fecundity (oocytes/g ovary weight), but the mean potential fecundity (oocytes/fish) of the low ration (6 mg/g/day) group

was 26% lower than that of the high ration (12 mg/g/day) group (see their Fig. 8). This body weight-dependent decrease in potential fecundity was due to the atresia of early stage oocytes, a common phenomenon in the herring; the atretic intensity of the oocytes was 3 and 6% in the high and low ration groups, respectively and the intensity was also a body weight-dependent phenomenon in both low and high ration groups (see their Fig. 7). Under better feeding regimes, both the herring and damselfish produce a large number of large/equal sized eggs but smaller numbers of small/equal sized eggs, when receiving low ration.

In general, fishes produce a large number of smaller eggs; their egg size ranges from 0.25 mm in diameter to >20 mm (Pinheiro et al., 2006) and batch fecundity ranges from 3 eggs (20 mm) in the bagrid *Bagre marinus* (Pinheiro et al., 2006) to 1,00,000 eggs in the striped weakfish *Cynoscion striatus* (Macchi, 1998). Hence, there is a trade-off between egg size and fecundity, and the trade off is also limited by the volume of the body cavity that can accommodate ripe eggs. It appears that the fecundity can be maximized by minimizing egg size. However, the fishes seem to strike at an optimum egg size, which shall ensure maximum survival of their progenies and fecundity that the female can afford to allocate from its reserves and can provide space for the ripe eggs in its body cavity. Hence, it may be possible to recognize the most important factors that are responsible for the variations in the egg size at intraspecies and interspecies levels.

Most pelagic spawners are prolific and produce smaller but a large number of eggs; for instance, the mean size of pelagic eggs of 21 labrids is 0.64 mm and that of the spindle shaped eggs of 6 scarid species is 0.54 mm diameter (Colin and Bell, 1991). Density of pelagic eggs is known not to decline with egg volume and production of large low-density eggs might be advantageous, when sperm concentrations are low (Robertson, 1996). Secondly, pelagic spawners release a few thousands (e.g. *Centropyge ferrugatus*, Sakai, 1996) to millions of eggs (e.g. *Lethrinus nebulosus*, Ebisawa, 1990). In contrast, the demersal spawners lay a few hundred (e.g. *Chandraranga*, Hunnam et al., 1991) to thousand (e.g. *Notropis leedsi*, Rabito and Heins, 1985) eggs only. The size of demersal eggs is also large; for instance, the egg size of *Tilapia zilli* is 1.46 mm (Coward and Bromage, 1999); it is 1.5 mm for 48% (10 of 21) fish species in the British waters (Russel, 1976). The structure of the demersal adhesive egg is more complex than that of planktonic eggs. Further, many demersal eggs have higher concentrations of respiratory pigments, presumably for anaerobic metabolism (Balon, 1975b) and also their vascular surfaces and associated structures are often elaborated to enhance gaseous exchange (Balon, 1975a).

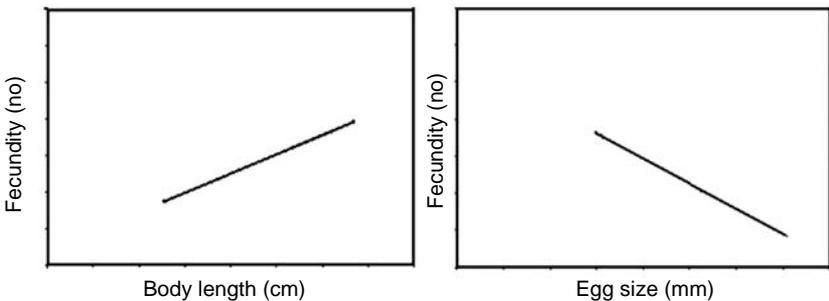
Smaller fishes opt for larger but a smaller number of eggs. For a 3-fold increase in body length from 3.5 cm in *Apogonichthys waikiki* to 12.9 cm in *A. menesmus*, the egg number decreases by 20% in the Hawaiian apogonidan

cardinalfishes (Barlow, 1981, see also Vagelli, 1999). Indeed smaller fishes make a heavy investment per egg; consequently, they are even less fecund than their size would predict.

Fishes, which display parental care with a wide range of investments in time and energy, have also opted for larger but smaller number of eggs. In maternal and/or paternal mouthbrooding fishes like the cichlids, apogonids, silurids and others, the trade-off between egg size and fecundity may also have to match the space available to accommodate the ripe eggs in the body cavity with that available to accommodate the developing embryos in the oral cavity. In general, fecundity increases with body size but decreases with egg size among the mouthbrooding fishes (Fig. 5). For 18 species of the mouthbrooding cichlids, despite wide scatter, the expected relations are apparent namely (i) a positive correlation between body size and fecundity and (ii) a negative one between egg size and fecundity. A reason for such a wide scattering can be the loss of eggs or brooded embryos. In biparental mouthbrooding cichlid *Haplotaxodon microlepis*, the egg losses are about 40% and 20% (totalling to 60%) by a mouthbrooding female and male, respectively (Kuwamura, 1988).

Interestingly, the maternal mouthbrooding, a highly energy demanding job, drives a few females of *Pseudotropheus lombardoi* to change the feminine body colour to that of masculine and even generate testicular oocytes. However, these females remain females (see Naish and Ribbnik, 1990).

In many syngnathids, the fecundity increases exponentially with body length of females (e.g. pipefish *Syngnathus typhle*, Berglund et al., 1986b, 1988; weedy seadragons *Phyllopteryx taeniolatus*, Forsgren and Lowe, 2006). The number of embryos gestated by the males is also positively correlated, although the data are widely scattered, owing to the loss of embryos during the period of gestation (e.g. *S. typhle*, Berglund et al., 1988); besides males are found to gestate more number of embryos than the fecundity of females of corresponding size (see also Fig. 3 of Berglund



**Fig. 5** Relations of body length to fecundity (left panel) and egg size to batch fecundity (right panel) of mouthbrooding fishes.

et al., 1988). In pipefishes, the males may provide nutrients to their broods; however, males of *Nerophis ophidion* invest more energy per zygote than do females, while the two sexes of *Syngnathus* (= *Siphonostoma*) *typhle* contribute about equal energy. Hence, it is likely that their progenies may be larger than the eggs (Berglund et al., 1986a). Clearly, the pouch in the male must have a larger space to accommodate the growing embryos than that is available to accommodate the ripe eggs in the body cavity of the female. However, many pipefishes are polygynous and accept eggs from more than one female (Berglund and Rosenqvist, 1990; Wilson et al., 2003). Using cloned microsatellite loci, Jones et al. (1999) have shown that the eggs from the pouch of a male *S. typhle* are contributed by one to six females; the females are 'bed-hedging' by spreading their eggs among multiple males to increase genetic variability in broods to benefit one or both parents; this sort of 'bed-hedging' mode of ovipositions does not provide the scope to check whether the space available in the male's pouch is larger than that available in the female's body cavity to accommodate the ripe eggs.

Yet information made available (Table 4) for the seahorse *Hippocampus kuda* collected from the Gulf of Mannar, India and reared in the laboratory provides an opportunity to match the space available to accommodate the ripe eggs in the body cavity of the female to that available to hold growing embryos in the pouch of the male. Accordingly, the female (8.3 g) provides adequate space in its body cavity to accommodate 192 eggs weighing 393 mg (GSI: 4.7%) but the male's pouch has space to hold only 153 progenies equivalent to 51 mg (0.56% body weight). The following may be inferred: 1. Of 192 eggs, 39 (20%) are lost during the transfer from female to male and/or gestation; Thangaraj and Lipton (2009) have also observed that many eggs are lost during the transfer and 2. Males do not provide nutrients to the broods, as the weight of a progeny is only 15% of an egg.

**Table 4** Reproductive traits of the seahorse *Hippocampus kuda* (from Thangaraj and Lipton, 2009 unpublished).

Traits	Mean
Female body mass (g)	8.31
Egg mass (mg)	2.05
Batch Fecundity (no)	192.00
Fecundity mass (mg)	393.00
GSI of female (%)	4.70
Male body mass (g)	8.98
Progeny mass (mg)	0.30
Brood size (no)	153.00
Brood mass (mg)	50.70
Brood space index, as (%)	0.56

More than 70% cichlids are mouthbrooders (Keenleyside, 1979). The number of eggs/embryos held in the mouth ranges from 6 to 139 (Kuwamura, 1986). The accidental loss of eggs/embryos by the mouthbrooding female is in the range of 27–32% of the acquired eggs (Kuwamura, 1986). Understandably, the losses (about 60%) are higher for the biparental mouthbrooding *Haplotaxodon microlepis* (Kuwamura, 1988).

Among silurids, males are the mouthbrooders. More than 54% adult males of *Tachysurus tenuispinis* are recorded to mouthbrood at any time during the breeding season. The egg losses by some Indian marine silurids range from 8 to 56% (Table 5). The values vary not only with species but also with places of collection. Raje and Vivekanandan (2008) suspect that the catfishes might have spewed some eggs held in their mouth, while suffering in the fishing gear. They also indicate that some of these Indian fishes starve and lose body weight, due to the oral gestation for about 2 months.

Mouthbrooding apogonidan males are known for filial cannibalism. The females produce surplus eggs as the source of nutrition for the mouthbrooding apogonidan males (Kume et al., 2000). Each clutch is held together by chorionic filament (Kume et al., 2000) and males mouthbrood an egg mass received from one female at a time (Okuda, 1999). The duration of mouthbrooding lasts from 5 days in *Apogon niger* (Kuwamura, 1985) to 19 days in *Pterapogon kauderni* (Vagelli, 1999). The proportion of cannibals ranges from 9% in *A. niger* (Okuda, 1999) to 50% in *A. deoderleini* (Okuda and Yanagisawa, 1996a); the males are known to consume 6% (*A. lineatus*) to 8% (*A. deoderleini*) eggs held in the mouth. Such filial cannibalism is also not uncommon among gobies ventilating their eggs in the

**Table 5** Fecundity, mouthbrooded eggs and egg loss during brooding in selected catfishes (from Raje and Vivekanandan, 2008; modified).

Species	Location	Length (cm)	Fecundity (no)	Mouth-brooded eggs (no)	Egg loss (%)
<i>Osteogeneiosus militaris</i>	Mumbai Mandapam	45	61	56	8
<i>Tachysurus caelatus</i>	Mumbai Mandapam	26 35	62 50	54 37	14 26
<i>T. thalassinus</i>	Mandapam	38–42	46	33	27
<i>T. dussumieri</i>	Mangalore Mandapam	– 70	188 136	101 63	47 56
<i>T. tenuispinis</i>	Vizag	28	55	28	50
<i>T. platysomus</i>	Mandapam	32	39	19	50

nest; 30 to 60% eggs are consumed by the male of *Pomatochistus microps* (Jones and Reynolds, 1999 a).

### 1.5 Sperm count and sperm cells

For fishes, information on sperm count per milting and total sperm count of a male during its life time is relatively less. Paucity of information on these aspects may be due to one or more of the following: 1. In general 95–98% of the eggs released by a female in its natural habitats is fertilized (e.g. *Thalassoma bifasciatum*, Marconato et al., 1997; *Acanthurus nigrofuscus*, Kiflawi et al., 1998). Hence, the contribution of a male to the reproductive success of a species may not be a critical limiting factor; for instance, there are unisexual species that are flourishing as successfully as bisexual species. 2. Energy required for normal development and functioning of testis is far less, as indicated by gonadosomatic index (GSI) (Table 6). It is shown to be 6–18 times less than that required for the development of the ovary (e.g. Diana, 1983). 3. Whereas the oocytes and eggs can readily be counted, sperm are not amenable for counting; their counting requires minimum of a microscope and haemocytometer; further, their separation from the rest of the earlier stages of spermatogenesis and also from the testicular tissues may prove an impossible task. 4. In a few species, the testes are embedded in fatty tissues; the dissection and clearing them from the fat masses are a difficult and time consuming task (e.g. brown butterflyfish *Chaetodon larvatus* (Zekeria and Videler, 2000).

Yet, the GSI values of males may indicate the relative sperm content of a fish species, as much as the GSI of a female may provide an idea on fecundity. Table 6 lists the GSI values reported for selected gonochoristic species. The values for the males range from 0.39 in the tropical cichlid *Oreochromis niloticus* (Gomez-Marquez et al., 2003) to 10–27 in the boreal *Boreogadus saida* (Craig et al., 1982). The GSI value of females is more than 3 (e.g. *O. niloticus*) to 10 (e.g. *Gasterosteus aculeatus*) times greater than the respective values of the males. The space available to accommodate the ripe eggs in the body cavity may have to match with that available to accommodate the growing wrigglers and larvae in the buccal cavity of mouthbrooding cichlid females and males; not surprisingly the GSI values are less, for instance 0.39 in the mouthbrooders like *O. niloticus* in comparison to 0.65 in the substrate brooders like *Telmatochromis vittatus*. The lowest value thus far reported seems to be for the ‘fragile’ male seadragons *Phyllopteryx taeniolatus*, which broods the eggs in his pouch (Forsgren and Lowe, 2006). Semelparous salmon like *Salmo salar* have higher GSI values than the multiple spawners like *Lethrinus nebulosus*. Understandably, the sturgeons *Acipenser schrenckii* and *Huso dauricus*, which are substrate spawners and are known to breed once in 3–4 years, have high (> 18–23) GSI values (Wei

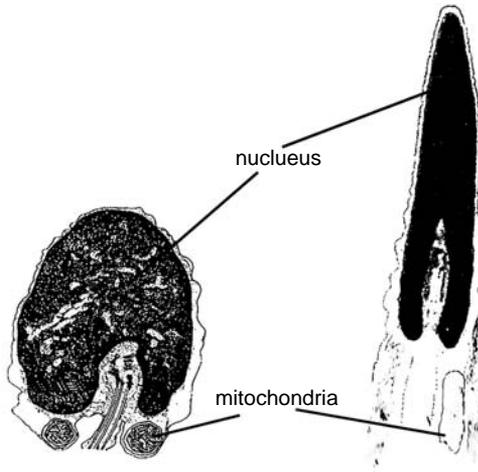
**Table 6** Gonadosomatic index (GSI) of selected teleostean fishes.

Species	GSI of ♂	GSI of ♀	Reference
<i>Telmatochromis vittatus</i> (substrate brooder)	0.65	–	Ota and Kohda (2006)
<i>Tilapia zilli</i> (substrate brooder)	–	1.60	Coward and Bromage (1999)
<i>Acipenser schrenckii</i>	–	18.4	Wei et al. (1997)
<i>Huso dauricus</i>	–	23.5	Wei et al. (1997)
<i>Oreochromis niloticus</i> (maternal mouthbrooder)	0.39	1.34	Gomez-Marquez et al. (2003)
<i>Haplotaxodon microlepis</i> (biparental mouthbrooder)	–	0.20	Kuwamura (1988)
<i>Phyllopteryx taeniolatus</i> (male brooder)	0.34	1.25	Forsgren and Lowe (2006)
<i>Hippocampus kuda</i>	–	4.7	Thangaraj and Lipton (2009)
<i>Lethrinus nebulosus</i> (pelagic spawner)	1.00	8.00	Ebisawa (1990)
<i>Gasterosteus aculeatus</i> (demersal spawner)	2.00	20.00	Wootton (1984)
<i>Salmo salar</i> (demersal spawner)	3–6	20–25	Fleming (1996)
<i>Boreogadus saida</i> (Arctic cod)	10–27	–	Craig et al. (1982)
<i>Galaxias maculatus</i>	35	35	Boy et al. (2007)

et al., 1997). Notably, the Arctic cod *Boreogadus saida* and the Argentina's puyen *Galaxias maculatus* have very high GSI of 27–35.

In coupling with diverse modes of fertilization in fishes, the spermatozoa display various morphological adaptations in size, shape and ultra-structure. The spermatozoan structure of more than 300 fish species spread over 100 families has been described (Mattei, 1988; Jamieson, 1991). The sperm cell consists of a small spherical head, a short collar and a single flagellum of 1.8 µm length (David and Pandian, 2006). Typically, the head lacks an acrosome and accommodates electron dense nucleus; the collar contains a few mitochondria (Fig. 6). With this basic morphology, the 'aquasperm' is adapted for external fertilization. The process of its spermatogenesis is characterized by short duration of spermiogenetic cycle and reduced energy expenditure. Consequently, these enable the externally fertilizing fishes to produce a large number of sperm cells (see Kweon et al., 1998).

On the other hand, the teleosts that employ the internal mode of fertilization have specialized spermatozoa with a relatively larger and elongated head containing nucleus and collar containing extensively modified mitochondria (Fig. 6). This kind of 'introsperm' with its elongated head ensures the passage of the sperm cell through more viscous fluids of the female reproductive tract than the aqueous medium (Gardiner,



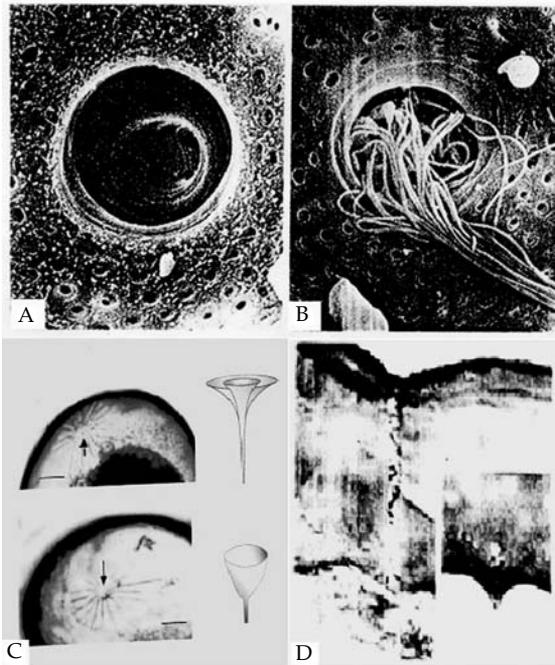
**Fig. 6** Longitudinal sections of spermatozoa of *Oncorhynchus tshawytscha* (left) and *Poecilia latipinna* (right). Note the few mitochondria in *O. tshawytscha* sperm and large modified ones in *P. latipinna* (from Jamieson, BGM, 1991 *Fish evolution and Systematic Evidence from Spermatozoa* (Cambridge University Press).

1978) and may also facilitate side-by-side alignment and thus the formation of sperm bundles (Burns et al., 1995). An enlarged collar containing numerous mitochondria prolongs the life of the sperm cells within the female reproductive tract (see Kweon et al., 1998).

The absence of acrosome in the spermatozoa (Fig. 6) coincides with the presence of micropyle in the eggs (Fig. 7). Since entry of the sperm is made through the micropyle during fertilization, fertilization in fishes may not necessarily be a species specific event. Thus the sperm of the common carp *Cyprinus carpio* can activate eggs of a dozen fishes crossing the borders of species, genus and family. Likewise, the eggs of *Oncorhynchus mykiss* may be activated by the sperm of a half dozen fish species (Table 7). The possibility of activation of teleostean eggs by heterologous sperm has facilitated natural hybridization in more than 130 species and artificial hybridization in 150 species of fishes (Argue and Dunham, 1999). The wide scope for hybridization has also enhanced the plasticity of sex determination and differentiation processes in fishes.

## 1.6 Breeding sites

For most oviparous species, selection of site(s) for mating and spawning is one of the most important events in the life history, as oviposition site directly affects egg survival, hatching success and progeny performance, and thereby parental fitness. Hence, oviposition site selection eventually



**Fig. 7** Apical view of unfertilized eggs of (C) *Gymnocorymbus ternetzi* (upper) and *Hemigrammus caudovittatus* (lower) and schematic views of their respective micropylar canal in the eggs (from David, 2004). Electron microscopic views (A) of egg showing micro-pyle and (B, D) entry of sperm through the micropylar canal in an egg of *Alcichthys alcicornis* (from Munehara et al., 1989).

**Table 7** Activation of teleostean eggs by heterologous sperm (from Pandian and Kirankumar, 2003; modified).

Sperm donor	Sperm recipient
<i>Cyprinus carpio</i>	<i>Ctenopharyngodon idella</i>
	<i>Carassius auratus</i>
	<i>Hypophthalmichthys molitrix</i>
	<i>Cirrhinus mirrgala</i>
	<i>Misgurnus anguillicaudatus</i>
	<i>Cobitis biwae</i>
	<i>Tinca tinca</i>
	<i>Oreochromis niloticus</i>
	<i>O. mossambicus</i>
	<i>Oncorhynchus mykiss</i>
	<i>Salmo salar</i>
	<i>S.trutta</i>
	<i>Salvelinus fontinalis</i>
<i>Oncorhynchus kisutch</i>	
<i>O.tshawytscha</i>	
<i>O.masou</i>	

affects recruitment (Watanabe et al., 1996), population dynamics, community structure and distribution patterns of species (Spencer et al., 2002). Females use both abiotic (substrate, oxygen level, water depth) and biotic (chemical cues emanating from con-specifics, competitors, predators) factors as cues to select appropriate oviposition sites. Balon (1975a, b, 1981) has comprehensively classified the reproductive guilds of fishes, broadly based on the site of spawning, and the degrees of parental care. Table 8 provides some representative breeding sites of selected fishes.

There is a basic division between pelagic and demersal eggs (see Pandian and Fluchter, 1968). Pelagic eggs are characteristic of offshore marine, coral reef and riverine species. In such species, spawning usually takes place at sites and at times in relation to the tidal cycle in marine, and faster flow and flooding of rivers, which ensure fertilization and dispersal of fertilized eggs. In many pelagic-spawning marine fishes, a female releases her entire clutch in one act that lasts only a few seconds. In contrast,

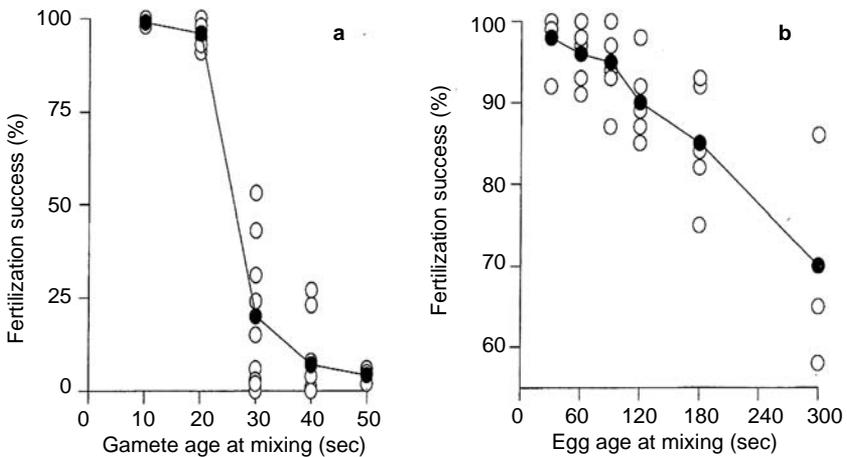
**Table 8** Breeding sites of fishes.

Spawning site	Examples
I. Pelagic spawners	
a) Pair spawners	<i>Zebrasoma scopas</i>
b) Group spawners	<i>Mycteroperca rosacea</i>
c) Mass spawners	<i>Theragra chalcogramma</i>
II. Benthic spawners	
a) Free spawners	<i>Phoxinus phoxinus</i>
b) Burrow spawners	<i>Zacco temmincki</i>
c) Nest spawners	<i>Pomatochistus microps</i>
III. Substrate spawners	
a) Cave spawners	<i>Anoptichthys jordani</i>
b) Spawners on others with irrigation	<i>Acheilognathus rhombeus</i>
c) Plant spawners	<i>Symphodus ocellatus</i>
d) Crevice spawners	<i>Notropis leedsii</i>
e) Beach spawners	<i>Galaxias maculatus</i>
f) Mud spawners	<i>Cynolabias nigripinnis</i>
g) Bubble nesters	<i>Betta splendens</i>
h) Anemone nesters	<i>Amphiprion clarkii</i>
i) Rock spawners with glue	<i>Lamprologus furcifer</i>
IV. Bearers	
1. External brooders	
a) Mouth brooders	<i>Oreochromis mossambicus</i>
b) Anal pouch brooders	<i>Corydoras aeneus</i>
c) Pouch brooders	<i>Hippocampus kuda</i>
2. Internal brooders	
a) Ovoviviparous	<i>Sebastes marinus</i>
b) Viviparous	<i>Poecilia reticulata</i>
V. Brood parasites	<i>Pungtungia herzi</i>

a benthic spawning female may release eggs in pulses and may require a longer duration to deposit her entire clutch. This may ensure dispersal and reduce predation but the female is more vulnerable to disruption and premature termination of spawning (see Robertson, 1991).

Marine pelagic-spawners may spawn as pairs, groups (4 to 15 individuals) and mass (500–2000 individuals; Kiflawi et al., 1998). With narrow bodies and the ability to swim rapidly, the spawners closely oppose male and female gonopores at the apex of ‘spawning rush’, when gametes are released. Consequently, gametes are diluted to a lesser degree. Group and mass spawning may enhance the chances of early fertilization. In group spawns, sperm release by each of 5 to 20 males together with the egg release of a single female may result in the release of 50 times more sperm than that in a pair-spawn (Shapiro et al., 1994). The gamete clouds by group- and mass-spawners span several cubic meters and are often highly visible for more than 2 minutes after spawning ceases (Kiflawi et al., 1998).

From experiments on artificial fertilization of the pelagic-spawning surgeonfish *Acanthurus nigrofuscus*, Kiflawi et al. (1998) showed that more than 60 and 90% of spawned eggs are fertilized within 5 seconds and 15 seconds of gamete release, respectively; the fertilizing ability of the activated sperm lasts for about 20 seconds only, whereas the egg viability lasts for longer (Fig. 8). Hence, eggs not fertilized by a female’s spawning



**Fig. 8** Artificial fertilization experiments in surgeonfish *Acanthurus nigrofuscus* (a) both gametes have been aged separately prior to mixing, (b) only eggs have been aged and mixed with fresh sperm. Open and filled symbols represent individual data points and mean, respectively. Note differences in scale along both axes (from Kiflawi et al., 1998).

partners may be fertilized by fish sperm released in subsequent and nearby matings within the aggregation.

In pelagic spawners, courtship culminates in so called 'spawning rush' and the partners may ascend to heights up to 9 m (Thresher, 1982). Releasing eggs at the surface water is synchronized with tidal cycles in most species for dispersal of fertilized eggs. There is a great variety of patterns of the daily, lunar and seasonal time scales (Robertson, 1991).

For the demersal spawners, the most important potential hazard is the deficiency of dissolved oxygen in the surrounding water to the metabolically active embryonic and larval stages, which can ill-afford to escape from it because of their inability to move away; the second hazard is siltation. To avoid both of them as well as predation, many fishes such as, the salmonids choose to bury their eggs in gravel substratum, through which water readily percolates and bathes the eggs and embryos with required oxygen. The female Atlantic salmon *Salmo salar* digs several nests and the area of disturbed gravel containing the nests forms a redd. A female may use more than one redd, and may move as far as 0.5 km to dig a new one (Fleming, 1996). Large male sunfish excavates a nest pit by mouth and by sweeping movements of the tail. Small holes and narrow crevices, which eliminate the entry of predators, are used as very important resources by the hole/crevices spawners. For instance, the crevice spawner *Notropis leedsi* prefers to spawn in crevices with a width of 1.5–3.0 cm (Rabito and Heins, 1985).

While selecting a nest as the site for oviposition, the nest-fidelic damselfishes use predation-specific olfactory cues in assessing earlier brood losses and avoid the nests that have suffered complete brood losses. In the bicourous damselfish *Stegastes partitus*, females mate repeatedly during the reproductive cycle and show high nest fidelity, with the majority of females laying more than 70% of their eggs in a single nest for an incubation period of 12 days. Of 831 clutches laid during five reproductive cycles, 146 (18%) were found to have disappeared before hatching (Knapp, 1993). Of the 146 clutches which disappeared, 22% disappeared during the day, perhaps owing to filial cannibalism, and the remaining 78% disappeared at night. Other damselfishes like *S. reucostictus* and *S. dorsopanicans* are also known to suffer 69 and 79% brood losses, respectively at night. The high frequency of nocturnal egg loss relative to day losses in these species may stem from the pattern of parental care. Although males of the bicourous damselfish vigorously defend their nests against all intruders during the day, they seem to provide no defense at night. This lack of nocturnal nest defense presumably allows easy nest access to night active egg predators like brittlestar *Ophiocoma echidna*. Females of the damselfish can detect the probability of future offspring survival directly by monitoring the survival of eggs in nests. They use olfactory cues specific to the brittlestar and

avoid the nests from which the brittlestar have removed the entire brood. By experimental removal of broods and addition of brittlestar odours to nests, Knapp (1993) demonstrated that brittlestar odours, which last for 5 days, are sufficient to cause females to avoid nests. Females may also respond to visual cues associated with brood losses. Therefore, the females of bicour damselfish are capable both directly and indirectly of assessing the expected future of egg survival from nests; they use this information to make mating and oviposition decisions that increase offspring survival and enhance their own fitness.

Large males of smallmouth bass *Micropterus dolomieu* are more site tenacious, a character desired for continued ventilation of eggs and chasing predators, and thus the size of the male provides a cue to the female to assess the future security of her eggs, to select him for mating and his nest for oviposition (Wiegmann and Baylis, 1995).

Other studies have reported that the presence of early stage eggs in the nests provides positive visual cues for the females to choose the nests as the site for oviposition. Using visual cue of colour of developing eggs, the females of *Amblyglyphidodon leucogaster* preferentially visited males that had eggs in their nest site, perhaps signalling no recent loss of broods. Males, that possessed nests with early embryonic stages, as indicated by the pink colour, were more frequently visited by females (134 times) and their nests were the more preferred sites for oviposition (206 spawns) than the nests with no eggs (163 visits but 55 spawns only) and nests with eggs at the late stage of embryonic development, as indicated by the black colour (28 visits but 25 spawns only). Clearly, the female assessed that the nests with eggs at the early stage of development are those that have not suffered brood loss due to predation. Thus, a male could acquire and care for as many as 4, 50,000 eggs in his nest during a breeding season (Goulet, 1998).

As indicated earlier, oxygen deficiency is another potential hazard. In the common goby *Pomatoschistus microps*, males provide parental care, which consists primarily of nest defense and egg ventilation by fanning and turning the eggs with fins. They build nests using mussel shells by piling different quantum of sand on the shells and excavating sand from beneath to provide an entrance hole; the larger the quantum of sand piled on the shells, the smaller shall be the entrance hole; such nests with narrow holes are less vulnerable to detection by predators like the shore crab *Carcinus maenas* but suffer reduced oxygen due to low water flow through the nest and demand ventilation by the males. Consequently, the mating pairs encounter intense trade-offs between low vulnerability of eggs to predation and high cost of parental care on egg ventilation. During her visit to the nest, the female receives visual cues from the nest built and assesses the male's strength to pile sand and possibly to ventilate eggs.

Under saturated oxygen conditions, she prefers to spawn in nests with small entrance holes. The mount heights of the nest and entrance width appear to provide visual cues to her not only to choose the mate but also the site for oviposition (Jones and Reynolds, 1999b). However it is also found that this preference to narrow entry is not significant under low oxygen conditions.

To understand this difference in the site selection to levels of oxygen, Jones and Reynolds (1999a) reared one series of 'control' gobies in fully saturated (95% oxygen) water and another experimental series in low oxygen (35% saturation) dissolved water for a period, during which the gobies completed the first and second incubation cycles, and estimated selected parameters of egg ventilation. The experimental males under low oxygen conditions ventilated eggs nearly 1½ times more frequently and spent 2 times longer durations on ventilation both during the first and second incubation cycles (see Fig. 1 of Jones and Reynolds, 1999a). Besides, the males had to turn the eggs to reduce competition for oxygen among the eggs in a clutch. That costed them nearly 5 times more work, i.e. the control males turned the eggs once every 5 minutes but the experimental males did it more than twice every 5 minutes. Further, the incubation period was also extended from 9 days in the control males to 9.7 days and 10.4 days during the first and second incubation, respectively in the experimental males. All these led to six times more loss of body weight of the experimental males during an incubation cycle in comparison to controls. Hence 30 and 60% males abandoned the ventilation and even ventured to eat the eggs from the 3rd to 7th day of incubation. Consequently, 65% eggs alone hatched under low oxygen conditions during the second incubation, whereas more than 80% eggs successfully hatched both from the nests of controls and experimentals, when the first incubation was completed. Thus, under low oxygen condition the nests with a narrow entrance led to egg cannibalism, despite providing low vulnerability of eggs to predators. This is perhaps the reason why the female gobies are not selective of the nests under harsh environments characterized by low oxygen. Incidentally, this may explain how critical the oxygen requirement is for the developing eggs and trying situations encountered by the fishes in selecting 'safe' sites for oviposition.

Many tropical and sub-tropical fishes respond to hypoxia by using aquatic surface respiration (ASR), whereby the oxygen-rich microfilm at water surface is pumped over the gills to increase oxygen availability (Kramer and Mehegan, 1981, Kramer and McClure, 1982). Consequently, the ASR may reduce ventilation rate. Chi-Corrie et al. (2008) quantified the behavioural response of the mouthbrooding African cichlid *Pseudocrenilabrus multicolor victoriae* to progressive hypoxia. The oxygen level (threshold), at which a brooding female initiated ASR, was (36.4

mm Hg) higher than that (15.4 mm Hg) at which a male switched to ASR and thereby clearly showed the greater demand for oxygen supply by the brooding female and it, in turn, reduced ventilation 0.7 times/second in the brooders and males. This suggests that ASR does increase the efficiency of oxygen uptake by facilitating a drop in gill ventilation rate. As aquatic oxygen level approaches zero, the brooding female spends most of her time at the surface, endeavouring to survive herself and her brood but greatly risking aerial predation by birds (see Maan et al., 2008).

Incidentally, under low oxygen condition, the guarding parent has to ventilate eggs by more frequent fanning with fins; simultaneously it has also to more frequently irrigate its own gills to secure enough oxygen from the low oxygen saturated water. Not surprisingly, many fishes belonging to Cichlidae, Siluridae, Bagridae and Apongonidae mouthbrood eggs, a process in which the ingressing water simultaneously irrigates first the eggs in mouth, and then the gills.

With reference to oxygen availability, even more challenging is the predicaments encountered by the bitterling to select a suitable site for oviposition. As an alternative, the bitterling has chosen the symbiotic partner, the mussel to irrigate their developing eggs under much secured habitat. They use the interlamellar space of the gills of live unionid freshwater mussels alone as the site of oviposition. Females of bitterling develop a long ovipositor to place their eggs on the gills of a mussel through the mussel's exhalent siphon. During a spawning season, they spawn in more than one mussel species. Multiple clutches from several females of the same and different bitterling species can be hosted in a single mussel (Fukuhara et al., 1998). Embryonic development of eggs of bitterling is completed inside the mussel's gill chamber in a month in spring-spawning species like *Tanakia lanceolata*, *T. limbata* and *Acheilognathus tabira tabira* but during a period of 6–8 months in autumn spawning species like *A.rhombeus* in Japan. Embryos of autumn spawning bitterling species undergo a sort of hibernation and resume development in spring (Kitamura and Uehara, 2005). During incubation in the host mussel, the bitterling embryos compete with each other for oxygen, which is known to lead to density-dependent mortality in *Rhodeus sericeus* (Smith et al., 2000) and *R. ocellatus kurumeus* (Kitamura, 2005). A chosen mussel species may not always be accessible to bitterling for spawning because of the 'wrong' orientation of the mussel in the substrate; during non-feeding and non-respiring period and when disturbed, the closure of siphons by the mussel renders not only inaccessible to bitterling but also subject the embryos to asphyxiation in the gill chamber of the mussel (Aldridge, 1997). Premature ejection of dead or live embryos is prevented by the relatively large size of eggs and embryos, and their possession of projections and tubercles, which ensure the eggs and embryos by fitting them securely between the gill lamellae

of the mussel (Smith et al., 2004). Thus the bitterling may choose mussels that are compatible with their eggs and embryos in terms of gill structure and conditions prevailing in the gill chamber of the mussels. The strategy of selection of a oviposition site in a mussel is so ingenious that there is evidence that the bitterling have co-evolved with mussels (Smith et al., 2004).

In preferring mussels that contain low number of eggs and placing eggs in the gill chambers, where the egg density is the lowest, females of bitterling make sophisticated oviposition decisions that maximize embryo survival. In an interesting and well designed investigation on reproductive ecology and host utilization of sympatric bitterling in low land Harai River in Japan, Kitamura (2007) showed that these sympatric bitterling do not directly compete against each other for the host mussel. All the bitterlings use *Inversidens brandti*, *Obovalis omiensis* and *Inversiumio jokohamensis* as spawning hosts. Of the four bitterlings investigated, the autumn spawner *A. rhombeus* is temporally isolated in its reproduction from the others. The ability of a bitterling to conduct siphon inspection in a fast flowing river may energetically be limited by its body shape. *T. limbata* has the highest relative body depth among the spring-spawning bitterlings and spawned largely in inshore area. Secondly, by virtue of its short ovipositor, *T. limbata*, which inhabits the inshore area, is effectively spatially separated from the other two spring spawners. Among the two spring-spawning bitterlings in the offshore area, *A. tabira tabira* significantly prefers the large *I. brandti*, while *T. lanceolata* prefers *O. omiensis*. Of course, there are considerable overlaps among the host mussels as oviposition sites for the bitterling in terms of space and time but competition is not intense or direct.

Benefits accruing from incubation of eggs in respiratory chambers are so high that fishes have explored all possibilities of utilizing every animal group possessing a system of irrigation for incubation of their eggs. Left with no other alternative group, the freshwater fishes exclusively utilize the mussels as the substratum for egg incubation. Marine fishes have a wide choice; they incubate their eggs in the canal system of sponges, and respiratory chambers of ascidians and crab (Table 9). The scope for using marine mussels for egg incubation is indeed very vast, but reports on such a relation are still awaited. Freshwater fishes have developed a long ovipositor to place the eggs between the gill lamellae and the males to fertilize the eggs by milting through inhalant siphon. Marine fishes deposit their fertilized eggs by a relatively shorter ovipositor (e.g. *Pseudoblennius percoides*) or by projecting their body into the host (e.g. *Aulichthys japonicus*). These fishes and their hosts provide an exciting area for biologists interested in coevolution.

**Table 9** Incubation of fish eggs in respiratory chambers of some invertebrate hosts.

Species	Host species	Reference
Cyprinidae	mussel <i>Unio</i>	Hunnam et al. (1991)
<i>Rhodeus sericeus</i>		
<i>R.ocellatus</i>	mussel <i>Anodonta woodiana</i>	Nagata (1985)
<i>Acheilognathus rhombeus</i>	mussel <i>Inversidens brandti</i>	Kitamura (2007)
<i>A. tabira tabira</i>	mussel <i>Obovalis omiensis</i>	
<i>Tanakia lanceolata</i>	mussel <i>Inversiunio jokohamensis</i>	
<i>T.limbata</i>		
<i>Tanacia</i>	mussel	
Aulorhynchidae	Ascidian <i>Halocynthia roretzi</i>	Akagawa et al. (2008)
<i>Aulichthys japonicus</i>		
Cottidae		
<i>Blepsias cirrhosus</i>	sponge <i>Mycale adhaerens</i>	Munehara et al. (1991)
<i>Pseudoblennius percoides</i>	ascidian	Shinomiya and Ikemoto(1987)
Liparidae	crab <i>Lopholithodes foraminatus</i>	Peden and Corbett (1973)
<i>Careproctus sp</i>		
Monacanthidae	sponges <i>Grantessa mitsukurii</i>	Akagawa et al. (1995)
<i>Brachaluteres ulvarum</i>		

Besides providing protection and irrigation, some fishes protect the eggs from potential predators by mimicking the predator of the potential predators of their eggs. For instance, the clown goby *Microgobius gulosus*, one of the gobies exhibiting biparental care, the female guards the eggs inside the burrow in which the eggs are tied to the roots of cattails *Typha angustifolia*, while the male at the entrance of the burrow may mimick the adult blue crab *Callinectes sapidus*, a known predator of the juvenile crabs, who approach the burrow to eat the eggs (Gaisner, 2005).

Voluminous literature is available on spawning migrations of fishes, especially on the migration of Atlantic and Pacific salmon and eel. There are publications on anadromous, amphidromous, catadromous, oceanodromous, and potamodromous migrations. There are also interesting publications such as, Glebe and Leggett (1981) on the energy cost of migrations and spawning in the context of semelparity and iteroparity. Attention has also been paid to the mechanisms that migratory fishes use to orient their migration (Smith, 1985). The title is good enough for a separate book series.

## 1.7 Breeding times

With more or less uniform temperature and day length prevailing in the tropics, many fishes breed almost throughout the year. But in many freshwater fishes of South and Southeast Asia, monsoon synchronizes the breeding to a season. Likewise, temperature and photoperiod seem to

synchronize breeding in fishes of high latitudes to a specific season, when abundance of food resources may ensure maximum survival of the young ones. In temperate waters, egg size-dependent reproductive patterns have been described. 1. Many salmonids spawn during autumn and early winter; buried in gravel, their large eggs (4–6 mm) develop slowly and hatch during spring. 2. Others like the pike are characterized by medium sized eggs (2.5 mm) and short window of spawning during spring and 3. Summer spawners are characterized by dynamics of small eggs of 1–2 mm (Wootton, 1998; see also Teletchea et al., 2008).

Coral reef fishes offer an excellent opportunity to study the dynamics of time selection and synchronization of spawning at the levels of pairs, groups and aggregates. They display a great variety of patterns of spawning on the daily, lunar and seasonal scales. Some species have fixed short diel periods, when they spawn, while others spawn throughout most of the days (Robertson, 1991). The timing of discrete diel spawning periods may also vary and track changes in the daily timing of the tides. For instance, fishes producing pelagic eggs at the lagoon-ocean channel (Marshall Islands) spawn: 1. At or after high tide (44 species), 2. At dusk without reference to tide (6 species) and 3. After slack low water (1 species). The first pattern is most common and occurs in at least 20 labrids, 10 scarids, 6 acanthurids, 1 zanclid and 1 caesionid, the second is found among pomacanthids and one labrid, and the third only from one mullid (Colin and Bell, 1991).

While synchronization of spawning to a specific lunar or tidal cycle offers wide scope for mating and spawning, it also provides an excellent opportunity for predators of spawning adults and eggs. Attacks by piscivores, especially on smaller adults' species occur once per 100–1,000 spawns. Predation on newly released eggs of labrids and scarids is uncommon, but occurs once or twice for every 1,000 spawns of acanthurids and pomacanthids. Increasing height of egg release, speed and length of the spawning ascend and trajectory alteration of ascending adults makes it more difficult for zooplanktivores to locate eggs after release. The ascend reflects strong selection for fishes to release their eggs as far off the bottom as possible. Thresher (1982) showed a conspicuous correlation between ascend height and body size in pomacanthids.

Two types of egg predation can occur over the reefs shortly after the release of 'gamete clouds'. 1. Cloud predators are those, who wait at the spawning site for the 'gamete cloud' and 2. Routine filter-feeding planktivores. The 'cloud predators' must find the 'gamete cloud' within a few seconds of release; otherwise they seem unable to locate the quickly diffusing clouds. Egg predation is less for pair spawners than for group spawners, possibly due to less conspicuous 'gamete clouds' and times of spawning by pairs. In general, the spawners quickly leave the site

after spawning without caring for the eggs. But females of *Labrichthys unilineatus* and *Anampses twistii* attempt to defend their eggs for a few seconds after release (Colin and Bell, 1991).

### 1.8 Breeding systems

Table 10 lists the sexuality and breeding components of fishes. As a group, the fishes display an almost complete range of sexuality from gonochorism to unisexuality and to different patterns of hermaphroditism. Remarkably, with reference to hermaphroditism, not all the species that belong to a family and even a genus may be hermaphrodites. Within the family Serranidae, all species belonging to *Serranus* and *Hypoplectrus* are simultaneous hermaphrodites. But most others are protogynous hermaphrodites. Among sparids, some species like *Sparus caeruleostictus*, *Spondylisoma cantharus*, *Taius tunifrons*, *Pagrus pagrus*, *Porphus*, *Pehrenbergi*, *Pauriga*, *Chrysoblephus laticeps*, *C.cristiceps*, *C.puniceus* and *Pagellus erythrinus* are protogynous hermaphrodites, but others like *Sparus longipinis*, *Pagellus mormyrus*, *Pascarne*, *Diplodus sargus*, *Sargus annularis*, *Boops salpa*, *Dentex gibbosus* and *Lithognathus aureti* are protandrous hermaphrodites, while still others (*Lithognathus lithognathus*, *Charax puntazzo*, *Cheimerius nufar*) exhibit all grades of rudimentary hermaphroditism ending with true gonochorists like *Oblata melanura* and *Dentex dentex* (Garratt, 1986).

In his list Garratt (1986) indicated that many sequential hermaphrodites could not be identified as protogynous or gonochoric. The bogue *Boops boops* may serve as an example for a hermaphrodite of doubtful identity. The first appearance of the male at the size of 15.5 cm and age of 3+ years, high female ratio of 0.62 and the presence of 5.5% hermaphroditic transitionals clearly show that *B.boops* is a (Monteiro et al., 2006) sequential hermaphrodite. While, no male larger than 28 cm is found in the Portuguese commercial capture, females persist even upto 31 cm body size and thereby introduce an element of unreliability on its protogyny. However, the number of females decreased from 3 at 27 cm size to 1 at 31cm body size. Hence, it is likely that a fraction of the female population does not change sex until death. Hence, *B. boops* may be placed as protogynous hermaphrodite, although Monteiro et al. (2006) considered it as a gonochore.

Among gonochorists, the attainment of sexual maturity is followed by more or less irreversible formation of sex specific structures and body colouration. In males, the elongation of gonopodium in Poeciliidae and gonadal papillae in Gobiidae are well known examples; the elongation of the second ventral ray in the sex changing males of *Cirrhilabrus temmincki* is another good example (Kobayashi and Suzuki, 1990). Expansion and beautification of the caudal fin (e.g. *Betta splendens*) and other fins (e.g.

**Table 10** Sexuality and breeding components of fishes.**1. Gender**

- a) Gonochorism
- b) Unisexuality
  - i) Gynogenesis *Poecilia formosa*
  - ii) Hybridogenesis *Phoxinus eos-neogaeus*
  - iii) Gynogenesis-Hybridogenesis—  
Gonochorism *Rutilus alburnoides*
- c) Hermaphroditism
  - i) Simultaneous hermaphroditism
    - α. Self-fertilizing-  
β. Out-crossing *Rivulus marmoratus*  
*Serranus nigricans*
  - ii) Sequential hermaphroditism
    - a) Protogynous
      - 1. Monandric *Thalassoma bifasciatum*
      - 2. Diandric *Synbranchus marmoratus*
    - b) Protandrous
      - 1. Monandric *Pagellus acarne*
      - 2. Diandric *Lates calcarifer*
  - iii) Serial hermaphroditism
    - α. Marian hermaphroditism *Lythrypnus dalli*
    - β. Okinawan hermaphroditism *Dascyllus aruanus*
    - γ. Bidirectional hermaphroditism *Gobiodon histrio*
    - δ. Cyclic hermaphroditism *Paragobiodon echinocephalus*

**2. Secondary sexual characteristics**

- a) Monomorphism *Clupea*
- b) Dimorphism
  - a) Permanent *Centropyge heraldi*
  - b) Temporary *Centropyge argi*
  - c) Polymorphism *Symphodus ocellatus*

**3. Mating system**

- a) Promiscuity *Sebastes alutus*
- b) Polygamy
  - i) Polygyny *Lamprologus ocellatus*
  - ii) Polyandry *Amphiprion akallopsis*
- c) Monogamy *Valenciennea longipinnis*

**4. Fertilization mode**

- a) External *Puntius conchoniis*
- b) Buccal *Corydoras aeneus*
- c) Internal *Poecilia reticulata*

**6. Parental care**

- a) No care *Puntius conchoniis*
- b) Paternal care *Pomatoschistus microps*
- c) Maternal care *Oreochromis mossombicus*
- d) Biparental care *Xenotilapia flavipinnis*
- e) Viviparity
  - 1. Paternal *Syngnathus typhli*
  - 2. Maternal *Poecilia reticulata*

*Carassius auratus*) are also well known. In the female *Nerophis ophidion*, the blue colouration consists of several deep blue stripes along the anterior sides of the body and the colouration is permanent; the blue colouration is known to be preferred by the male (Rosenqvist, 1990). A circular or ellipsoidal pearl organ is developed on the head and anal fin of the dark chub *Zacco temmincki* (Katano, 1990b); they are used in aggressive encounters and to bury the eggs, respectively. Even more interesting is the presence of Light Organ System (LOS) in 40 species belonging to 3 genera of leiognathids. A circumo-esophageal light organ, in which a monospecific extracellular culture of the luminescent bacterium *Photobacterium leiognathi*, is maintained. On sexual maturity, the leiognathid fish *Photoplagios rivulatus* controls the intensity and duration of the bacterial luminescence by coordinating the LOS and surrounding tissues, including the muscular shutters, chromatophores, reflective layer of the gas bladder and the transparent skin. A significant positive correlation has also been found between the males GSI and LOS of males (Ikejima et al., 2008).

Temporary structures and colouration are also developed for a short period covering the duration courtship. *N. ophidion* develops skin folds, which are shown to enhance the attractiveness of a female (Rosenqvist, 1990). *S. typhle* also develops such heavy ornamentation (Berglund, 2000). With regard to nuptial colouration, no other description is as vivid as that depicted for the European bitterling *Rhodeus sericeus* by Hunnam et al. (1991). Spawning occurs between April and June, when sexes of the bitterling develop special colouration. The male becomes olive and emerald green on the back, iridescent with many colours on the flanks, orange-red on the throat and belly, bright red on the dorsal and anal fins, and green on the caudal fin, while large white tubercles develop about the upper lip and eye. The female becomes more yellowish green and grows a fleshy egg laying tube of 6 cm long.

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## Sexual Pattern

A vast majority of fishes are gonochores; individuals develop and remain as either female or male throughout their life time. A few fishes are unisexual (Fig. 9); they are females; their haploid or diploid eggs are activated by sperm 'borrowed' from males of the sympatric species. Hermaphrodites are bisexuals and this sexual pattern is broadly categorized into those in which individuals to a greater or lesser degree synchronously function as both sexes, i.e., simultaneous hermaphroditism and those, in which individuals develop and function as one sex or the other and subsequently change to the opposite sex, are sequential hermaphrodites. The latter form of hermaphroditism may further be divided into sexual patterns that involve either one or two pathways of sexual development for the second sex. For example, in the case of protogyny, i.e., female to male sex change, either all males are derived from adult females (monandry or a single pathway of male development, Fig. 9) or some males develop directly as males from a juvenile phase, while others are derived from adult females (diandry or two pathways of male development). The reverse is true to protandry, i.e. male to female sex change, either all females are derived from adult males (monogyny or a single pathway of female development) or some females develop directly as females from a juvenile phase, while others are derived from adult males (digyny or two pathways of female development). Serial (Grober, 1998) sex change includes the bidirectional and cyclical (Kuwamura et al., 1994) sex changes during the life time of adults, and the most extreme sexual plasticity is characterized by back and forth sex change. This has come to light only during the recent years. A population or species is considered to be functionally hermaphroditic, if a substantial proportion of individuals functions as both sexes, either simultaneously or sequentially or serially, at some point during their life, the emphasis is placed on reproductive function to distinguish between gonochoristic and non-gonochoristic strategies (Sadovy and Colin, 1995).

Gonochorism is the existence of one sex either male or female in an individual. Two types of gonochorism can be distinguished. Primary

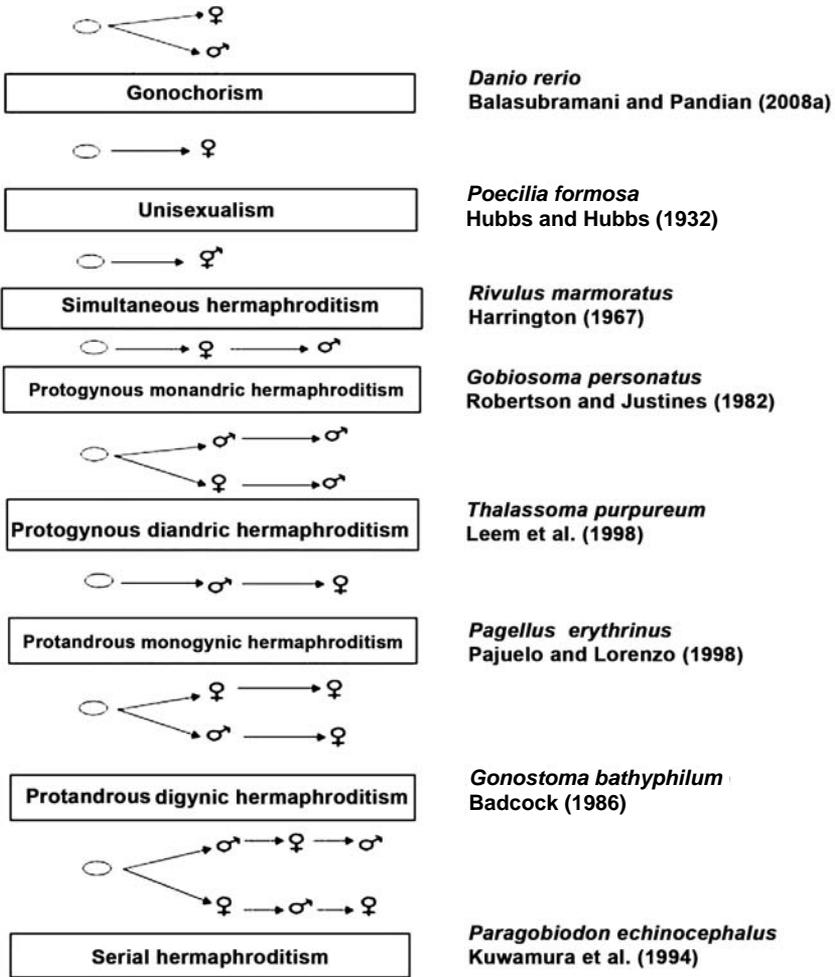


Fig. 9 Ontogenetic pathways of sexual development in fishes.

gonochorism occurs, when the physiological process of sex differentiation corresponds with genetically determined sex and the same is established at the onset of gonadal development. Secondary gonochorism occurs, when genotype and phenotype do not correspond with each other. The gonochorism is established after a delay for a short period, during which the gonad passes through a stage of intersexuality.

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## Intersexuality

Intersexuality is the simultaneous existence of male and female characteristics in a single individual. In fact, all hermaphrodites are intersexes, but some intersexes are not hermaphrodites. Essentially, there is mutual tolerance between the two opposing sexual tendencies in the hermaphrodites. Conversely, intersexuality is the result of interference between the two opposing sexual tendencies, neither of which can therefore express itself decisively (Atz, 1964). In fact, intersexuality is a state from which many fishes pass through during the early stage of their gonadal development (e.g. threadfin bream *Nemipterus bathybius*, Takahashi et al., 1989) and during the adult stage of sequential and serial hermaphrodites (Cole, 2008). However, permanently functional intersexuality in adult fishes has so far not been reported, although very rarely partially functioning intersexual abnormality has been reported only once. A valuable list summarizing the available records dating from 1867 to 1963 on abnormal intersexuality in teleosts is summarized by Atz (1964). Of 90 and odd reports on the simultaneous presence of ovary and testis in an individual, the presence of an ovotestis is the most frequent; the number of reports available is: ovotestis 3, functional ovotestis 1, ovotestis + ovary 8, ovotestis + testis 8. In those with ovary and testis (3 cases), the abnormality ranges from a pair of ovaries with another pair of testes (e.g. tiger buffer *Takifugu rubripes*, Suzuki, 1997) to three gonads and to a testis on one side with an ovary on the other; in the others with ovotestis, a pair of asymmetrical ovotestes is the most common arrangement; however, the testicular portions are mainly located posteriorly and in at least five cases, the testicular portion is only attached to the ovary. Oocytes and eggs are found in a testis or testes in nine cases. It is not clear how the anatomical structure of one functional ovotestis was accommodated in the superfoetid viviparous guppy *Poecilia reticulata*. A similar apparently functional intersex was also described in *Rutilus rutilus* (Jafri and Ensor, 1979). From a simultaneous hermaphroditic carp maintained under controlled conditions, Kossman (1971) stripped sperm and eggs and successfully fertilized them.

Understandably, abnormal intersexuality is reported from as many as 23 families and has been more frequently reported from commercially important fishes belonging to families like Cyprinidae (12+1 added), Salmonidae (12), Clupeidae (6+2 added), Gadidae (9), Percidae (7), Poeciliidae (6), Cyprinodontidae (5), Mugilidae (4), Gobiidae (3), Scombridae (3), Siluridae (3), Acipenseridae (2), Centrarchidae (2) and Channidae (2 including Reddy 1978). It may equally be common among others but has not been brought to light. The prevalence of abnormal sexuality may be low for the present, but it is likely to be more common in future with increasing use of endocrine disruptors. Frequent reports on wide deviations in sex ratio from unity and occurrence of intersexuals are not uncommon (e.g. roach *Rutilus rutilus*, Schultz, 1996). Large scale use of endosulfan in agricultural fields of many developing countries tempted Balasubramani and Pandian (2008a) to test its effect on the zebrafish *Danio rerio*; it was found that a discrete immersion of post-hatchlings of the zebrafish for 14 hours in 1400ng endosulfan/l resulted in the production of 33% females and 44% intersexuals. These abnormal intersexual fishes may provide excellent opportunities to physiologists, cytologists and geneticists for better understanding of the high plasticity encountered in sexuality of teleostean fishes.

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## Gonochorism

In teleosts, the problem of sex determination (Pandian, 2nd volume of this book series) and differentiation (Pandian, 3rd volume) is more puzzling than in any other vertebrates. The following features of sex differentiation are striking examples of teleost's peculiarity: 1. In both the sexes there are no structural connections between the genital and excretory systems, 2. The somatic tissue of the gonad lacks a dual origin from cortex and medulla, 3. The chromosomes are not differentiated into autosomes and heterosomes and 4. Teleosts are the only vertebrates, among which simultaneous, sequential and serial hermaphrodites are found (Reinboth, 1983).

### 4.1 Gonadal differentiation

With reference to the lack of a dual origin of gonadal somatic tissues, the chronological sequences of gonadal differentiation in a typical gonochoric fish may explain the plasticity of the sex differentiation process. Incidentally, the techniques developed for an understanding of the sex differentiation process seem to provide scope for *ex-situ* conservation of fishes and to mass produce seedlings of fishes, which are not readily amenable for aquaculture. Through a series of publications, Yoshizaki and his colleagues have demonstrated that the Primordial Germ Cell (PGC) is the progenitor of the germ cell lineage and is committed to differentiate into either spermatogonia or oogonia after the completion of gonadal differentiation. As such, the PGCs have the potential to develop into a complete individual (Yoshizaki et al., 2002). Marking the PGC with Green Fluorescent Protein (GFP) gene, they extracted and transferred the marked PGC into host fry of the same species or closely related species. As the *vasa* transcripts are restricted to the germ cell lineage, the transgenic strain carrying GFP gene driven by the *vasa* regulatory regions was generated (Takeuchi et al., 2002). Consequently, the expression of GFP gene was limited to the PGCs alone (Yoshizaki et al., 2000; Takeuchi et al., 2003). The genital ridge, isolated from the transgenic embryos, was dissociated by

trypsin and flowcytometrically sorted into GFP-positive and GFP-negative cells. On transplantation of these exogenous GFP-positive cells into the peritoneal cavity of the recipient hatchling, the exogenous GFP-positive cells were incorporated into the genital ridge of about 20% host fry, very much like the endogenous PGCs. Subsequently, 4% of the exogenous PGCs proliferated, underwent meiosis and differentiated into eggs and sperm, in synchrony with the endogenous PGCs. Thus, the donor-driven gametes produced normal progenies through fertilization. This new technique provides the scope for *ex-situ* conservation of PGCs, from which progeny of the concerned species can be derived. In fact, Yoshizaki proposes to rapidly mass produce tuna by transplanting its PGCs into *sterile* mackerels, which are easier and cheaper to maintain, and to conserve the tuna by cryopreserving their PGCs (see Pandian, 2003).

The gonads of higher vertebrates are known to have dual embryological origin and the undifferentiated gonad consists of the cortex, originated from the stromal cells and the medulla derived from peritoneal wall, which itself is drawn from the mesonephric blastema. The cortex develops and the medulla degenerates during ovarian differentiation. Conversely the cortex degenerates and the medulla develops during testicular differentiation. However, the gonad of teleost fishes is composed of somatic tissues that are embryologically unitary in origin, i.e. from the medulla derived from the peritoneal wall alone (Hoar, 1969). This unitary origin of gonad is attributed as one of the causes for the observed plasticity in sex differentiation of teleosts. In other words, the undifferentiated gonads of teleosts are sexually bipotential and can differentiate into either ovary or testis. During the gonad differentiation, the PGCs differentiate into oogonia or spermatogonia, while as the germ cell-supporting cells also differentiate into female- or male-type cells namely granulose or Sertoli cells. Germ cells and their supporting cells in a given gonad differentiate into the same sex so that either type of cells must have a primary effect on sex differentiation.

To know whether the germ cells performed any function in gonadal differentiation, Shinomiya et al. (2001) suppressed proliferation of early germ cells (PGCs), by exposing the embryos (stage 17–37) of the medaka *Oryzias latipes* to bosulfan solution (25  $\mu\text{g}/\text{ml}$ ) that significantly reduced the number of germ cells generating regions without germ cells in the developing gonads. Globular structures were observed in these regions. The structure was male specific and developed as acinus, the precursor of seminiferous tubule. In the ovary, no follicle developed, although an ovarian cavity was formed. From these, Shinomiya et al. concluded that differentiation of gonadal parenchyma cells is not germ cell-dependent, though morphological differentiation of somatic cells followed the differentiation of germ cells.

To further investigate the role of the germ cell supporting cells namely the gonadal cells, Shinomiya et al. (2002) generated chimeras of the medaka *O. latipes* containing male germ cells (XY) or female germ cells (XX), by transplanting them into the blastula embryos. Thus the chimeras of all combinations containing gonads with (1) XY/XY (blastular recipient/donor of germ cells) male, (2) XY/XX male, (3) XX/XY female and (4) XX/XX female were produced. Genotyping these progenies and strains specific DNA fragments, Shinomiya et al. traced the role of supporting cells in gonadal differentiation. All the chimeras (groups 1 and 2) with XY recipient (Table 11) grew into males in accordance with the genetic sex of the recipient, irrespective of the genetic sex of the (donors) germ cells. All of XX/XX (group 4) and 81% of XX/XY (group 3) chimeras developed into females in accordance with their respective recipient sex.

In all the groups, secondary sexual characters coincided with the gonadal sites and the histology of testes and ovaries were completely normal even in the group 3 of XX/XY female that had not taken the donor's sex. These results showed that 1. The sex chromosome composition of the germ cell has no effect on the sex determination process, 2. The sex of germ cells is determined by the sex of the surrounding somatic gonadal cells, 3. The XX somatic cells can induce XY germ cells to follow the female pathway and 4. Even in the absence of germ cells, the sex differentiation process is initiated by the sex of the gonadal cells (Shinomiya et al., 2002). Incidentally, in mammals, the XY germ cells that mismigrated out of the testis, differentiated into oocytes. Hence the germ cells basically tend to differentiate into oocytes, regardless of their genetic sex and only in the presence of precursor of Sertoli cells differentiate into male cells (Upadhyay and Zamboni, 1982).

These findings of Shinomiya et al. (2001, 2002) support the hypothesis of Yamamoto (1969) that the endogenous estrogens and androgens act as the natural ovarian and testicular inducers, respectively. Hence, it is required to trace the key events of gonadal induction process. In tilapia *Oreochromis mossambicus*, gonadal ridges are formed 3 days after hatching and germ cells in these ridges are enclosed by somatic cells derived from

**Table 11** Phenotypic sex of chimeras generated in *Oryzias latipes* (from Shinomiya et al., 2002).

Genotype	Female		Male	
	(no)	(%)	(no)	(%)
XY / XY	0	0	9	100
XY / XX	0	0	12	100
XX / XY	13	81	3	19
XX/ XX	12	100	0	0

XX or XY sex of recipient blastula; XY or XX sex of donors of germ cells.

the peritoneal wall (Nakamura and Takahashi, 1973). Finding no recognizable dual somatic structure of the gonad before or after sex differentiation period in six other teleosts, Nakamura (1978) confirmed the unitary origin of the somatic cells in the gonad. Subsequently, the number of somatic cells in these genital ridges increases by mitotic division. Thus, it is certain that the stromal cells of gonad originate from the peritoneal wall. Secondly, sexually undifferentiated gonad can be divided into two types on the basis of number of gonial germ cells; germ cells destined to become ovaries always outnumber those in gonads destined to become testes. Accordingly, a notable increase in the number of germ cells prior to their meiotic changes is one of the important signs of the ovarian differentiation. Thirdly, the histological evidence for the formation of ovarian cavity is the next reliable criterion to identify the gonad as an ovary (see Fig. 1 of Nakamura et al., 1998).

Germ cells in gonad destined to become testis remain quiescent for a long time. The first sign of testicular differentiation is the formation of efferent ducts (see Fig. 2 of Nakamura et al., 1998). The second one is the appearance of blood capillaries in the lateral region, whereas they appear on the distal and proximal regions of the ovary. Finally, the aggregation of stromal cells in the gonads provides the valid criterion for the differentiation of gonad as testis.

Endocrine studies support the hypothesis of Yamamoto (1969) that endogenous steroid hormones are produced in the gonads at the stage of sex differentiation in salmonids (see Nakamura et al., 1998). However, there are also claims that the endogenous steroid hormones do not act as natural sex inducer in tilapia and medaka. To quantify the ultra-trace quantity of endogenous steroids, Nakamura and Nagahama (1985) have developed an immuno-histochemical detection technique. They have cloned and sequenced the DNA encoding steroidogenic enzymes. Using these newly acquired nucleotide sequences, corresponding peptides have been synthesized and used to raise polyclonal antibodies against four steroidogenic enzymes, essential for the biosynthesis of all major sex steroid hormones including androgens and estrogens (Kobayashi et al., 1996, Chang et al., 1997). Of them, the cytochrome P450 aromatase antibody stains theca cells and granulosa cells in the ovary but it does not stain Leydig cells in the testis. Thus, steroid producing cells in the presumptive ovaries at the undifferentiated and in differentiating stages express the steroidogenic enzymes required for  $17\beta$ -estradiol biosynthesis from cholesterol. Recent studies in *Trimma okinawae* have also shown that steroidogenic enzymes are important for development and serial sex change (Kobayashi et al., 2005; Sunobe et al., 2005a,b). All these findings have shown that the endogenous estrogens act as natural inducers of ovarian differentiation in fishes and support the hypothesis of Yamamoto.

## 4.2 Morphotypes

Many hermaphrodites and a few gonochores commence their adult life in an Initial Colour Phase (IP); then they undergo a radical change into Terminal Colour Phase (TP), in which they end their life. Sexually dichromatic species contain both colour phases; females and small (rather young) males belong to the IP, while the large (old) males belong to the TP. Barlow (1975a) introduced 'midphase' and 'termphase' as equivalents of IP and TP, respectively and these terms have not been used subsequently. Sexually monochromatic species have only one, the IP colour.

As a prelude to diverse mating systems and breeding tactics adopted by fishes, there is a wide range of morphotypes within males and females. Among the gonochores, there are typical males, equivalent to that in other vertebrate taxa, but the other morphotypes are: solitary and paired males, primary and secondary males, cuckolders and parentals (Neff, 2004), hooknoses and jacks (Gross, 1984), sneakers and satellites, bachelors and helpers. There are typical females, comparable to that in other vertebrate taxa; the other morphotypes are primary and secondary females among the digynic protandrous hermaphrodites. The nest of the harem cichlid *Lamprologus ocellatus* is visited by two females one after another to spawn and guard the eggs. To distinguish the first female from the second, Walter and Trillmich (1994) wrongly named them as primary and secondary females; however, the terms must be restricted to the females in the digynic protandrous hermaphrodites.

A territorial male constructs a nest or defends a territory and courts with the female(s) in his territory; he fertilizes and cares for the eggs. Sneakers are relatively small males, which dart and steal spawning with territorial females, who are waiting to spawn with the territorial male (Warner and Robertson, 1978); satellites are a bit larger males and are tolerated by the territorial males; they fertilize a portion of the eggs. Sneakers are also known to occur among the gonochores in and around the harems (e.g. American sunfishes, Gross, 1982; Japanese dark chub, *Zacco temminckii*, Katano, 1983; Mediterranean labrid, *Symphodus* spp, Warner and Lejeune, 1985; African cichlid, *Pelvicachromis pulcher*, Martin and Taborisky, 1997). Incidentally, streaking males are those, who join the territorial male and female at the climax of spawning (Warner and Robertson, 1978). Table 12 shows that body growth from 4–6 cm to 6–8 cm transforms the sneakers to satellites and that to 8 cm–9.5 cm the satellite to territorial male status in *Symphodus ocellatus* (Bentivegna and Benedetto, 1989). This is also true of *S. roissali* and *S. tinca* (Warner and Lejeune, 1985). Likewise, the body growth from 2.3 cm to 4.6 cm transforms the sneaker to satellite, from 4.6 cm to 5.1 cm the satellite to territorial and from 6.2 cm to 6.4 cm transforms the territorial to piracy male in the shell-brooding cichlid *Telmatochromis*

**Table 12** Ontogenetic growth and transformation from sneaker to satellite/helper and to male status in *Symphodus* spp, *Julidochromis*, *Telmatochromis*, and *Leptoscarus* (source Warner and Lejeune 1985; Bentivegna and Benedetto, 1989; Yamagishi and Kohda, 1996; Ota and Kohda, 2006, Robertson et al., 1982).

	Sneaker ♂	Satellite ♂	Territory ♂
<i>Symphodus ocellatus</i>			
Size (cm)	3.5–6.0	6.1–8.0	8.1–9.5
Body colour	Brown	Greenish	Red-Blue
Urinary papillae	White	White	White
<i>S. tinca</i>			
Size (cm)	8.0–12.0	12.1–20.0	20.1–27.0
Colour	Dull	Dull	Bright
<i>S. roissali</i>			
Size (cm)		4.5–8.0	8.1–14.0
Colour		Dull	Bright
<i>Julidochromis marlieri</i>			
Size (cm)		4.0–4.4*	4.8–6.7
<i>Telmatochromis vittatus</i>			
Size (cm)	2.3–3.4	4.6–5.5	5.1–6.2 <sup>+</sup>
<i>Leptoscarus vaigiensis</i>			
Size (cm)		6–8**	8–18***

\* helper, + Piracy ♂ 6.4–6.7 cm, \*\* IP, \*\*\*TP

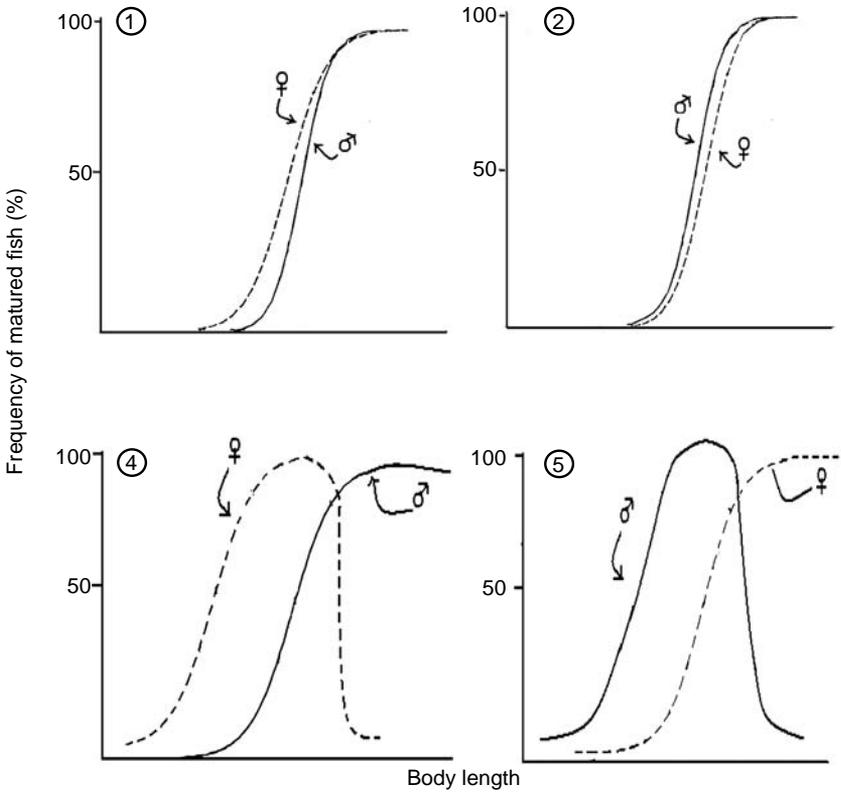
*vittatus* (Ota and Kohda, 2006). Similarly, ‘helpers’ may attain the adult male status, when they grow; for instance, the body length of the helpers in *Julidochromis marlieri* ranges between 4.0 and 4.4 cm but when grown to more than 4.8 cm, they attain the adult male status (4.8 cm to 6.7 cm; see Yamagishi and Kohda, 1996). The scarid *Leptoscarus vaigiensis* is a secondary gonochore and perhaps reflects its past monandric protogyny; the males switch from ‘IP’ to ‘TP’ status, when they attain a body size of 7.2 cm (Robertson et al., 1982). Except in *P. pulcher*, in which the satellites are known to be genetically determined, in all other cases, the sneakers and satellites represent only the transitory ontogenetic stages in the life history of the respective species concerned.

Apparently, the primary and secondary males, among the diandric protogynous hermaphrodite species (Lo Nostro and Guerero, 1996), satellite and harem males in *P. pulcher*, have been shown to be genotypes, rather than phenotypes and have a genetic basis. Cuckolders of the bluegill sunfish *Lepomis macrochirus* and jacks of the coho salmon *Oncorhynchus kisutch* are precociously matured males, which stealthily sneak into the nests of the ‘parentals’ and territories of the ‘hooknoses’, respectively and spawn with the females. The trait(s) responsible for rapid growth and precocious maturation appear to have a genetic basis (see Wootton, 1998); hence these terms are retained.

It is not clear whether these male genotypes represent the expression of the alleles of the same sex determining gene or different genes operate to determine these male genotypes. The available descriptions for the sex specific molecular markers is limited to few gonochoristic fish species, in which only a single phenotype exists within a sex; for instance, a sex specific molecular marker is reported for the rosy barb *Puntius conchonius* (Kirankumar et al., 2003). Stein et al. (2002) used a microdissection library to isolate a microsatellite locus designated as Yp136, which maps 37 centimorgans (cM) from the sex determining locus (sex) and 19.5 cM from the centromere of the X chromosome of the lake charr *Salvelinus namaycush*. The combination of linkage data and microdissection information places the sex-determining locus near the telomere of the Y chromosome. Using the male-linked growth hormone pseudogene (GHP) by real time PCR, Nagler et al. (2004) have found that the GHP is present as a single copy in the genome of the male chinook salmon *Oncorhynchus tshawytscha* but it is absent in the female. An array of these male genotypes present in fishes may indeed pose a huge challenge to the molecular biologists searching for the sex determining gene(s) in human and other mammals. Yet a comparative study on the sex determining gene(s) from some fishes with many genotypes within a sex to mammals with a single genotype within a sex may prove rewarding.

### 4.3 Sex ratio

From the point of reproduction adult sex ratio may be more relevant in a population of fish species, though sex may be identified early. The number or percentage of sexually mature males and females increases, as the fish grows. Among gonochores, differences in sex-dependent growth may result in the attainment of sexual maturity first by females or by males in different species. Hence five models for the relationship between body size and sexual maturity are possible. In model 1, 100% females mature prior to the attainment of sexual maturity by the male (e.g. the jewfish *Epinephelus itajara*, Bullock et al., 1992, Fig. 10). The reverse is true in model 2, in which males mature first (e.g. widow tetra *Gymnocorymbus ternetzi*, David, 2004). In model 3 (not shown) males and females attain sexual maturity at the same age or size (e.g. Buenos Aires tetra *Hemigrammus caudovittatus*, David, 2004). Understandably, 100% female of protogynous hermaphrodites in a population first attain sexual maturity (model 4, e.g. *Pagrus auriga*, Pajuelo et al., 2006b). However as sex changed females became males, the size or age interval between the attainment of sexual maturity of females and males is much wider than that between females and males of gonochores. The reverse is true for protandric hermaphrodites, in which males mature first (model 5 e.g. *Sarpa salpa*, Villamil et al., 2002). A specific example for



**Fig. 10** Relations of body size at sexual maturity of males and females (1) Gonochores: females mature earlier than males (2) Gonochores: males mature earlier than females. (4) Protogynous hermaphrodites: matured males are produced by sex changed mature females. (5) Protandrous hermaphrodites: matured females are produced by sex changed mature males.

gonochores is: 50% and 100% females of the sea garfish *Hyporhamphus australis* in a population become sexually mature, only when the fish attain body lengths of 21 cm and 28 cm, respectively (Hughes and Stewart, 2006). Likewise 50% and 100% males mature at the size of 21 cm and 30 cm. Within a species, the age of sexual maturity may vary. The age at which 50% of the American plaice *Hippoglossoides platessoides* varies from 5.3 to 7.5 years for males and from 7.8 to 15.2 years for females in the sea around Newfoundland (Pitt, 1966). Spatial variations for the age of maturity are also known. The age of maturation for the orange roughy *Hoplostethus atlanticus* around the Porcupine Bank, Northeast Atlantic is 27.5 years but is 25 years at the eastern Tasmania and 29 years at the Chatham Bank off New Zealand (Minto and Nolan, 2006).

Fowler (1991) made a rare record of  $0.15\♂ : 0.85\♀$  sex ratio in the population of the butterflyfish *Chaetodon rainfordi* in the One Tree Reef of the southern Great Barrier Reef but the ratio was  $1\♂ : 0\♀$  in the lagoon. The reverse  $0\♂ : 1\♀$  was true for *C. plebius*. In a large sample of the chaetodontid *Chelmon rostratus* at the same One Tree Reef, no female showed any sign of sexual maturity, while males were mature and the sex ratio was  $0.5\♂ : 0.5\♀$ . From the western province of the Solomon Islands, Hamilton et al. (2008) recorded spatial variations of sex ratio of the green humped parrotfish *Bolbometopon muricatum*; the ratios were  $0.43\♂ : 0.57\♀$ ,  $0.49\♂ : 0.51\♀$ , and  $0.88\♂ : 0.22\♀$  for the populations at Munda, Nusabanga and Tetepara, respectively. Seghers (1973) reported that the adult sex ratio of the guppy *Poecilia reticulata* from the Paria River of the West Indies varies spatially from  $0.35\♂ : 0.65\♀$  to  $0.40\♂ : 0.60\♀$  and temporally from  $0.40\♂ : 0.60\♀$  to  $0.53\♂ : 0.47\♀$ . These examples show that the spatial and temporal variations in sex ratio are not uncommon among marine and freshwater fishes.

The migrating yellowfin bream *Acanthopagrus australis* shows dynamic changes in sex ratio during the breeding season, as age advances. From the turbid mangrove fringed Deception Bay, Australia, the bream undertakes spawning migration to the surf-bar, Jumpinpin at a distance of 80 km every year during May to August (Pollock, 1984). The actual or Operational Sex Ratio (OSR) of the bream at Jumpinpin progressively changes from  $0.9\♂ : 0.1\♀$  at the age of 1+ to  $0.7\♂ : 0.3\♀$  and  $0.53\♂ : 0.43\♀$  at the age of 2+ and 3+, respectively. Clearly, the OSR within the migratory population of the bream remained male biased every year, when the bream grows from the age 1 to 3+. The OSR of the 1+ year old bream at the Deception Bay also remains male biased ( $0.8\♂ : 0.2\♀$ ) during the period September to April but female biased during the 2nd ( $0.27\♂ : 0.63\♀$ ) and 3rd year ( $0.0\♂ : 1.0\♀$ ) of its age.

Exposure of early embryonic stages to changes in temperature is known to induce considerable alterations in sex ratio of fishes, especially, in *Rivulus marmoratus* (Harrington, 1968, 1971; for others, see Strussmann et al., 2005). However, exposure of juvenile and adult stages does not alter the sex ratio; for instance, the sex ratio remains  $0.5 : 0.5$ , when the juvenile Atlantic halibut *Hippoglossus hippoglossus* is reared at 7, 12 and 15°C (Hughes et al., 2008). Other factors are listed by Chan and Yeung (1983) like water quality, crowding, handling stress and so on are all elaborately discussed in the proposed Volume 3 of this book series. However, an overview on the effect of social factors altering sex ratio of gonochores is presented here in this volume.

Sporadic reports on pair formation, which alters the sex ratio, are available. In the hawkfish *Oxycirrhites typus* 40% are found in pairs, while the others are 'solos'; in *Neocirrhites armatus* 45% are paired, 11% are 'trios',

i.e.  $1\sigma + 2\phi$  and another 0.7% are 'tetras', i.e.  $1\sigma + 3\phi$  (Donaldson, 1989). Robertson et al. (1979) found 14% more females than males among the adults of the surgeonfish *Acanthurus leucosternon*; the population consisted of 'solos', 'pairs' and 'trios'. Interestingly, the fat reserve, obligately required for gonadal maturity, was low, i.e. 0.56% of body weight in the 'solos' but 2.06% in the pairs. This was also true of *Zebrasoma scopas*. In *A. nigrofuscus* and *A. trigostegus* too, the 'trios' are not uncommon. A reason for the formation of 'trios' may be the presence of excessive females. This pattern of female biased OSR may represent an alternate strategy of mating, especially in places, where the resources are scarce.

Among the burrow-inhabiting gobiids, whose mobility is limited to a few meters, the need for finding mates within their respective home ranges has driven them to the formation of groups in 'pairs', 'trios' ( $1\sigma + 2\phi$ ) and 'tetras' ( $1\sigma + 3\phi$ ); the need is intensified, when availability of males is interrupted by the obligate nest-guarding. Hernaman and Munday (2007) quantified the groups among the three Australian gobiids *Valenciennesa muralis*, *Amblygobius bynoensis* and *A. phalaena*. The group formation commences from the juvenile stage and the frequency increases in adults. For instance, *A. bynoensis*, the frequency of pairs increases from 24% to 47%, 'trios' from 4% to 9% and 'tetras' to 1% ; consequently, such group formation is linked to the reduced male ratio from 0.5 to 0.41.

Another social structure that is responsible for female biased sex ratio is the presence of a stabilized harem, in which a dominant territorial male holds 2–9 females, more or less permanently or at least for a breeding season. Table 13 lists some examples for the stabilized harem type of social organization in Acanthuridae (Robertson et al., 1979), Pomacanthidae (Moyer et al., 1983), Ostraciidae (Moyer, 1979), Labridae (Clark and Petzold, 1998) and Cichlidae (Kuwamura, 1997). Interestingly, the number of females held in the harem of the angelfish *Centropyge argi* decreases from 7 at 8 m depth to 2 at 14 m depth (see p 85).

While classifying the mating systems of 122 Tanganyikan cichlids, Kuwamura (1997) recognized 20 species as harem and 5 of them hold one sub-adult male as a helper; surprisingly, in one harem cichlid, the helper belongs to another cichlid species. Incidentally, in the biparental mouthbrooding cichlid *Julidochromis marlieri*, some females are larger (6.0–9.1 cm) than the males (4.8–6.7 cm) and helpers (3.9–4.8 cm). Though known to be monogamous, two of eight large females are found to hold and maintain two males in adjacent territories and each with a helper. This kind of a polyandrous 'harem-like' mating system has been reported by Yamagishi and Kohda (1996).

Oldfield (2005) has summarized available information on the social influence in sex differentiation of cichlid fishes. Zupanc (1985) describes the sex changing process in cichlids, almost similar to that reported for

**Table 13** Sexual size dimorphism and harem gonochochoristic teleostean fishes.

Family & Species	Heavier ♂ by (%)	Harem ♀	Reference
Acanthuridae			Roberston et al. (1979)
<i>Acanthurus gahhm</i>	63	2	
<i>Ctenochaetus striatus</i>	43	3	
<i>C. strigosus</i>	25	4	
Pomacanthidae			Moyer et al. (1983)
<i>Holocanthus clariorensis</i>	25	1+	
<i>H. passer</i>	300	7–9	
<i>Centropyge argi</i>			
8 m depth		7	
12 m depth		2–3	
14 m depth		2	
Ostraciidae			Moyer (1979)
<i>Lactoria fornasimi</i>		4+ helper	
<i>L. diaphanus</i>		2–4	
<i>Ostracion cubicus</i>		2–4	
Labridae			
<i>Cymolutes torquatus</i>		2–4	Clark and Petzold (1998)
Cichlidae			Kuwamura (1997)
Tanganyikan 122 species		20 species	

protogynous hermaphrodites. All individuals of the checkerboard cichlid *Crenicara puntula* raised in isolation pass through the female phase, but then change into males at the age of 7–10 months. But in a group, some 2-year old females differentiate into sub-males. They are not strongly territorial but have the male's colouration. When the  $\alpha$ -male is removed, a sub-male begins to reproduce with the females. In a territory, the most dominant  $\alpha$ -female, on removal of the territorial male, changes sex and begins to mate normally with the remaining females. Both on removal of the male and in the absence of males (cf Shapiro, 1979; Ross et al., 1983), the checkerboard cichlid changes its sex at the age of 1–2 years depending on the social influence. However the transformed males have no gonadal lumen (Carruth, 2000), a diagnostic feature typical of protogynous hermaphrodites (Sadovy and Shapiro, 1987).

In a harem cichlid, the dominant male maintains a small (e.g. 2 m<sup>2</sup>, *Neolamprologus savoryi*) or large (e.g. 100 m<sup>2</sup>, *N. furcifer*) territory, in which more than one female stay longer or at least for one breeding cycle (Kuwamura, 1997). The territory of a harem, for instance, in *N. savoryi* includes several smaller territories of females and still smaller ones of the helpers. The harem consists of a few females, whose number ranges from 2 as in *Lamprologus ocellatus* (Walter and Trillmich, 1994) to 16, of which 7 are brooding and the others non-brooding (e.g. *N. furcifer*, Kuwamura, 1997). Almost all-harem females mouthbrood and guard their eggs (Table 14)

**Table 14** Mating system and brooding habits of the Tanganyikan cichlids (from Kuwamura 1997).

Mating system & brooding habits	Species	
1. Monogamy		52
a) Monogamy+biparental guarding		26
i) Monogamy+biparental guarding	12	
ii) Monogamy+biparental guarding?	5	
iii) Monobigamy+biparental guarding	4	
iv) Monobigamy+biparental guarding + helper	1	
v) Monogamy + helper	4	
b) Monogamy + mouthbrooding		5
c) Maternal guarding with paternal mouthbrooding + biparental guarding		12
d) Maternal+paternal mouthbrooding +biparental guarding		7
e) Maternal mouthbrooding with biparental care		2
2. Haremic		
a) Maternal mouthbrooding		20
3. Male territory visiting polygamy		50
a) with maternal mouthbrooding	43	
b) with paternal mouthbrooding	7	
4. Non territorial polygyny		5
a) with maternal mouthbrooding		4
b) maternal mouthbrooding + guarding		1

and the males monopolize the females in their respective harems; these males are polygynic. Hence, the sex ratio in these 20 haremic cichlid species is likely to be female-biased. It will be interesting to masculinize amenable haremic cichlids to produce a range of male-biased OSR to know whether the harems are still formed, and to feminize and produce a range of female-biased OSR to know, whether the harem shall have more females with enlarged territory. In *N. savoryi*, known to include sub-adult helpers in its harem, no experiment seems to have been made to know whether the removal of the dominant harem-owner male, one or more of the helpers shall still hold the harem. The experiments may also help us to know whether all the phenotypic males or only the genotypic males hold the harem. It may also be interesting to know whether the trait of pair-forming males, 'solo' and 'trio' males of acanthurids (Robertson et al., 1979) and gobiids (Hernaman and Munday, 2007) have a genetic basis. For it is presently known that at least in one cichlid species haremic *vs.* pair-forming male trait has a genetic basis and the trait is inherited.

Perhaps the most interesting cichlid is the Nigerian cichlid *Pelvicachromis pulcher*. They are small, sexually dimorphic with colour polymorphism. The sex ratio ranges from 0.33♂ : 0.67♀ in the upper part of the Sombreiro River to 0.5♂ : 0.5♀ in the other locations. Females are intensely coloured with purple bellies. In the males, there are yellow and red colour morphs. The colour is genetically determined (Heiligenberg, 1965). Besides, the red

morphic males are more aggressive and hence are harem; they defend their territories more intensely than the yellow morphic males.

According to Martin and Taborsky (1997), 50% of the red morphic males are pair-forming ones, while the other 50% obtain two or more females each and establish their respective harem. Individual males of this morph change between pair and harem status but cannot change into the status of yellow morph.

The yellow morphs develop into pair forming males, or become satellites, when the partner or territory is lost. Depending on their size and behaviour, the satellites are ranked as dominant and submissive. A few dominant satellites may regain the mate and territory. The satellites can sneak and spawn with the paired females. Briefly, the yellow morphic males can change their status from paired to satellite and satellite to the paired one. However, they can not cross into the status of the red morph. When assessed for the reproductive success, a harem male produces 3.3 times more progenies than the non-territorial paired male and 7 times more than the satellites. Whereas the dominant satellites have 29% of mating success, the submissive ones have hardly 5% success.

Because colour morphism of a male is heritable, the reproductive potential of males appears to be genetically determined. The colour morphism of males is proved to be irreversible after its expression at an early stage of ontogeny. The mating system of the morphs is also genetically fixed. When water levels are high, the red morphic harem males may produce a large number of progenies but during dry spells, the yellow morphic males may be at an advantage. A highly productive wet season with increasing population of red males may alternate with dry seasons, in which the proportion of yellow morph may be restored due to the latter's less demanding and more flexible reproductive tactics. The whole situation recalls the alternation of parthenogenetic with sexual reproduction in some tropical branchiopod crustaceans (see Pandian, 1994).

#### 4.4 Operational sex ratio

Operational sex ratio (OSR) is the ratio of males to females ready to mate at a given moment (Emlen and Oring, 1977). It is determined by three factors: i) the adult sex ratio in the population, ii) the spatial and temporal distribution of the two sexes and iii) their potential reproductive rates. The rate at which a sex can potentially reproduce depends on the investment in gamete replenishment and parental care. Even in species with no parental care, the OSR is usually skewed because females are limited by the number of eggs. Thus, males typically compete among each other for mating, whereas the females are selective about mating partners. Competition among males for mating takes various forms, including

i) contest ii) alternative mating tactics, iii) mate guarding and iv) sperm competition (Spence and Smith, 2005).

During the breeding season from May to October at the Shikoku Island, Japan, the OSR (receptive males: matured females) greatly deviates from the apparent sex ratio in the mouthbrooding cardinalfish *Apogon niger* (Okuda, 1999). The breeding behaviour of the cardinalfish is likely to change from June to August, when the OSR is male biased to September, when the ratio is almost at equality.

Some experimental observations made from offer of more females or males to an opposite sex partner within a limited space seem not to quite agree with the OSR concept of Emlen and Oring (1977). For instance, when the male and female were kept together, the zebrafish *Danio rerio* spawned 45 eggs/day, in comparison to 150 eggs of a female kept separated for 4 days and then mated with one male and 180 eggs with two males (Delaney et al., 2002). Hence, a period of separation from male-biased OSR resulted in the production of more eggs, although the egg output did not increase in proportion with the two males or with the number of days of separation.

In the oviparous zebrafish with no parental care, Spence and Smith (2005) designed an experiment to investigate the effects of both density and OSR on aggression of males and egg output by females. The design was to have four groups: the first two named 'low-density' groups consisted of  $1\text{♂} + 2\text{♀}$  and  $2\text{♂} + 1\text{♀}$  and the next two called 'high density' groups with  $5\text{♂} + 10\text{♀}$  and  $10\text{♂} + 5\text{♀}$ . At low density with female bias, the male displayed no aggression and the output was 70 eggs per female but at the same density with male-biased OSR, the egg output was reduced to 52 eggs per female and the males were aggressive (3.8 times/minute/male). At high density, the males were more aggressive (10.4 times/minute/male), when the OSR was male-biased than that (7.4 times/minute/male) with the female-biased OSR; the egg production increased from 36 eggs/female in the group with 10 females to 42 eggs/female in the group with 5 females (Table 15). Briefly, the males intensely competed for mating, as evidenced by increased aggression from 0 to 10.4 times/minute/male; the increased availability of females ensured greater availability of mates at low density but the same had a negative effect at high density. These observations confirm the report of Grant et al. (1995), who have shown that in *Oryzias latipes*, the frequency of male-male aggression, sneaking and disruption of courtship is increased, as the OSR became more male biased. Clearly, simultaneous availability of more than two females per male affects reproductive success in the oviparous fish with no parental care.

The viviparous poeciliids like *Poecilia sphenops* and *P. velifera* realize their maximum reproductive potential, when they receive the first

impregnation as virgins at the age of about 7 months and the second one at the age of about 12 months. These two impregnations are found adequate for their reproductive life time of 20 and 25 months, respectively (George and Pandian, 1997). In *P. reticulata* too, Jirotkul (1999) found that the females are ready to mate, when they are virgin, and then once or twice after giving birth. Hence the number of receptive females (FFR) at any given time is very low, resulting in very strongly male-biased OSR. For this reason, he perhaps has defined the OSR a little different from the one stated earlier. His definition states that the OSR is the number of sexually active males divided by the total number of sexually active adults of both sexes. He also assessed the reproductive success as the number of copulations rather than eggs or progenies.

Manipulating male-biased and female-biased OSR, Jirotkul (1999) found that the number of sigmoid courtship display, the percentage of receptive females and the number of copulations per male decreased with an increase in the male-biased OSR group. Conversely, the sigmoid display was stabilized from 0.5 OSR group onwards, the percentage of receptive females exponentially increased and the copulations also progressively increased in the female biased OSR. Briefly, the reproductive success of a male guppy, as indicated by the copulations per male, was limited by the availability of females only (Table 15).

The oviparous Japanese minnow *Pseudorasbora parva* with paternal care is sexually dimorphic. During the breeding season, females usually deposit eggs in several batches in territories of several males during the day time only. Subsequently, the 'father' takes care of the eggs. Katano

**Table 15** Effect of altered OSR and mode of reproduction on egg production in selected teleostean fishes.

Mode of reproduction/ Species	Experimental design	Eggs/copulations (no)	Male reproductive success
Oviparous <i>Danio rerio</i>	i) 2 ♂ + 1 ♀	52	high upto 1 ♂ + 2 ♀♀
	ii) 1 ♂ + 2 ♀	70	
	iii) 10 ♂ + 5 ♀	42	
	iv) 5 ♂ + 10 ♀	36	
Oviparous: ♂egg guarding <i>Pseudorasbora parva</i>	i) 1 ♂ + 1 ♀	1996	high with 3 ♂♂ + 1 ♀
	ii) 3 ♂ + 1 ♀	2053	
	iii) 10 ♂ + 1 ♀	1717	
	iv) 1 ♂ + 3 ♀	1170	
	v) 1 ♂ + 10 ♀	632	
Oviparous with ♂ gestation <i>Syngnathus typhle</i>	i) 4 ♂ + 2 ♀	85	high with 2 ♂♂ + 1 ♀
	ii) 2 ♂ + 4 ♀*	58	
Viviparous <i>Poecilia reticulata</i>	♂ biased		low with ♂ bias
	♀ biased		high with ♀ bias

\* males took 702 minutes in courting against 121 minutes in the male bias series

and Maekawa (1997) made an interesting but perhaps inadvertently a little ill-designed experiment without providing information on aggression and egg predation. The objective of the experiment was to test the effect of manipulated sex ratios on fecundity; accordingly, almost equal sized males (8 cm) and females (7.4 cm) were allowed to mate in separate aquaria and to produce eggs during a period of 124 days, when one male was simultaneously allowed to mate 1, 3 or 10 females in the first female biased series; in the second male-biased series, one female was allowed simultaneously to mate with 1, 3 or 10 males. With increased availability of females from 1 to 3 and to 10, however, the number of females, who did not participate in the mating increased to 35% and the fecundity progressively decreased from 1,996 eggs/female in the group with 1♂ + 1♀ to 632 eggs/female in the group with one male and 10 females (Table 15). On the other hand, with increased availability of males from 1 to 3 and to 10, the fecundity initially increased from 1,996 eggs/female in the 1♂ + 1♀ group to 2,053 eggs/female in the 3♂ + 1♀ group but subsequently decreased to 1,717 eggs/female in the group with 10 males. Briefly, with abundance of females, many of them remained unmated and the fecundity of the mated females also significantly decreased. Clearly, in an oviparous fish with the male guarding the eggs, the presence of 3 males per female increases the egg output of a female.

In the pipefish *Syngnathus typhle*, known for paternal gestation of eggs, Berglund (1993) manipulated sex ratio with 4♂ (3 small plus one large) + 2♀ (one small plus one large) in the first male-biased OSR series and in the second female-biased OSR series, the combination was 2♂ (one small and one large) + 4♀ (3 small plus one large) to study the effect of size and OSR. In the male-biased OSR series, the males did not select the large or small females, took shorter durations to court (121 minutes) and mated for longer (440 minutes) duration than in the female-biased series. Consequently, each male in the first series received more (85) eggs and got his pouch filled to 93% capacity, whereas in the second series, each female produced 58 eggs only and filled the male's pouch only to 63% of its capacity. With increased availability of females, the male spent a longer duration of 702 minutes to select and court rather than to spawn and acquire eggs. Thus in the oviparous fish with male gestation, the presence of 2 males per female increases the progeny output.

Table 15 represents a comprehensive summary to show the effect of experimentally manipulated OSR and mode of reproduction on the reproductive success of fishes, for which relevant information is available. In oviparous species with no parental care, which constitute 78% of teleosts, the reproductive output of a male increases with increasing availability of females, but only upto a maximum of 2 females, beyond which the increased availability of females itself has a negative effect on the male

reproductive success. Incidentally, the frequency of courting attempts terminating in successful spawning in *Cirrhitichthys falco*, a harem protogynous hermaphroditic fish was 76%, when a male courted 3 females against 64%, when 5 females were courted by the male (Donaldson, 1987). Besides 7% teleostean fishes, which gestate the developing embryos, the availability of upto 5 females increases the copulation frequency and hence may increase reproductive output. It is known that the sex ratio among the viviparous poeciliids is female-biased, providing 2–3 females to every male.

However, in oviparous fish with the male guarding or gestating (as in syngnathids) eggs, the presence of 2–3 males for every female results in (i) provision of a wider choice to the females, (ii) quick courting of the females by the intensely competing males and (iii) higher reproductive output. Incidentally, male guarding/gestating species constitute 11% of teleosts; in these fishes, it is the availability of males that limits the reproductive output. But in oviparous species with no parental care and in viviparous species ( $78 + 7 = 85\%$  teleosts), it is the availability of females per male that limits reproductive output. No information is yet available in this regard for 4% teleosts, in which both male and female afford protection to the eggs. Further research work is required, however, to confirm this generalization.

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## Unisexualism

Approximately 70 unisexual biotypes of fishes, amphibians and reptiles are presently recognized (Vrijenhoek et al., 1989). Although the greatest number of unisexuals recorded are lizards, the rearing difficulties limit the possibilities of experimental manipulations. Only seven unisexual biotypes of fishes are described and all of them are readily amenable for rearing and experimentation (Table 16). In fact Hubbs and Hubbs (1932), the first to discover the very existence of unisexual vertebrates, have reared and experimented with the Amazon molly *Poecilia formosa* for 15 years (Schultz, 1973). At the Museum of Zoology Department, University of Michigan, *Poeciliopsis monacha latidens* were reared over a period of 29 years during which the fish underwent 60 generations (Schultz, 1989). According to Wu et al. (2003), the unisexual complex of the crucian carp *Carassius auratus* was reared at the Institute of Hydrology, Wuhan for a minimum of 4 generations, covering a period of not less than 5 years. Not surprisingly, in relation to the small number of species, a relatively large volume of literature is available for unisexual fishes.

### 5.1 Hybridization

Essentially, all unisexuals are hybrids between two or more biparentally reproducing sexual species. Interspecific hybridization generally leads to either  $F_1$  sterile progenies or hybrids with some measures of fertility. Occasionally, however, hybridization disrupts oogenesis so that hybrids produce viable eggs without recombination and often without a reduction in ploidy. Incidentally, by virtue of their hybrid nature, the unisexuals already possess a high degree of heterozygosity. Their unisexual mode of reproduction maintains this heterozygosity without incurring the cost of segregational load paid by the bisexual amphimictic species. However, the absence of genetic recombination constitutes an evolutionary constraint on uniparental fishes. Consequently, the exclusion of recombinant source of variation appears to have driven them to triploidization and hybridogenesis; the maternal genomes of hybridogens in each generation draw

**Table 16** Hybridization, polyploidization and reproductive modes in unisexual fishes.

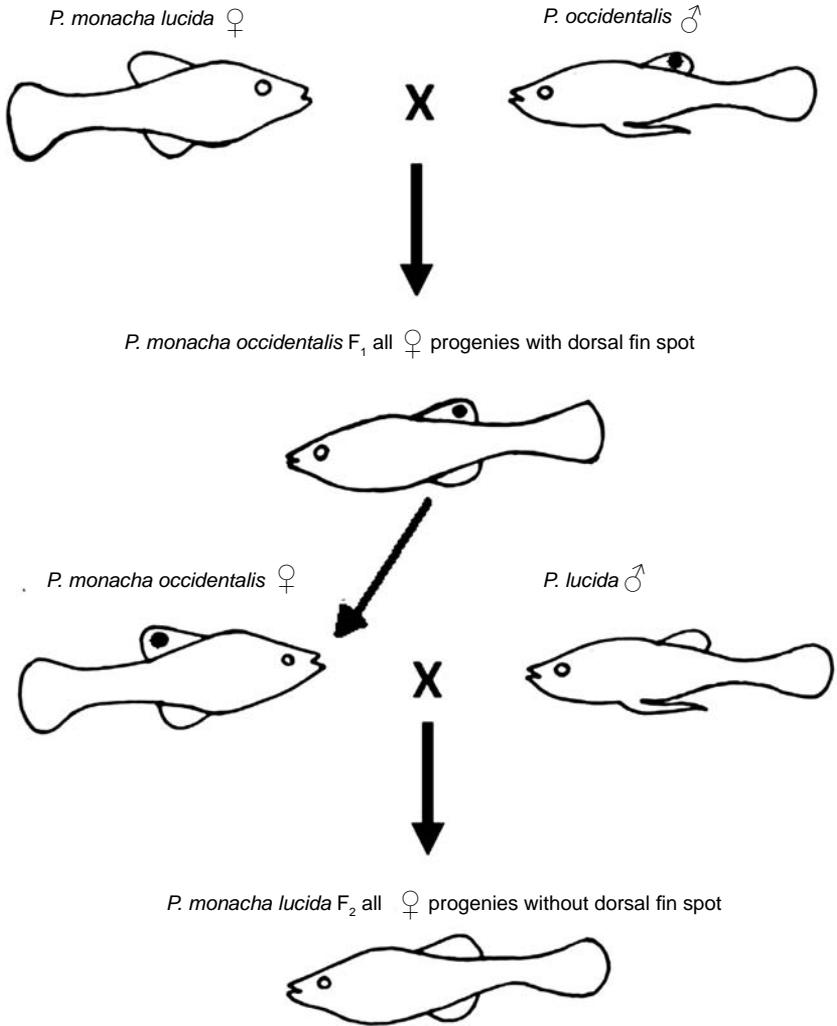
Unisexual complex	Sexual pattern	Mode of reproduction	Sperm source
Poecilia formosa	2n ♀ only	Gynogenesis	P. mexicana
Poeciliidae Texas, northeast Mexico	3n ♀ only	Gynogenesis	P. latipinna
Menidia clarkhubbsi	2n ♀	Gynogenesis	M. beryllina
Athernidae Capano Bay, Texas	3n ♀		M. peninsulae
Carassius auratus Cyprinidae gibelio, north China	2n ♀ only	Gynogenesis	Cyprinus carpio, C. auratus red,
langsdorfi, Japan	3n ♀ only		
sugu, south China	4n ♀ only		
Cobitis granoei + taenia	3n ♀	Gynogenesis	Misgurnus fossilis
Cobitidae	4n ♀	Gynogenesis	
Moscow River	4n ♂, ♀	Gonochorism	
Poeciliopsis	2n ♀ only	Gynogenesis	P. lucida, P. monacha
Poeciliidae, Mexico	3n ♀ only	Hybridogenesis	P. occidentalis
Phoxinus eos-neogaeus	2n ♀ only	Gynogenesis, Variant	P. eos; P. neogaeus
Cyprinidae East Inlet pond, New Hampshire	3n non-clonal 3n clonal, non-clonal	in hybridogenetic direction	
Rutilus alburnoides	2n ♂, ♀	Gynogenesis	Leuciscus carolitertii (Duero Basin)
Cyprinidae	3n ♀ only	Hybridogenesis	
West Spain, Iberia	4n	Gonochorism	L. cf pyrenaicus (Guadiana Basin)

their variations from the gene pool of the bisexual host species (Vrijenhoek and Schultz, 1974).

## 5.2 Polyploidization

With a single exception of inter-racial hybrids of *C. auratus* complex, all the other six unisexual fishes are essentially interspecific hybrids. Sterility associated with hybridization provides strong selection pressure for any oogenetic mechanism that rescues egg production and retains or restores diploidy (White, 1978) or tetraploidy, as in *Rutilus alburnoides* (Alves *et al.*, 1999). Assuming such hybrids are viable and fertile, an oogenetic 'rescue mechanism' will rapidly be fixed, if demographic advantage also arises with female reproduction. Hybridization brings together specific alleles that in combination cause changes in regulation of oogenesis and allows sustenance of unisexual reproduction (Balance hypothesis; Moritz *et al.*, 1989). Schultz (1969) conceived that hybridization and unisexuality provide a new avenue to evolution of polyploidy. Consequently, gynogenetic and hybridogenetic modes of clonal and hemiclonal reproduction with polyploidization are common among unisexual fishes. Surprisingly, the cytological mechanisms underlying these reproductive modes are unique to each of the seven unisexual complexes of fishes. Hence, the unisexual fishes pose highly provocative problems to cytologists, geneticists and evolutionary biologists, and demand long term research to understand the oogenetic mechanisms, levels and routes of polyploidization, and clonal and other modes of reproduction. Indeed, it took longer than 12 years of experimentation for Hubbs and Hubbs to discover that *P. formosa* is a unisexual taxon and reproduces gynogenetically (see Schultz, 1989).

Gynogenesis is strictly a clonal mode of reproduction that faithfully replicates the maternal genotype. Unlike in parthenogenesis, the gynogenetic females require sperm from males of one or more sexually reproducing host species to activate embryogenesis of their unreduced diploid or triploid ova. In contrast, hybridogenesis is a hemiclonal mode of reproduction that delivers only one genome of a hybrid to the functional oocytes (Cimino, 1972a). For example, MP hybrid would produce haploid eggs containing only the maternal M genome. The P genome is replaced in each generation by mating with the male of sexual species P and the P gene products are usually expressed (Fig. 11). In contrast, clonal reproduction of gynogenesis is expected to result in diploid expression of the original maternal alleles.



**Fig. 11** Series of matings that led to the discovery of hybridogenesis in which the haploid sperm of a related species *Poeciliopsis occidentalis* fertilize the eggs of *P. monacha-lucida* and the character dorsal fin spot of *P. occidentalis* is expressed in F<sub>1</sub> progenies but is lost in F<sub>2</sub> when its egg is fertilized by *P. lucida*.

### 5.3 *Poecilia formosa*

It occurs within the coastal rivers of Texas and the Soto la Mariana drainage system, Mexico, where it is sympatric with its bisexual congeners (sperm donors) *P. latipinna* and *P. mexicana*, respectively. The observations of Hubbs and Hubbs (1932, 1946) may be briefly summarized: 1. In the laboratory, crosses between the shortfin molly *P. mexicana* (2n=46; 1.62–1.67

pg DNA/cell) and the sailfin molly *P. latipinna* ( $2n=46$ ; 1.6 pg DNA/cell), the progenitors of *P. formosa*, resulted in the production of offspring of both sexes, males and females, 2. Matings of *P. formosa* to these hybrids or to other species, even as distantly related as *Gambusia affinis* generated *P. formosa* only, and 3. Interestingly, no  $F_1$  hybrids are found in Mexico and Texas, where *P. mexicana* and *P. latipinna* occur.

The hybrid origin of *P. formosa* and its gynogenetic mode of reproduction are confirmed by a number of cytological and biochemical studies. The karyotype of *P. formosa* is consistent with one that would be predicted in the  $F_1$  hybrid of *P. latipinna* and *P. mexicana*, except that the largest chromosome in *P. formosa* complement is distinctly sub-telocentric, while that of *P. latipinna* lacks discernible short arm and must be regarded as telocentric. The apparent 'homologue' of the largest chromosome in *P. formosa* is slightly smaller acrocentric and is identical in relative size and shape to the largest chromosome of the karyotype of *P. mexicana* (Turner et al., 1980). Electrophoretic serum protein analysis revealed that gynogenetic species *P. formosa* possesses two albumin bands that are identical with those produced by hybrids of its sympatric species *P. latipinna* and *P. mexicana* (Abramoff et al., 1968).

Further studies of Hubbs and Hubbs (1946) and Hubbs (1955) revealed that mating males from several different species invariably produced all female progenies with strictly matroclinous inheritance. Tissue transplant experiments gave similar results; all the female offsprings were genetically identical to both their mothers and sisters (Kallman, 1970). The pattern of PAGE for several generations of pedigree stocks of *P. formosa* strictly showed matrilineal inheritance of a diagnostic muscle protein phenotype (Monaco et al., 1982). These findings led to the conclusion that unisexual reproduction in *P. formosa* is the result of gynogenesis.

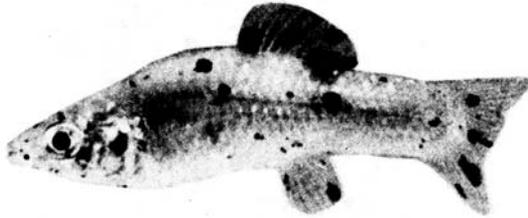
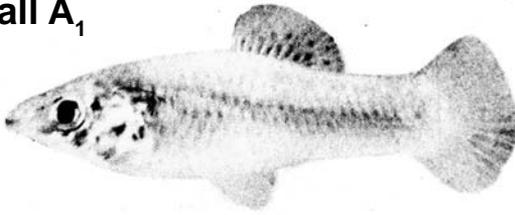
The *de nova* appearance of triploid unisexuals derived from *P. formosa* in nature and the laboratory indicates that the mechanism of sperm exclusion can sometimes fail (Rasch and Balsano, 1989). Adequate biochemical and cytological evidences are available to support the hypothesis that these triploid unisexuals arise by syngamy of the diploid egg of *P. formosa* and a sperm of its sexual host species to yield a zygote, where  $3n=69$ . For the formation of *P. formosa* triploid, the sperm is contributed by mostly limantouri race of *P. mexicana*. Hence, the Mexican triploid is designated as *P. formosa-limantouri*, while that of Texas is named *P. formosa-latipinna*. However, it is not known how the 50% male specific sperm of *P. mexicana limantouri* or *P. latipinna* are excluded, and when included how the male specific gene of XY-carrying sperm of *P. mexicana* or *P. latipinna* is not allowed to express in progenies sired by them.

Production of diploid or triploid offspring by gynogenesis requires the maintenance of correct ploidy level in the developing embryo without the contribution of the paternal genome. This may be achieved by *apomixis*, where meiosis is aborted, or by *automixis*, where meiosis is normal. In most unisexual amphibians and reptiles, for which oogenic pathways are known, isogenic matroclinal inheritance is achieved by automictic production of unreduced ova following a premeiotic endoduplication of the chromosome complement, formation of pseudo bivalent and two conventional meiotic divisions (cf Uzzel, 1970). However, cytophotometric analyses of DNA and DNA Feulgen levels in the nuclei of oogonia and primary oocytes of unisexual diploid and triploid *P. formosa* have shown that functional apomixis is the cytological basis of gynogenetic reproduction. Briefly, the first meiotic division is replaced by mitotic division, bivalents are not formed and there is no reduction of the chromosome number (Rasch et al., 1982). Consequently, all female gene pools remain isolated and 'frozen' from the gene pool of the species.

Incidentally, the frozen-niche variation hypothesis assumes that many clones of unisexual forms co-exist in a population, having arisen on several occasions through hybridization between sexual species. As new clones arise, they 'freeze' and maintain the genotypes that affect their use of food and spatial resources, with each clone expressing different phenotypes. As the clones differ in their mixture, the hypothesis assumes that the sexual forms can co-exist with the clones for two reasons: The genetic diversity of sexual forms allows them to use the wider range of resources than the clones and the clones depend on the presence of sexual forms for the sperm.

Many recent studies have posed challenges to the traditional assumption that genomes of uniparental vertebrates are inherited *en bloc*, without the incorporation of even subgenomic amounts of DNA from the bisexual host in gynogenetic fish (e.g. Schartl et al., 1995). The possibility of such 'leakage' of small amounts of male host genome into some gynogenetic fish was suspected by Rasch and Balsano (1989) but their failure to detect the same was related to the wide variations in the DNA estimates made by them. As the genes concerned with regulation of phenol oxidase systems, which account for the variety of patterns of melanin deposition, are found in  $F_1$  progeny (Fig. 12) of *Poecilia formosa* collected from Brownsville, Texas. The haploid genome of *Poecilia* is roughly 0.8 pg DNA. Thus, the average poeciliid chromosome contains roughly 0.035 pg DNA, which is about 3–4% of the total genome. Because the cytophotometric determinations of DNA levels in fish blood cells routinely show coefficient of variation of 3–6%, the determinations might have failed to detect the incorporation of at least one paternal chromosome into the diploid set of maternal chromosomes produced by apomixis in *P. formosa*: If genes for melanization

**all  $A_1$**



**all black male**



**Fig. 12** Progenies from matings of wild-type *Poecilia formosa* (top) to all-black male of *Poecilia* sp (bottom). Progenies of  $A_1$  are (middle ones) indicating the incorporation of sub-genomic amounts of DNA from black male host (from Rasch and Balsano, 1989 printed with kind permission of the New York State Museum, Albany, N.Y. 12230).

of the integument were of paternal (from '*P. latipinna* hybrid') origin, as indeed seems to be the case, they were apparently 'on loan' for just one generation, then that would appear to be like hybridogenesis (Rasch and Balsano, 1989).

Investigations by Balsano and his colleagues have shown that the triploid unisexuals of *P. formosa* are more stable and their clonal diversity increases from the head waters on the Soto la Mariana drainage to

downstream, where there is greater habitat diversity. Laboratory data suggest that the triploids are continuously generated at the rate of 1.5% of offspring of diploid unisexuals (Balsano et al., 1989). The triploids have higher survival, as their adults constitute 2–3 times more in a population than that of juveniles (Balsano et al., 1984). The frequency of triploids may comprise up to 90% of the poeciliid females and it fluctuates from year to year and site to site (Rasch and Balsano, 1974). Of 82 collections totalling 8, 800 females made on the Soto la Mariana drainage from 1970 to 1978, 11.3% were diploid and 20.7% were triploid unisexuals (Balsano et al., 1981). On the average, the unisexuals comprise about a third of *Poecilia* females (Balsano et al. 1985).

In general, the sex ratio among the poeciliids is skewed in favour of females; several field studies suggest that *Poecilia* males do not exceed 26.9% in a population (Monaco et al., 1981). Assuming 25% males for *P. mexicana*, its females would make up 50%, and the diploid and triploid *formosa* females would jointly make up the rest of 25%, i.e. for every *P. mexicana* male, there would be 2 *mexicana* females and 1 *formosa* female. Further experimental studies indicate the preference of poeciliids for their respective congenerer (Schlupp and Ryan, 1996) and the ultimate choice of *P. formosa* to *P. latipinna* over other poeciliids like *Xiphophorus multilineatus* (Marler et al., 1997). Hence, it would be interesting to know the chances of *P. formosa* in acquiring sperm from a non-congenerer. Despite such a skewed sex ratio, males are found not in short supply, for: 1. *P. mexicana* males maintain essentially constant levels of sperm production throughout the year and show no significant variation in testicular maturation index values associated with season, microhabitat, body length or rank in male dominance hierarchies (Monaco et al., 1981), and 2. *Poecilia* females show striking asynchrony in their reproductive readiness. Such asynchrony limits the proportion of receptive females at any one time (Balsano et al., 1985). Consequently there are more males ready to mate than there are receptive females and no dearth of sperm, despite the relative low frequencies of males in natural populations. Given a direct choice to a *P. mexicana* male between *P. mexicana* female and diploid *P. formosa* or between *P. mexicana* and triploid *P. formosa*, the number of copulation thrusts gained by the *formosa* females is more (8.6 for 2n♀ and 9.5 for 3n♀ than that by *P. mexicana* female (8.0, Balsano et al., 1981). Hence sperm is found in ovaries of both bisexual and unisexual females indicating that the unisexuals efficiently compete with females of *P. mexicana* for mating with *mexicana* males (Monaco et al, 1978). The frequency of gestating females is 50% for *P. mexicana*, but 68% and 52% for 2n and 3n *P. formosa* and embryos produced are 7–8 per female of both species. Consequently, the relative reproductive output of the unisexuals is as high (0.9–0.94) as the *mexicana* (1.0). The ability of *P. formosa* to acquire sperm from *P. latipinna* has also

been tested; *P. latipinna* males also mate preferentially with conspecific females; however, *P. formosa* receives more gonopodial thrusts in tests than *P. latipinna* females (Balsano et al., 1981). In the presence of conspecific mates, males are less discriminating than in tests, when only gynogens are present (Foran and Ryan, 1994).

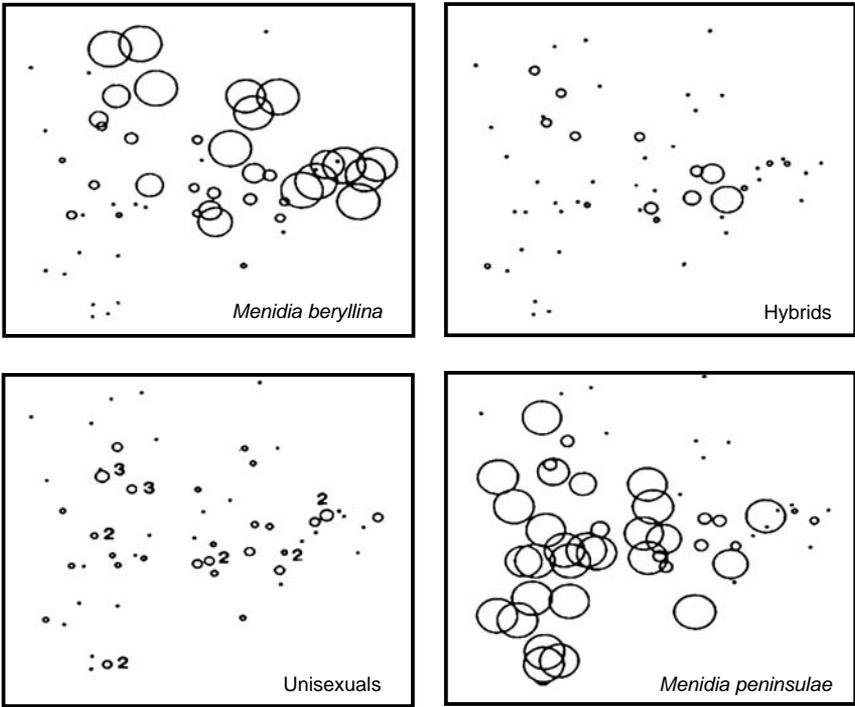
#### 5.4 *Menidia clarkhubbsi*

The *Menidia* complex includes two bisexual species *M. beryllina*, *M. peninsulae*, their diploid and triploid (mostly  $F_1$ ) hybrids (Echelle et al., 1988) and the unisexual *M. clarkhubbsi*. The complex occurs from Texas to Alabama on the coast of the Gulf of Mexico. *M. clarkhubbsi* reproduce gynogenetically and their unreduced diploid eggs require the trigger from the sperm of the sympatric bisexual species. Genetic analyses of the unisexuals indicate multiple origin of clones from separate zygotes produced during hybridizations between *M. beryllina* and allozymically distinguishable 'missing ancestor' similar to *M. peninsulae* female (Echelle and Mosier, 1981). These unisexuals are "a species complex, as each gynogenetic lineage of clones originating from a single hybrid zygote is a self-perpetuating, sexually parasitic, historical entity separated from other clones of similar origin" (Echelle and Echelle, 1997).

From samples of 54 collections totalling to 4,144 specimens of *Menidia* from the Capano Bay, Texas during June–August, 1985, Echelle et al., (1989b) reported that the occurrence frequency of *Menidia* was 37.3% for *M. beryllina*, 54% for *M. peninsulae*, 5.8% diploid and triploid *M. beryllina* x *M. peninsulae*  $F_1$  hybrids and 2.8% unisexuals. Representative of the unisexual *M. clarkhubbsi* species complex occurred in all the 27 samples. The unisexuals were composed of 108 individuals belonging to 6 allozymically described clones (Echelle et al., 1989a). Clone A was the most abundant occurring in all 27 collections and constituted 89% of the unisexuals; the remaining 11% specimens represented clone C (7 in 5 samples and clones B, H, I and J (1% for each clone) (Fig. 13). A cause for the abundance of other unisexuals but the presence of very few clones, most of which are represented by merely one individual is traced to the absence of one of its apparent progenitors; consequently no new individuals are produced. The existing *M. clarkhubbsi* species complex may be a relic of one more diverse and abundant assemblage of clones.

#### 5.5 *Carassius auratus* complex

Unlike the others, the unisexual of *Carassius auratus* is characterized by the presence of sterile males and is a racial but not a species complex. Fertile hybrid females (unisexuals) arising from the common carp *Cyprinus carpio*



**Fig. 13** Contribution by each of four biotypes to the *Menidia*. Dots signify sites, where the indicated biotype was absent; diameter of circle is proportional to percent contributed to the collected *Menidia*. Unisexuals included only clone A, and one or two other clones, indicated by numerals 2 and 3, respectively (from Echelle and Echelle, 1997).

♂ fertilizing the eggs of the crucian carp *C. auratus gibelio* from Russia, ginbuna *C. auratus langsdorfi* from Japan and *C. auratus* var *sugu* from southern China were first described by Cherfas (1966), Kobayashi et al. (1970) and Yu et al. (1987), respectively. In Japan, M. Namura is the first to distinguish the unisexual ginbuna from bisexual funa. Subsequently, Kojima et al. (1984) reported that as the first meiosis is abortive, the ginbuna produce unreduced  $2n$  and  $3n$  eggs. Recent scanning electron microscopic studies of Liu et al. (2001; see also Zhang et al., 1992 b) have shown that the natural production of unreduced gametes by male and female  $F_2$  hybrids of the common carp ♂ x red crucian carp ♀ is not unusual.

Wu et al. (2003) have experimentally generated a lineage from the sexual diploid carp transforming into allotriploid and tetraploid unisexual clones by genome addition. The hybridization between the common carp ♀ x red crucian carp ♂ produces  $F_1$  hybrids of fertile females and sterile males in ratio of 0.5 : 0.5. However, the  $F_1$  female hybrid produces unreduced diploid eggs with two maternal genomes (Fig.14); the eggs pass

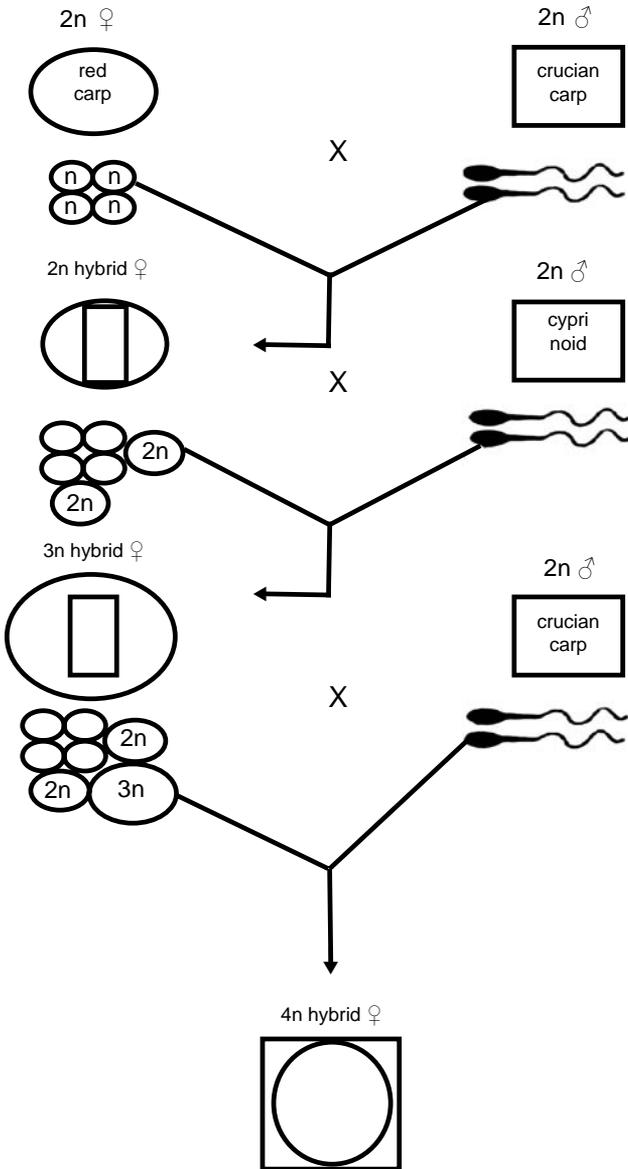
through the entire sequence of oogenesis, including yolk accumulation, the formation of follicular layer, zona radiata, chorion and micropyle. These diploid eggs are activated by the haploid sperm of the red crucian carp ( $2n=100$ ), mirror carp (*Cyprinus carpio*,  $2n=100$ ), Chinese blunt snout bream (*Megalobrama amblycephala*,  $2n=48$ ), gudgeon topmouth (*Pseudorasbora parva* ( $2n = 50$ ) or *Hemiculter leucisculus* ( $2n=48$ )). However, these eggs, when fertilized by the sperm, are hatched out as allotriploids with  $3n=150$ , each of which consists of 2 genomes from the common carp and 1 genome from the crucian carp.

Sex ratio of the allotriploids is  $0.5\text{♀} : 0.5\text{♂}$  but the males are sterile (see also Flajshans et al., 2008). The fertile females produce unreduced triploid eggs; although these eggs may be activated by the cyprinids listed above, only those eggs fertilized by the haploid sperm of the red crucian carp hatched out as allotetraploids. All others develop gynogenetically and hatch out as allotriploids (Fig. 14).

Sex ratio of the allotetraploid too is  $0.5\text{♀} : 0.5\text{♂}$  but the males are sterile (see also Flajshans et al., 2008). The females produce unreduced  $4n$  eggs. They can be activated by the inactive haploid sperm of any one the cyprinids listed above, i.e. the allotetraploids reproduce gynogenetically. Consequently, the offspring resemble the mother, irrespective of any of the cyprinid sperm used to initiate embryogenesis (Wu et al., 2003). However, the allotriploid females gynogenetically reproducing in natural aquatic systems are limited. For instance, using multilocus DNA fingerprint technique, Umino et al. (1997) reported that 86% of the triploid ginbuna are all the clonal progenies of only 3 females in the Kurose River, Japan.

The testes of  $2n$  hybrid male are pink in colour instead of white. An electron microscopic study shows the presence of primary and secondary spermatocytes and abnormal spermatids owing to abnormal spermatogenesis. No functional spermatozoa are ever produced by  $3n$  and  $4n$  males. Consequently, despite the presence of males, which are completely sterile, the females of these crucian carp race remain sexual parasites and have to depend on parental or related species to reproduce and perpetuate the race.

The possibility of leakage of small amounts of genes from the host *Poecilia latipinna* to the genome of *P. formosa* (Rasch and Balsano, 1989) was earlier indicated. From their electron microscopic and electrophoretic studies, Zhang et al. (1992a) have shown that synopsis and recombination occur between at least some homologous chromosomes in the triploid female ginbuna and the sperm donor gongoroubuna *Carassius auratus cuvieri*. Hence, the scope for introduction of heterozygosity does exist in the gynogenetic reproduction too. Recently, Jia et al. (2008) have quantified the paternal leakage to the progenies of gynogenetic unisexual *Carassius auratus gibelio* from China. Using microsatellite genotyping at

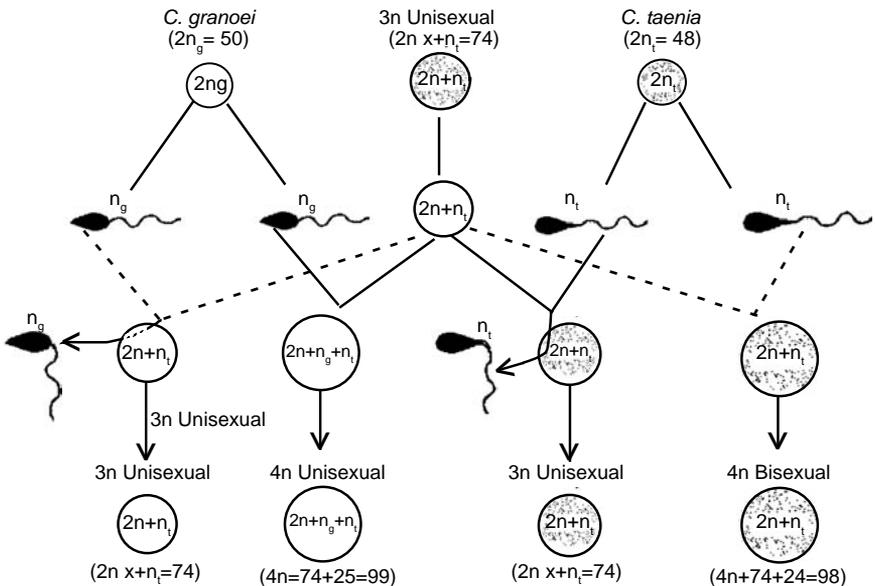


**Fig. 14** Generalised scheme of genome addition by hybridization between red common carp and crucian carp. Note the production of unreduced eggs by diploid and triploid carp (from different sources).

15 loci, they have shown that 0.63% of progenies have incorporated the paternal genetic material. Frequent reports on the paternal leakage in the totally gynogenesis-dependent unisexuals *Poecilia formosa* and *Carassius auratus* show that they may not be trapped into the Muller's ratchet and gradually meet the evolutionary dead ends, for lack of genetic recombination and re-assortment.

### 5.6 *Cobitis* complex

Vasil'ev et al. (1990) have reported the discovery of *Cobitis* complex from the Dubeshnya River, Moscow. The complex consists of 2 diploid bisexuals *Cobitis granoei* ( $2n_g=50$ ) and *C. taenia* ( $2n_t=48$ ), a triploid unisexual ( $3n=74$ ) and 2 different tetraploid forms (Fig. 15). The triploid unisexual ( $2n_x+n_t$ ) is a hybrid between an unknown progenitor and *C. taenia*, and produces unreduced  $3n$  eggs ( $50+24=74$ ). If the  $3n$  egg is fertilized by the haploid sperm of *C. taenia*, bisexual tetraploid ( $2n_x+2n_t$ ;  $74+24=98$ ) is generated (Fig.15). On the other hand, unisexual tetraploid ( $2n_x+n_t+n_g$ ;  $74+25=99$ ) is produced, when the unreduced  $3n$  egg is fertilized by the haploid sperm of *C. granoei*.



**Fig. 15** Tentative pathways of gynogenetic and sexual reproduction in *Cobitis* complex. Note the gynogenetic production of  $3n$  unisexual involves activation of embryonic development by sperm, as shown by the outgoing arrows from the eggs (based on the data of Vasil'eva et al., 1989, Vasil'ev et al., 1990).

Vasil'eva et al. (1989) and Vasil'yev et al. (1990) have provided a long list of morphological characters that may be used to distinguish these five different forms of *Cobitis* complex. They have also indicated that  $3n+n_g$  tetraploid consists of two electrophoretic clones. During the spawning period in June (14–20°C), the frequency, in which they occur, is 28% for  $2n$  *C. taenia*, 38% for triploids, 16% for the  $2n+n_t$  and 18% for the  $2n+n_g$ . Hence, the chances of the polyploidy forms to acquire sperm from bisexual diploids or tetraploid are abundant.

However, the results on reproduction of polyploids, as reported by Vasil'yev et al. (1990) are not clear, especially with regard to the origin of  $(2n+n_g)$  triploids. About 85% of eggs produced by the tetraploid  $3n+n_g$  were successfully triggered by the sperm of the spiny loach *Misgurnus fossilis*. "None of the larvae in the experiments with the sperm of *M. fossilis* appeared to be hybrid". Apparently, the unisexual tetraploids reproduce gynogenetically.

Likewise, the  $3n$  eggs were also successfully triggered by the sperm of diploid males. Apparently, the triploids too reproduce gynogenetically, borrowing the sperm from  $2n$  *C. taenia* or *C. granoei*. Whereas the sperm of diploids (*C. taenia*, *C. granoei*?) readily triggered embryogenesis in  $3n$  eggs, the sperm of tetraploid failed to do it. Further, the tetraploid sperm also failed to activate or fertilize the eggs of  $2n$  female in 9 of 10 experiments and the eggs of  $4n$  female in 2 of 3 experiments. Even in those experiments with success, the success was limited to 5–13% hatchlings only. The tetraploid  $(2n_x+n_t)$  males seem to encounter a major cytological problem in their spermatogenesis. No mention is made on the fertilizability of eggs of the tetraploids  $(2n_x+2n_t)$ .

### 5.7 *Poeciliopsis monacha-lucida*

Among the unisexuality, the *Poeciliopsis* complex is the second discovery from the Rio Fuerte of north western Mexico (Miller and Schultz, 1959). The complex consists of two bisexual species *P. monacha* and *P. lucida* and three unisexual hybrids. Of these hybrids, one is a hybridogenetic diploid *P. monacha-lucida* and the other two are gynogenetic triploids: *P. 2monacha-lucida* and *P. monacha-2lucida*. Apparently, hybridization between *P. monacha* female and *P. lucida* male, the progenitors of the unisexual hybrid, *P. monacha-lucida* was generated. However, unlike *Poecilia formosa*, which produces diploid eggs and reproduces gynogenetically, *P. monacha-lucida* normally produces haploid eggs, which are normally activated by the sperm of *P. lucida* or in some cases by *P. occidentalis* or *P. infans*. These hybrid progenies share characteristics of both parents (Fig. 11). During oogenesis, however, the paternally derived *P. lucida* chromosomes are lost; thus primarily the *monacha* set alone is transmitted to the egg. This hemiclinal mode of reproduction that

delivers one genome of a hybrid to the functional oocyte is called hybridogenesis (Schultz, 1969). As long as the male is *lucida*, *occidentalis* or *infans*, the female determining genes of *monacha* overrides the male determining genes of the paternal contribution (Schultz, 1989). Yet, *P. monacha-lucida* provides an excellent opportunity to show whether the male specific chromosome is excluded from syngamy or the exact molecular mechanism by which the male specific genes are inhibited from expression by some *monacha* gene(s).

The hybridogenetic 2n *P. monacha-lucida* hybrid evidently provides the stepping stone to the formation of the triploids. Thus, the other two triploid forms stem from the occasional failure of the mechanism that excludes the transfer of paternal genome into the oocyte and the consequent production of 2n ova. Such ova, when combined with the *lucida* sperm, result in a triploid form that is designated as *Poeciliopsis monacha-2lucida*. Alternatively, if the egg is fertilized by the sperm of *P. monacha*, it then brings out what is called *P. 2monacha-lucida* (Schultz, 1969).

In *P. monacha-lucida*, hybridogenesis involves the formation of a unipolar spindle before vitellogenesis. During meiosis, the maternal (*monacha*) chromosomes align unpaired on the metaphase plate, where they attach to a unipolar spindle; they are then drawn to the single pole, where they become the sole contributor to the egg nucleus. Since the paternal chromosomes do not pair with the maternal chromosomes and are not attached to spindle fibres, no segregation or crossing over occurs. Thus, the maternal genes are transmitted as a single unit and the inheritance is actually hemiclinal. The paternal chromosomes are not attached to spindle fibres but remain scattered in the cytoplasm and eventually get lost. The paternal chromosomes are regarded as incompatible with the ooplasm and hence the paternal half-spindle fails to form properly (Cimino, 1972a).

Triploid forms of *Poeciliopsis*, *P. 2monacha-lucida*, and *P. P. monacha-2lucida* undergo a process of endoduplication prior to meiosis, during which the somatic chromosome number is increased from 3n to 6n (Cumino, 1972b). This is followed by the formation of 'pseudo bivalents' and two meiotic divisions, Recombination does not occur, so the 3n eggs produced are isogenic to each other and to the mother (see Schultz, 1989).

Despite conspecific preference by males of the donor by sexual species, the females of *Poecilia formosa* were successful in acquiring adequate sperm. Equally, the females of *Poeciliopsis* are also successful. For activation/fertilization, the triploid gynogen *P. monacha-2 lucida* and the diploid hybridogen *P. monacha lucida* depend on the sperm of *P. lucida*. But *P.2 monacha lucida* females depend on *P. monacha*. Mating frequency experiments of Lima et al. (2002) have shown that the uncommon all-female biotype has a mating advantage over the common sexual *P. monacha* female.

Restriction site analyses of mtDNA revealed the *P.monacha* is the maternal ancestor of five complex allozyme/mtDNA genotype (Quattro et al., 1992a). The high levels of mtDNA diversity are also mirrored by similar high levels in *P. monacha-lucida*; thus hybridizations giving rise to unisexual lineage have occurred many times (Quattro et al., 1991). Despite having to remain dependent on 'borrowed' sperm from sympatric species, hybridogenesis has perpetuated them over 100,000 generations (Quattro et al., 1992b). With hybrid vigour, the unisexual *Poeciliopsis monacha-lucida* are known to self-replicate and are extremely successful in outnumbering their parental species at some localities by as much as 20 : 1 (Schultz, 1977). Their success is also attributed to their clonal diversity and ability to store genetic polymorphism, which contrasts strikingly with sexual species. Laboratory studies have shown the natural existence of a minimum of eight clonally inherited genomes and each of these eight clones may include additional variations (up to 30, see Angus and Schultz, 1979) at other loci. The unisexual hybrids maintain significantly high levels of heterozygosity ranging from 36% to 50% (Vrijenhoek et al., 1978). These indicate that multiple hybrid origins have been the most significant source of clonal diversity in these unisexuals (Angus and Schultz, 1979).

### 5.8 *Phoxinus eos neogaeus*

The hybrids between the red belly dace *Phoxinus eos* ( $2n=50$ ; 3.28 pg DNA/cell) and the fine scale dace *P.neogaeus* ( $2n=50$ ; 3.65 pg/cell) are always females, abundant and in some localities outnumber their parental species, and are found within the northern states of Maine, New Hampshire and New York of the USA and Quebec of southern Canada. They constitute 53% to 83% of the *Phoxinus* complex (Dawley et al., 1987). In New Hampshire, the hybrids include diploids ( $2n=50$ ; 3.45 pg/cell), triploids ( $3n=75$ ; Dawley et al. 1987; 4.98 pg/cell; Dawley and Goddard, 1988) and mosaics ( $2-3n=50-75$ ; 3.45-4.98 pg/cell). The heterozygous diploid hybrids carry one haploid *eos* and one haploid genome of *neogaeus*. The triploids carry an additional genome of mostly *eos* or rarely *neogaeus* and are designated as *P. 2eos-neogaeus* or *P. eos-2neogaeus* (Goddard and Schultz, 1993). Tissue graft studies have also confirmed that the triploids and mosaics are derived from the diploids and the third genome present in the triploids and mosaics originates from the males of *P. eos* or *P. neogaeus* (Dawley and Goddard, 1988).

Diploid unisexual *P. eos neogaeus* reproduces gynogenetically and 50% of diploid eggs are only activated by the sperm of bisexual species and the other 50% of eggs undergo syngamy with the sperm; this 50% value is exceptionally high compared to 1% to 6% gynogens of other unisexual

species (e.g. 1.5% *P. formosa*, Balsano et al., 1981; Echelle et al., 1989a). Hence *Phoxinus* complex continuously generates triploids and mosaics. Thus the triploids (30%) together with mosaics (29%) constitute 59% of the unisexuals of *Phoxinus* complex, for instance in Orange River, Maine (Dawley and Goodard, 1988).

Among the diploid-triploid, the presence of mosaics in the *Phoxinus* complex adds an extra element of complexity and is extremely rare among the unisexual vertebrates. At the East Inlet Pond, New Hampshire, the diploid-triploid-mosaics constitute 34% of the unisexuals and at other localities mosaics make 11% to 38% of the collected unisexuals. Within the individual mosaics, some have equal number of diploid and triploid cells, while others have mostly diploid or mostly triploid cells, and in fact the mosaics form a continuum between pure diploids and triploids forms. However, mosaics with diploid cells are the most common.

In contrast to the other unisexual fishes, which remain as independently reproducing lineage, each hybrid type of *Phoxinus* is linked to the other types, as the diploids and mosaics continuously generate all the three types, and as all of them inherit the same *eos-neogaeus* clonal genome; accordingly, the diploid hybrid *eos-neogaeus* may generate i) diploid hybrid (*en*) through gynogenesis, ii) mosaics (*en/ene*) and iii) triploid (*ene*) progenies, when the eggs of *en* diploid is fertilized by *eos* sperm (see Fig. 3 of Goddard and Dawley, 1990). Whatever the underlying meiotic mechanism, reproduction in *Phoxinus* mosaics resembles only superficially the hybridogenesis in that the *eos-neogaeus* genome is transmitted intact and a sperm genome is incorporated into some cells of some progenies alone for one generation and is discarded in the subsequent generation. Thus, the mosaics may reproduce clonally as well as non-clonally through its diploid egg, produced after undergoing a sort of modified hybridogenesis. According to Goddard and Schultz (1993), the triploids appear to undergo a normal hybridogenesis, and subsequently regular meiosis to produce a hybrid egg.

As *Phoxinus* hybrids require sperm of a bisexual species to fertilize their eggs, male hybrids are likely to have been produced by incorporation of sperm bearing male-determining genes, as they are all triploids or mosaics. Goddard and Dawley (1990) found 5 males out 1,000 fish from East Inlet Pond. Though, they may frequently be produced, no hybrid males ever fathered a progeny in the laboratory.

Briefly, reproduction in *P. eos-neogaeus*, although basically clonal and gynogenetic, has elements that make it complicated. These elements include 1. A much higher level (50%) of sperm incorporation than is typical for gynogenetic vertebrates, causing the continual production of polyploid individuals, 2. The presence of diploid-triploid mosaics and 3. The difference in reproductive mechanism—diploids and some mosaics

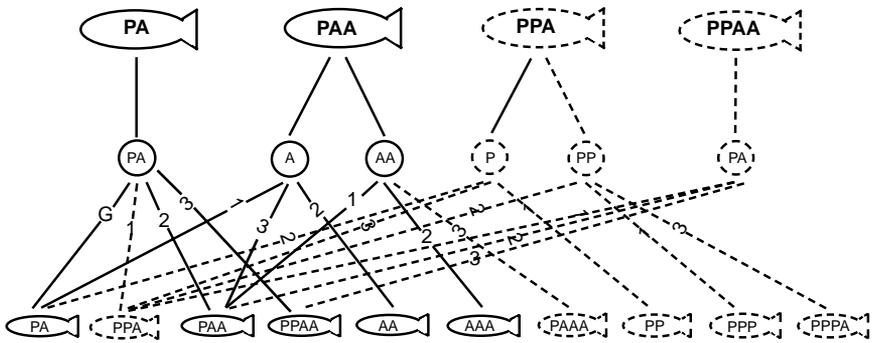
reproduce clonally by gynogenesis, while triploids and other mosaics reproduce non-clonally by modified hybridogenesis (Goddard and Dawley, 1990). These seem to have in a way compensated the extreme clonal uniformity (Elder and Schlosser, 1995) of *Phoxinus eos neogaeus*.

### 5.9 *Rutilus alburnoides*

Hybrid minnows collectively known as the *Rutilus alburnoides* complex are found throughout most of the Iberian Peninsula and include diploid and polyploid forms with female skewed sex ratio. Collares-Pereira (1985) was the first to describe this complex. Carmona et al. (1997) examined the nuclear (allozymes) and cytoplasmic genes (mtDNA) to assess the evolutionary origins, relationships and reproductive modes of *R. alburnoides* complex. The multilocus allozyme analyses revealed the hybrid origin of all the diploid and polyploid forms of this fish. Diagnostic markers identified a fish from the genus *Leuciscus* as the paternal ancestor of hybrids in the Duero and Guadiana basins in western Spain. Nuclear marker analysis established the occurrence of hybridogenetic mode of reproduction. Hybridizations in *R. alburnoides* have apparently 'opened' many novel reproductive pathways altering both oogenesis and spermatogenesis. Contrasting with all other unisexual vertebrates, hybrids of *R. alburnoides* are fertile and the hybrid males seem to have played a particularly important role in the dynamics, perpetuation and evolution of *R. alburnoides*. A series of publications by Collares-Pereira (1989), Collares-Pereira et al. (1998), Carmona et al. (1997) and Alves et al. (1997a, b, 1998, 1999) has unravelled perhaps the most diverse modes of reproduction of *R. alburnoides* involving hybridization, hybridogenesis and gynogenesis.

The diploid males of *R. alburnoides* are comprised of two distinct genotypes: 1. The non-hybrid genotype possesses the *L. pyrenaicus*-like mitochondria, exhibit normal Mendelian meiosis and produces haploid sperm, and 2. The hybrid genotype produces unreduced clonal sperm, a very rare event among vertebrates. Diploid females of *R. alburnoides* clonally transmit their hybrid genome to eggs.

Triploid males of *R. alburnoides* also produce unreduced  $3n$  sperm. Triploid females undergo a modified meiotic hybridogenesis, in which the *L. pyrenaicus* (P) genome is discarded in each generation without recombination, but the inheritance is hemiclinal (Fig. 16). Meiosis of the remaining AA' genome involves random segregation and recombination between a homospecific genome, and genetically distinct haploid (A+) and diploid (A-A') eggs are produced. Moreover, unlike in normal hybridogenesis, a sperm genome that is incorporated in the diploid egg may remain in the hybrid lineage longer than one generation and the sperm genome incorporated in the haploid egg is clonally transmitted by the diploid female to its



**Fig. 16** The putative reproductive modes and interrelationships between different forms of the *Rutilus alburnoides* complex. The continuous lines (—) indicate experimentally confirmed forms and pathways and the dotted lines (---) the hypothetical forms and pathways. P is the genome of *Leuciscus pyrenaicus* and A the genome of the other ancestor. 1. Fertilization by P sperm produced by normal meiosis in *L. pyrenaicus*; 2. Fertilization of A sperm produced by normal meiosis in nonhybrid males; 3. Fertilization by PA sperm produced either clonally by diploid hybrid males or by normal meiosis by tetraploid males. G represents the occurrence of gynogenesis (from Alves et al., 1999).

egg. The eggs produced by the diploids and triploids require syngamy to initiate development. However, 3% of eggs produced by diploid females develop directly without the need for syngamy into diploid clones. The female progenies, which inherit the intact hybrid genome of the female parent exhibit no fingerprinting bands of the male parent. This is the first direct evidence for the occurrence of gynogenesis in *R. alburnoides* complex. Thus “reproduction by the types of diploid and triploid has introduced high genetic diversity into the hybrid populations of *R. alburnoides* and allows purging of deleterious genes and incorporation of beneficial mutations in the same genome, characteristics believed to be major advantages of sexual reproduction” (Alves et al., 1998).

Restoration of an even ploidy value in tetraploids may allow a return of normal meiosis and serve as a stepping-stone to biparental reproduction. A typical route to tetraploidy in clonal vertebrates seems to be the syngamy of unreduced triploid eggs and haploid sperm produced by a male of a bisexual species, as has been seen with *Carassius auratus* complex. Here too, the *R. alburnoides* complex presents an alternate route to tetraploidy. The experimental hybridizations between diploid hybrids (cross  $2n_{\text{♀}}122 \times 2n_{\text{♂}}121$ ) (Table 17) produced all male PPAA progenies. However, the other cross between  $2n_{\text{♀}}122 \times 4n_{\text{♂}}130$  produced tetraploid female progenies with PPAA, as they received a clonal PA genome from  $130_{\text{♂}}$  and a recombined PA genome from  $122_{\text{♀}}$  (Alves et al., 1999).

According to Alves et al. (1999), the mechanism of sex determination in *R. alburnoides* remains a puzzle. If female heterogametic (ZW/ZZ)

**Table 17** Experimental crosses between diploids and polyploids of *Rutilus alburnoides* collected from the Tejo basin of western Spain (from Alves et al., 1999).

Crosses	Analyzed progeny			
	Ploidy	♀	♂	?
2n ♀ 122 X 2n ♂ 121	4n	–	9	4
3n ♀ 116 X 2n ♂ 117	3n	1	–	1
3n ♀ 132 X 2n ♂ 121	3n	4	3	–
3n ♀ 136 X 2n ♂ 121	3n	–	4	–
2n ♀ 122 X 4n ♂ 130	2n	1	–	–
	4n	3	6	15
3n ♀ 110 X 4n ♂ 112	3n	2	1	–
3n ♀ 111 X 4n ♂ 112	3n	2	4	1
3n ♀ 114 X 4n ♂ 115	3n	10	–	2
3n ♀ 133 X 4n ♂ 134	3n	1	4	–

mechanism of sex determination is to be applied, all the tetraploid progenies of the cross between 2n ♀122 x 2n ♂121 should have been females, but they were all males (Table 17). However, the male heterogametic (XX/XY) sex determining mechanism cannot explain the presence of female progenies in the crosses between 3n ♀116 x 2n ♂117 and 3n ♀132 x 2n ♂121, as they would have received the clonal sperm carrying X and Y from the hybrid males. Therefore, the mechanism of sex determination in the *R.alburnoides* complex remains not yet well understood.

Interestingly, having arisen as mostly interspecific and rarely interracial hybrids on multiple occasions, each unisexual fish species has explored its own uncommon modes of reproduction and course of evolution. Gynogenesis has been a chosen mode of reproduction by almost all of them but to escape the consequent clonal uniformity, each one has explored alternate avenues. Perhaps, *Poecilia formosa* and *Menidia charkhubbsi* have adopted gynogenesis as the only mode of reproduction and exhibit it in its simplest form. To introduce clonal diversity, *Gobitis* and *Carassius auratus* have included a component of 'genome addition' and polyploidization in their gynogenetic mode of reproduction. Besides gynogenesis, *Poeciliopsis* have opted for the hybridogenetic mode of reproduction, which provides them an opportunity to try and test a new genome at every generation. While most unisexual clones remain 'frozen' and isolated, *Phoxinus eos neogaeus* has chosen to link them together and introduce clonal diversity by discovering the 'mosaic' forms. In contrast to all these unisexual fish species, who have experimented by modifying oogenesis one way or another and a variety of (ploidy. dependent) hybrid female forms, *Rutilus alburnoides* has uniquely chosen to examine the possibility of altering spermatogenesis and the usage of hybrid males. It has adopted sexual, hybridogenetic and gynogenetic modes of reproduction.

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## Simultaneous Hermaphroditism

Among vertebrates, the existence of simultaneous hermaphroditism is a big puzzle. There are three kinds of simultaneous hermaphrodites: 1. Self-fertilizing hermaphrodites, 2. Gamete exchanging hermaphrodites and 3. Potential self-fertilizing hermaphrodites. The presence of the third group is based on recent reports on the occurrence of simultaneous hermaphroditism in *Chlorophthalmus*, *Bathypterois*, *Benthalbella* and *Bathysaurus* (see Sadovy de Mitcheson and Liu, 2008) and a few muraenids (Fishelson, 1992). The first group is represented by a single species *Rivulus marmoratus* (Cyprinodontidae) and the latter by a few species belonging to the genera *Serranus* and *Hypoplectrus* (Serranidae). These hermaphrodites possess functional distinct ovary and testis, and their corresponding ducts to release gametes or fertilized embryos (*R. marmoratus*). Though they are limited to only a few species, their very existence poses many challenges to geneticists and endocrinologists. Information on their general biology is available; molecular biologists have made an attempt to understand the sustenance of different clones of *R. marmoratus*; behaviour specialists have described the mating and mating system of serranids. However, the puzzling simultaneous hermaphrodites seem to have long remained unnoticed and unattended by endocrinologists and geneticists.

There are two ontogenetic model pathways through which the simultaneous hermaphrodites pass through (Fig. 17). In the '*Rivulus*' model, majority of them remain as simultaneous self-fertilizing hermaphrodites throughout their life. However, pure males can be hatched out in the laboratory and they remain as males throughout their life. In the '*Serranus*' model too, majority of them are hatched out and remain as 'egg-trading' hermaphrodites throughout their lives. However, a few large hermaphrodites may lose the female function and become male in their natural habitats.

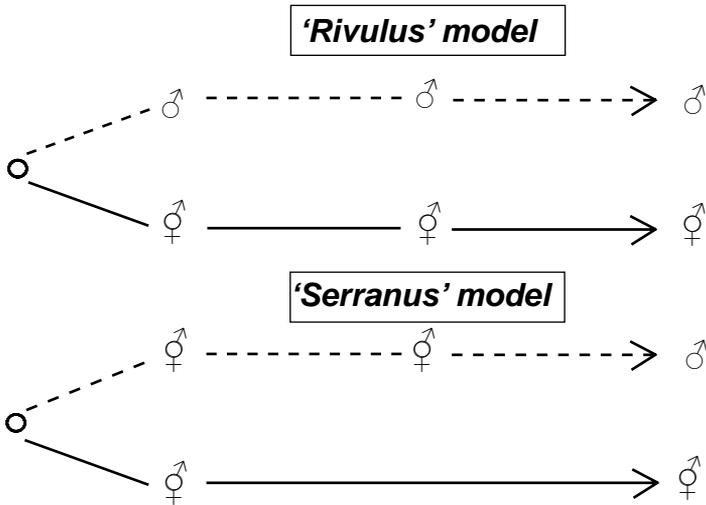


Fig. 17 Ontogenetic pathways of sexual development in simultaneous hermaphrodites. Dashed lines indicate artificial induction of male in Rivulus model or rare occurrence of male in natural habitats in Serranus model.

### 6.1 Self-fertilizing hermaphroditism

The cyprinodontid killifish *Rivulus marmoratus* is unique, as it is the only vertebrate with simultaneous internally self-fertilizing hermaphroditism. It is distributed from southern Florida, USA along the Yucatan, Mexico through much of the West Indies and Central America to Venezuela. True females have never been recorded and males are almost absent in Florida and are exceedingly rare in some Caribbean Islands. Hence most of its populations consist exclusively of obligate automatic selfers with a breeding system equivalent to cleistogamous plants. As outcrossing is thus far unknown, its natural populations are apparently clonal and homozygous.

However, using DNA fingerprinting with an array of microsatellite [e.g. (CT)<sub>n</sub>] and minisatellite (e.g. the 33.5 core sequence) probes, Turner et al. (1992) have found very high clonal diversity; within the samples of 7 Floridan populations, 5 showed as many clones as there were individuals. In all, there were 42 clones among 58 individuals (1.4 individuals/clone), a level of genetic diversity unprecedented among clonal animals. Hence, the data of Turner et al. imply that despite the clonal mode of reproduction, most individuals in a typical population of *R. marmoratus* have unique genotypic 'lottery tickets' bearing rather different numbers and do not have redundant copies of the same ticket, as may be expected (Williams, 1975). Turner et al. also argue that the mixing of unrelated individuals from different populations occurs to an extent previously unappreciated and/

or that divergence of clones is mediated by natural selection. If confirmed Turner et al. (1992) consider that the latter may pose a serious challenge to current ideas on the predominant role of recombination in promoting the evolution of biological diversity.

*R. marmoratus* matures within 3 months and can produce eggs almost throughout the year. Rearing the fish at below 20°C through 10 uniparental generations, Harrington (1967) noted the appearance of less than 5% primary males among the progenies. The temperature threshold for incubation of eggs for the generation of a primary male is 19°C or below, but above which only hermaphrodites are produced. The so called 'thermolabile phenocritical period' or labile period of sex determination lasts between the stage of formation of neural-haemal arches on caudal vertebrae and ends before hatching (Harrington, 1968). Harrington (1971) further showed that continuous exposure of post-hatchlings to temperatures below 20°C for 3–6 months fails to induce sex change in secondary males. The exposure to light for 12 hours or less at higher temperatures (~27°C), induces the hermaphrodites to change into secondary males. Hence, the response of the killifish to thermal-photoperiod changes is highly plastic.

If plasticity of the phenotype is important for most sexual individuals for survival and reproduction in a variable environment, it should be critical for 'clonal' individuals of *R. marmoratus* inhabiting estuaries and intertidal zones, in which biotic and abiotic factors are continuously changing. Lin and Dunson (1995, 1999) subjected five strains of the killifish to different rations and salinities to test whether these changing environmental factors induce significant differences in the overall fitness of each strain. Food ration had a significant effect on all reproductive traits like body length, body weight and age at sexual maturity as well as fecundity; there were no significant differences among strains in reproductive traits except for the egg size. Salinity too affected the reproductive traits. The effect of ration not only affected the reproductive traits of the tested generation of the killifish but also their F<sub>1</sub> progenies. The killifish matured earlier at a smaller body size at higher salinity and also produced a larger number of eggs, when food was abundant (Fig. 18). However higher salinity also led prolonged incubation period (29 days at 40 g/kg vs 19 days at 1 g/kg) and to low hatching success of the eggs. In contrast, individuals at low salinity (1 g/kg) matured later with a larger body size and produced fewer eggs; yet the hatching success of eggs incubated at 1 g/kg was almost 3 times higher (75%) than that (27%) at 40 g/kg. Thus, the advantages and disadvantages of exposure to a specific salinity were reversed between parental and their eggs. Besides, each strain responded to environmental factors somewhat differently. Hence, no strain was able to maintain higher fitness than the others across all the tested factors. The similarity in overall fitness

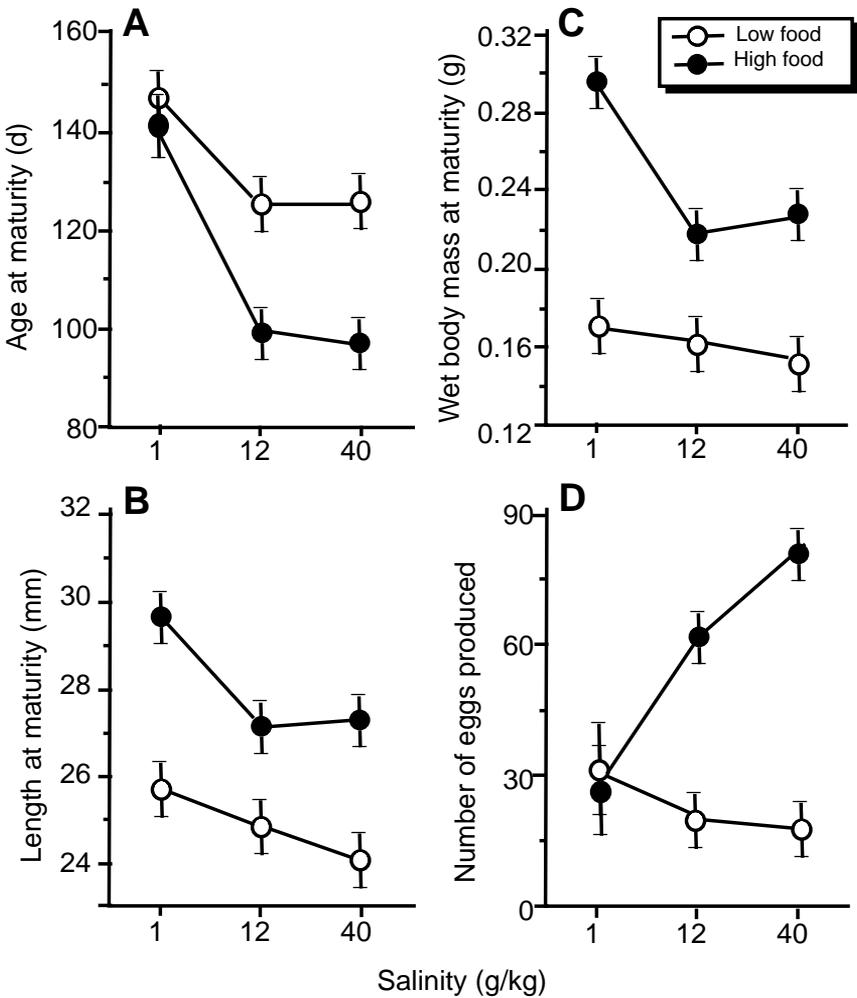


Fig. 18 Effect of ration and salinity on the reproductive traits of the parental generation of *Rivulus marmoratus*. The tested traits are A) age at maturity, B) body length at maturity, C) weight at maturity and D) fecundity (from Lin and Dunson, 1995).

indicated that each strain is primarily suited for a particular realized niche that is considerably smaller than the fundamental niche for the species as a whole. Therefore, the heterogeneity of abiotic and biotic environmental factors is matched by the bounds of phenotypic plasticity of the strain (Lin and Dunson, 1995). Thus, the magnitude of phenotypic plasticity encountered among the individuals of different strains of the killifish appears to neutralize the lack of recombination on genetic diversity.

## 6.2 Egg trading hermaphroditism

The presence of simultaneous hermaphroditism is limited to all species belonging to the two genera *Serranus* and *Hypoplectrus*. However, a tendency for simultaneous hermaphroditism among smaller serranids has also been noted (e.g. *Serraniculus pumilio*, 5–6 cm standard length, Hastings, 1973). Among the gonochores and sequential hermaphrodites, whose members function as either male or female, the presence of simultaneously functional hermaphrodites is regarded as a historical artifact, for these simultaneous hermaphrodites exhibit a rare alternate and hence may be at a disadvantage (Maynard-Smith and Price, 1973). There are at least two disadvantages that could prevent simultaneous hermaphroditism from being evolutionarily stable. Firstly, hermaphrodites should bear the costs of investing in the morphological apparatus and functional mechanism of both sexes and competitive superiority of pure males could easily render hermaphroditism evolutionarily unstable. Not surprisingly, large hermaphrodites like *S. fasciatus* lose the female function and become males. Secondly, reproductive cost invested in female function by a hermaphrodite (77% in *S. tortugarum*, Fischer and Petersen, 1987) shall not be less than that of a pure female. However, the serranines seem to have nullified these disadvantages by adopting to egg trading and dispersed spawning by parcels.

Among the serranids, the serranines alone are simultaneous hermaphrodites; however, their testicular and ovarian tissues are well separated with their respective ducts to the outside. They are not known to ordinarily self-fertilize; the serranine hermaphrodites mate only as a male or female during any given spawning but have the capacity to switch sex roles between matings. Since fertilization is external, such behaviour would effectively prevent selfing. In the serranines characterized by the mating system of serial monogamy (see Fischer and Petersen, 1987), the black hamlet *Hypoplectrus nigricans* (Fischer, 1980) and the chalk bass *Serranus tortugarum* (Fischer and Hardison, 1987) spawn only in pairs, one partner, the 'initiator' (Barlow, 1975b) releasing eggs and the 'follower' fertilizing them. Hence there is no selfing. Eggs are planktonic and there is no parental care. A clutch is not released in a single spawn, as in the other serranine characterized by the permanent monogamy, the harlequin bass *S. tigrinus* (Pressley, 1981) but in 4–9 parcels spawned sequentially and alternately usually with the same partner (Table 18). Partners generally alternate sex roles with each spawn by taking turns to give up parcel/eggs to be fertilized. This active reciprocative release of eggs, called 'egg trading' means that the reproductive success of a male depends upon the ability to reproduce as a 'female'. Since most of the reproductive effort of an individual is spent on female functions, egg trading provides a fecundity advantage

Table 18 Attributes of the three mating systems of co-sexual serranines (source: Fischer and Petersen, 1987. The evolution of sexual patterns in the sea basses. *Bioscience*, 37: 482–488; copyright permission by American Institute of Biological Sciences is duly acknowledged).

Characteristics	Serial monogamy	Permanent monogamy	Harem Polygyny
Species	<i>H.nigricans</i> <i>S.tortugarum</i>	<i>S.tigrinus</i>	<i>S.fasciatus</i>
Sexual pattern	♂	♀	♂♂
Territoriality	Part-time	Full time	Full time
Parceling of clutch	Yes	No	No
Reciprocation	Yes	Yes	No
Female courtship	Yes	Yes	No
Sperm competition	Yes, No	Yes	Yes

to hermaphroditism, making it evolutionarily stable, relative to dioecy. Repeated alternation of male and female sex roles in the mating bout has also been reported in the hermaphrodites sea slug *Navanax inermis* and the polychaete worm *Ophryotrocha diadema*. These convergences suggest that 'egg trading' is an evolutionary response to simultaneous hermaphroditism rather than some incidental aspect of serranine biology (Fischer and Petersen, 1987). Secondly, barring the only one permanently monogamous species *S.tigrinus*, all other serranines prepare their eggs to be spawned gradually parcel by parcel over the course of the daily spawning period and thereby constrain the hermaphrodite's mating success as a male.

The serial monogamous basses do not usually spawn, where they feed and sleep and have a larger (~750 m<sup>2</sup>) home range. Streaking indicative of intense sperm competition occurs in all serranines except in *H. nigricans*. A combination of low density, limitation of space use, reduced allocation for female function to 5–6% from 77% in an atypical simultaneous hermaphrodite such as the chalk bass, and a shortened spawning period appears to have driven a few simultaneous hermaphrodites like *S. fasciatus* and *S. baldwini* to switch over to pure male, when they attain a larger body size (Fischer and Petersen, 1987, see also Erisman et al., 2008).

Thus, all individuals of *S. fasciatus* commence life as simultaneous hermaphrodites but the largest individuals in any location lose the female function and become functional males. These males maintain territories, each of which may hold one to seven smaller hermaphrodites that they defend against other males. They obtain high levels of mating success by pair spawning with all the hermaphrodites within the harem. Playing the role of females, the hermaphrodites obtain low levels of mating success; the other larger hermaphrodites within the harem may spawn by streaking. Males do not streak and the hermaphrodites except for the occasional pairing with streakers (10.3% frequency) prefer to release eggs in spawns without being disturbed by the streakers (Petersen, 1987).

Incidentally, the occurrence of directly developed functional primary males or secondary males derived from hermaphrodites in internally self-fertilizing *Rivulus marmoratus* following the loss of ovarian function has also been reported from the laboratory (Harrington, 1971) and natural population (Davis et al., 1990). Likewise, the presence of large males of serranines following the loss of ovarian function has also been reported. Despite the sustenance of numerous clones by *R.marmoratus* and ‘egg-trading’ by the serranines, these simultaneous hermaphrodites show a tendency to generate males. It is not clear whether these hermaphrodites are not able to withstand the selection pressure to evolve into gonochores.

### 6.3 Potential self-fertilizing hermaphroditism

From collections of muraenids Fishelson (1992) reported the presence of possible simultaneous hermaphroditism in *Siderea grisea* (Gulf Aqaba), *S.picta* (Maldives and Palau Islands) and *S. thyrzoidea* (Great Barrier Reef). All the gonads of *S.grisea* from the smallest (14 cm) to the largest (92 cm ) have ovarian and testicular portions extending alongside each other (Fig. 19). The ovarian parts look identical to the normal ovaries of the gonochoristic eels with long extensive lamellae on both sides of the gut. The testes are situated laterodorsally, merging with the ovaries on both sides of the gut but are separated from each other by dorsal suspension. They are whitish, compact organs densely criss-crossed by septa

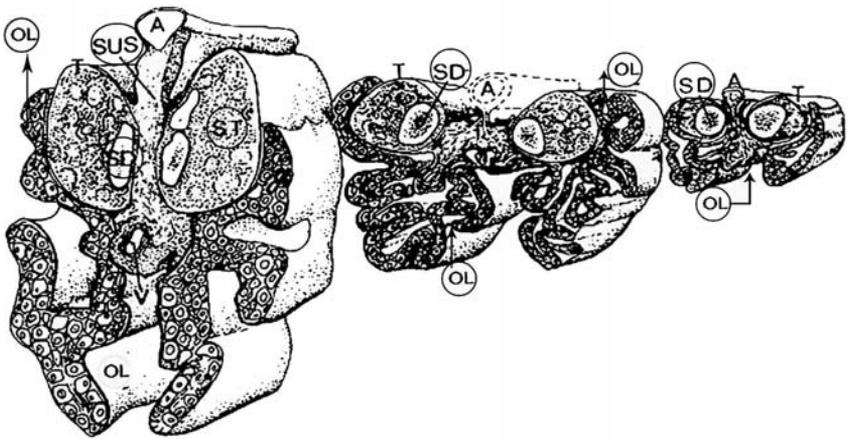


Fig. 19 Bisexual gonad of *Siderea grisea* (58 cm TL) as schematically viewed from the left side. Right, the most posterior, left, the most anterior. OL- ovarian lamella; T- testis; SUS- vein; A- artery; ST- spermatogonial tissue; SD-“sperm duct” (from Fishelson, 1992).

containing numerous sperm cysts. The ripe sperm and ova accumulate in the secondary 'sperm duct'. Both sperm and ova are released into the body cavity and are conducted towards the same genital opening on each side of the anus. The absence of gonadal ducts directly opening into the outside raises the possibilities of self-fertilization in these simultaneous hermaphrodites. Unlike the simultaneous hermaphroditic serranids, these muraenids have remained unattended by physiologists, endocrinologists, and behaviour scientists. For simultaneous hermaphrodites with a topographic separation between heterologous germinal tissues, there is no difficulty in their differentiation at an early gonad development, which leads to more or less simultaneous proliferation of testicular and ovarian elements. But it is for the endocrinologists to find out how the ovary and testis are able to co-exist and function side by side without mutual interference in these potential simultaneous hermaphrodites (see Reinboth, 1983).

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## Sequential Hermaphroditism

The evolution of sex change, as a life history strategic alternate to gonochorism, has been explained by the size advantage model (Ghiselin, 1969; Charnov, 1982). The model has been successful in explaining the adaptive significance of protogynous hermaphroditism in animals. If reproductive success increases with size or age more rapidly for one sex, an individual that changes sex in the appropriate direction will have a longer life of reproductive success (see also van Rooij and Videler, 1997) than that which does not change sex. If males gain in reproductive success with size or age faster than females, as in most cases, protogynous sex change is favoured; the opposite situation favours protandry. The model predicts protogyny, where large males monopolize mating. In most protogynous harem social systems, large males control access to females (Munoz and Warner, 2003).

Sequential hermaphroditism has been reported from at least 350 species belonging to 34 families (Nakazono and Kuwamura, 1987; see also Sakai et al., 2003; Sadovy de Mitcheson and Liu, 2008). Since then more species characterized by sequential hermaphroditism have also been reported. For example, Kusen et al. (1991) identified *Trichonotus filamentosus* to be protogynous and thereby added the Trichonotidae. Likewise, Fishelson (1992) reported the presence of nine muraenid species characterized by sequential hermaphroditism and thereby added not only species but also the family Muraenidae. Interestingly, Lo Nostro and Guerrero (1996) confirmed diandry by histological examination of the freshwater swamp eel *Synbranchus marmoratus* and added Synbranchidae to the list. However, except for *S.marmoratus*, almost all sequential hermaphrodites are marine and most of them occur on coral reefs. From the Enewetak Atoll, Marshall Islands alone 32 labrid species and 13 scarid species are reported (Colin and Bell, 1991). The density of scarids is 1,170 individuals equivalent to 276kg per hectare at the Aldabra, Indian Ocean (Robertson et al., 1979). A survey made on the Lizard Island, Great Barrier Reef indicated the presence of 64 labrid species within an area 13,500 m<sup>2</sup> at the density of 13 individuals per 100 m<sup>2</sup> (Green, 1996). By virtue of their abundance, the labrids and scarids have received much attention.

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## Protogynous Hermaphroditism

Due to plasticity of sexuality and flexibility in the mating system, the identification of sexuality among hermaphrodites is a highly complex issue: 1. Within a species, some females do not change sex at all (e.g. Scaridae: *Sparisoma radians*, Robertson and Warner, 1978; Sparidae: *Pagrus pagrus*, Kokokris et al., 1999; Serranidae: *Mycteroperca bonaci*, Crabtree and Bullock, 1998), 2. The presence of small males, which are not homologues of primary males in some species, suggests the development of secondary gonochorism. But their testes are derived from ovaries (e.g. Scaridae: *Sparisoma rubripinne*, Robertson and Warner, 1978; Serranidae: *Hypoplectrodes maccullochi*, Webb and Kingsford, 1992; *Paralabrax maculatofasciatus*, Hastings, 1989; Sparidae: *Pagrus pagrus*, Kokokris et al., 1999), 3. Within a species, some are harem owners, while others are group spawners (e.g. *Sparisoma cretense*, de Girolamo et al., 1999), 4. Within a species, there are single-male groups restricted to <3 m depth and others are multi-male groups residing at depths between 3 and 22 m (van Rooij et al., 1996), 5. Within a species, the mating system changes from place to place; *Halichoeres maculipinna* has a harem mating system in Florida but not in Panama; *H. garnoti* is suggested to be harem in Panama but is found not to be harem in Florida (Robertson, 1981), 6. Apart from these, there are also reports on the gonochores such as, the Nassau grouper *Epinephelus striatus* (Serranidae, Sadovy and Colin, 1995) and the Mediterranean wrasse *Symphodous* spp (Labridae), who are on the 'wings' to switch over to protogyny. For instance, Warner and Lejeune (1985) have recorded that 4% and 23 % males of *S. tinca* and *S. melanocercus* are secondary males, and 7. Variability in sexuality is further complicated by contradictory reports from several authors (see also Garratt, 1986; Moyer, 1990).

Histological examination of gonads remains a powerful method of determining whether a fish is a protogynous hermaphrodite or not (Sadovy and Shapiro, 1987). During sex change, ovarian tissues degenerate leaving only the ovarian wall, while the testicular tissues may develop from the former ovarian lamellae, or proliferate from the wall bordering the lumen radially or linearly, as in many gobiids (Cole and Shapiro, 1992). Hence,

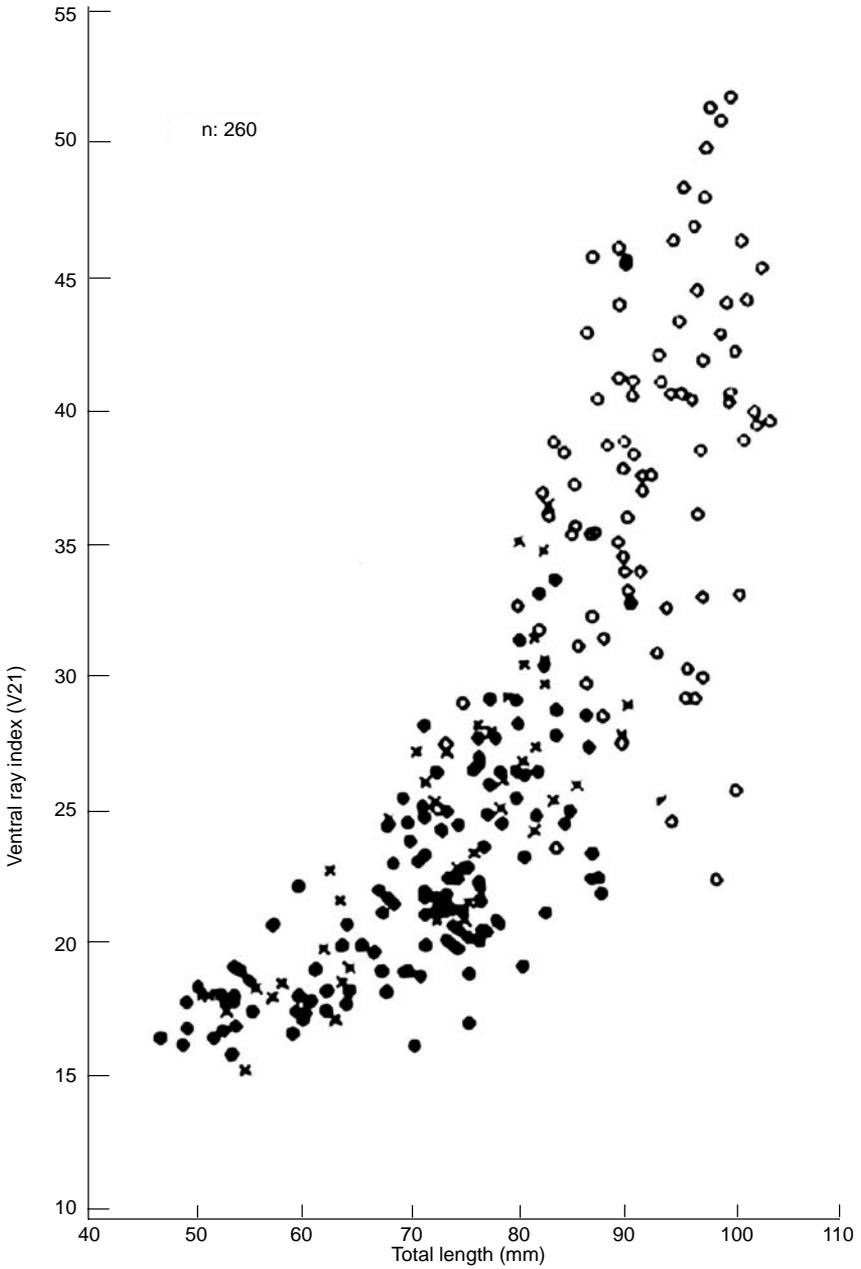
the secondary testes, transformed from the ovaries, possess the structure corresponding to the former ovarian wall and an ovarian lumen (Reinboth, 1962). For example, sex change has been histologically confirmed in many protogynous hermaphrodites like *Chrysoblephus puniceus* (Sparidae: Garratt, 1986), *Thalassoma cupido* (Labridae: Kobayashi, 1999; for more examples see Leem et al., 1998), *Lethrinus rubrioperculatus* (Lethrinidae: Ebisawa, 1997), *Neocirrhites armatus* (Cirrhitidae: Sadovy and Donaldson, 1995) and *Safflamen chrysopterus* (Balistidae: Takamota et al., 2003).

However, the absence of an ovarian lumen in the secondary testis has been described in *Sparisoma aurofrenatum* (Clavijo, 1982, cf Oldfield, 2005). In the gobiids too, the ovarian features are not retained in sex-changed testis beyond the newly transformed stage (Cole and Shapiro, 1992). But, a unique feature of the male gobiid's reproductive system is the presence of a paired, secretory accessory gonadal structure (AGS) associated with testis. At the time of sex change, it arises from the precursive tissue masses (pAGS) associated with the ventral portion of the ovarian wall in the region of the common genital sinus.

One or other morphological features may help to distinguish females from males and to identify the sex changing hermaphroditic transitionals, as well. The elongation of the second ventral fin ray indicates the sex change in Japanese peacock wrasse *Cirrhilabrus temmincki* (Fig. 20). In an attempt to quantify the elongation of the ray, Kobayashi and Suzuki (1990) developed the Ventral ray Index ( $V_2I$ ) based on  $V_2I = (V_2L/TL) \times 100$ , where  $V_2L$  is the length of second ventral ray and TL is the length of the fish. Of 260 specimens tested by them, 128 were females with the index of 15–33 (= 4.6–10.6 cm TL), 45 were transitional with 15–36 (= 5.1–9.4 cm TL) and 87 were males with 22–52 (= 7.4–10.5 cm TL). Thus they obtained a positive correlation between the second ventral fin ray and gonadal sex.

The Caribbean reef goby *Coryphopterus personatus* is known to develop an elongated second dorsal spine. From two of their collections from the Panama coast, Cole and Robertson (1988) used the elongated dorsal spine to identify 12% males (Table 19). As Cole and Robertson have not developed a quantitative index, as has been done by Kobayashi and Suzuki (1990), their estimate of males involved 6% errors.

In gobiids, the shape of the genital papilla may serve to distinguish a female from male. In females, the papilla is broad and oval with ridged margins surrounding the urinogenital opening but it is narrow and pointed in the male. Cole and Robertson subjected 379 specimens from 3 other collections to histological examination and the results were correlated with the shape of the genital papilla. Except for a single specimen, which was wrongly identified as male with genital papilla, the papilla served as the correct index of the sex in the goby.



**Fig. 20** Relationship between the index of the second ventral ray and total length in *Cirrhitlabrus temmincki*. Closed circle, female; open circle, male; cross marks, transitional fish (from Kobayashi and Suzuki, 1990).

**Table 19** Identification and confirmation of protogynous hermaphroditism in the Caribbean goby *Coryphopterus personatus* (from Cole and Robertson, 1988).

Sex marker	(No)		(Sex ratio)	
	♀	♂	♀	♂
Elongated dorsal spine	374	51	0.88	0.12
Genital papilla	379	85	0.82	0.18
Ovarian lumen	379	84	0.82	0.18

Apart from these structural markers indicating sex change, colour change can also be used as a biomarker. For instance, as the protogynous sex change advances in the Japanese wrasse *Pseudolabrus sieboldi*, the colour of the anal fin changes dramatically from yellow to red and is a useful visual biomarker showing the degree of sex change (Ohta et al., 2003).

The testes of primary males of the diandric swamp eel *Synbranchus marmoratus* consist of a paired organ joined medially by a connective tissue. In a cross section the testes are heart-shaped in immature individuals. Conversely, the testes of secondary males develop within a single ovary of a female during sex reversal. The appearance of the male tissue is concurrent with the replacement of the female tissue. Consequently, the anatomical shape of the gonad is very similar to that of the females except for the spermatic ducts (Fig. 2a, b 5a, 9, 10a Lo Nostro and Guerrero, 1996).

In primary males, spermatogenesis and spermiogenesis take place in spermatocysts. The sperm pass through efferent ducts, which extend the length of the gonads, fusing posteriorly and giving rise to the deferent duct, joins the urinary duct and thereby the urinogenital duct is formed. In secondary males, the lobular testes terminate at the efferent ducts, which follow one of the two ways. Those of the posterior region open directly into the urinary duct, while those of the anterior region lead into an intratesticular collector duct forming urinogenital duct (Fig. 6 & 12 of Lo Nostro and Guerrero, 1996).

### 8.1 Transitional load

The presence of hermaphroditic transitional(s), recognizable by the shape of the gonad (Fig. 21) and histological examination (Fig. 22), may prove the most reliable indicator of the protogynous hermaphroditism. Many authors have clearly quantified these transitionals, which range from 0.002% to 20.8% of their respective collections (Table 20). The fraction of transitionals, which may intrude into the breeding site (Shibuno et al., 1993a) and time is kept relatively low. Crabtree and Bullock (1998) alone have specifically mentioned that sex change in *Mycteroperca bonaci* occurs

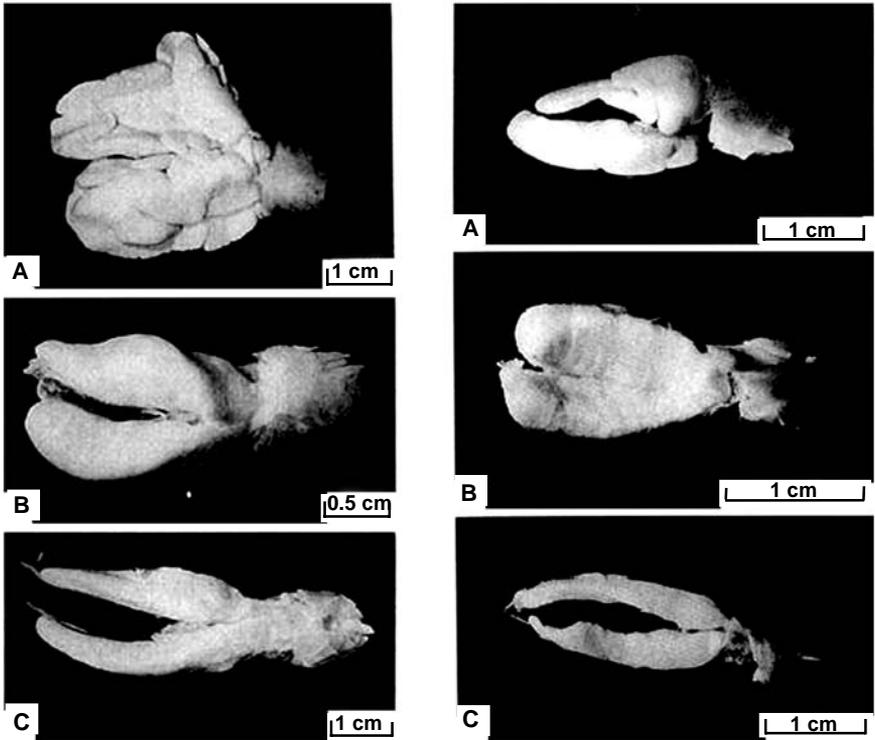
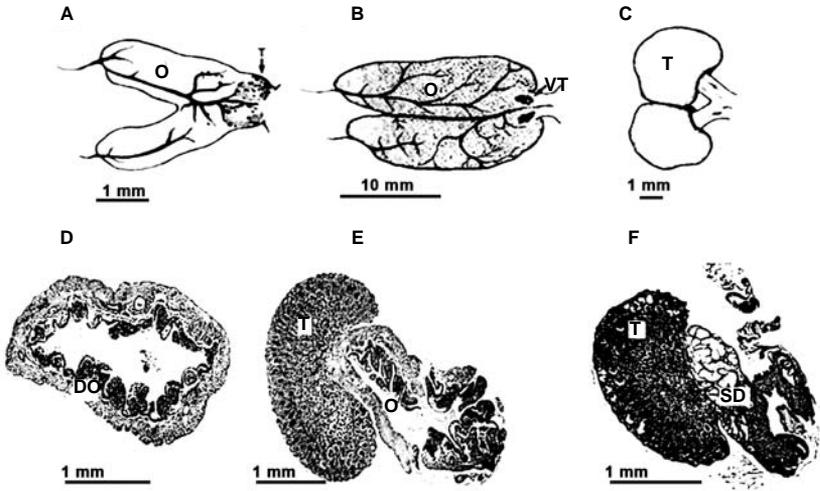


Fig. 21 Left panel: External appearance of the gonad in adult fish of *Halichoeres poecilopterus*. A: primary testis, B: ovary and C: secondary testis (from Kobayashi and Suzuki, 1994). Right panel: External appearance of the gonad in adult fish of *Suezichthys gracilis* from Suruga Bay. A: primary testis, B: ovary, C: secondary testis (from Kobayashi, 1999).

so quickly that it was not quantifiable. However, many others have chosen not to speak about it.

Apart from the sexually non-performing transitional load on the sexually active population of a protogynous hermaphrodite, the fraction of its life span, during which the transitionals persist in the population, should be an important consideration. On the basis of age, the transitional load lasts for only 3% life span of the black grouper *M. bonaci* (life span: 33 years; TL = 1.6 m) to 87% of its life span (10 years; TL = 11.5 cm) of the half-banded sea perch *Hypoplectrodes maccullochi* (Table 20). The values range from 9% to 47%, when body sized life span was considered. Incidentally, sex change is always more closely related to size than age (e.g. Jones, 1980). Hence size-based life span values may be considered more precise. It is not clear why the transitional load of the perch lasted for such a long period (87%) of its life span, though the examined 178 perches might have encountered more or less the same eco-social situation in the Jervis Bay,



**Fig. 22** Safflamen chrysopterus: Upper panel: Schematic representation of gonads of a juvenile with female coloration (A) a breeding female (B) and a breeding male (C). O. ovarian part; VT. Vestigial testicular tissue; T. testicular part. Lower panel: Gonadal structure of sex changed individual. D. ovarian part, DO : degenerated oocytes; E. Ovarian (O) part connected with testicular (T) part and F: Ovarian (O) part connected with testicular (T) part and sperm duct (SD) (from Takamoto et al., 2003).

Australia from January–November 1989. Also, why the load of the black grouper lasted only for a short period of 3% of its life span, although all the 888 groupers were captured from the Florida's Gulf coast from November 1994 to November 1996, a longer period, during which relatively more eco-social changes could have happened.

Notably, for every male, there was only 0.83 female in the sea perch (Webb and Kingsford, 1992) but there were as many as 15.4 female black groupers in Florida (Crabtree and Bullock, 1998), 30.3 in Cuba (Garcia-Cagidia and Garcia, 1996) and 76.6 in Mexico (Coleman et al., 1996), clearly indicating the intense fishing pressure on the large, long-living males. In the gag *M. microlepis*, the number of available females per male increased from 6 during 1977–80 to 50 during 1991–93 and in the scamp *M. phenax* from 5 during 1976–82 to 17 in 1995 (Matheson et al., 1986). In the brown marbled grouper *Epinephelus fuscoguttatus* at the Great Barrier Reef too there were eight females per male showing intense fishing pressure (Pears et al., 2007) as in the Gulf of Mexico. Hence size-specific and sex-specific fishing has induced earlier maturation at smaller size and thereby reduced individual fecundity and increased initial male ratio in the large, long living serranids (Jorgensen, 1990). Besides, such overexploitation may lead to inbreeding and ultimate collapse of the stock (Hoarau et al., 2005).

**Table 20** Percents of transitionals and life span of some protogynous hermaphrodites. Male ratio indicates the number of males available per female.

Species	Transitional (%)	Life span (%)	♂ ratio	Reference
<b>Serranidae</b>				
<i>Mycteroperca bonaci</i>	0.002	3 <sup>+</sup>	0.06	Crabtree and Bullock (1998)
<i>M. interstitialis</i>	1.5	32 <sup>+</sup>	0.37	Bullock and Murphy (1994)
<i>Epinephelus flavolimbatus</i>	0.004	31 <sup>+</sup>	0.37	Bullock et al. (1996)
<i>Hypoplectrodes maccullochi</i>	20.8	87 <sup>+</sup>	0.54	Webb and Kingsford (1992)
<i>Serranus baldwini</i> *	25	9	0.23	Petersen and Fischer (1986)
<b>Labridae</b>				
<i>Choerodon schoenleinii</i>	4.6	13	0.15	Ebisawa et al. (1995)
<i>Halichoeres poecilopterus</i>	14.3	36	0.52	Kobayashi and Suzuki (1994)
<i>Thalassoma bifasciatum</i>	13.3	17	0.17	Shapiro and Rasotto (1993)
<i>Suezichthys gracilis</i>	14.6	47	0.33	Kobayashi (1999)
<i>Cirrhilabrus temmincki</i>	17.3	41	0.39	Kobayashi and Suzuki (1990)
<b>Sparidae</b>				
<i>Chrysolephus puniceus</i>	13.5	28	0.36	Garratt (1986)
<b>Lethrinidae</b>				
<i>Lethrinus rubrioperculatus</i>	0.01	33	0.31	Ebisawa (1997)
<b>Gobiidae</b>				
<i>Gobiosoma multifasciatum</i> **	5.6	22	0.47	Cole and Justines (1982)
<i>Coryphopterus glaucofraenum</i>	10	-	0.27	Cole and Shapirio (1992)
<b>Pomacentridae</b>				
<i>Dascyllus reticulatus</i>	20	23	0.39	Schwarz and Smith (1990)
<b>Parapercidae</b>				
<i>Parapercis snyderi</i>	20	53	0.33	Kobayashi et al. (1993)
Mean	10.2	32.1	0.33	

\*\*Transitionals not histologically detected; + age based; \* Transitionals are transforming functional hermaphrodites.

The mean transitional load for 16 protogynous species belonging to 7 families is 10.4% and that for the percentage of life span, during which the transitionals persisted in the population is 31.6% at the mean male ratio level of 0.33. Despite wide scatter, the male ratio appears to have a direct relationship to the percentage of the life span, during which transitionals persisted (Fig. 23). Likewise, the male ratio also has a direct relationship to transitional load. Hence, these three components have a close relationship to each other. In fact, the male ratio appears to regulate the transitional load through the percentage of life span, during which transitionals persist.

Whereas the absence of large male (Inhibition hypothesis, Fishelson, 1970), the presence of smaller females (sex ratio hypothesis, Ross et al., 1983) or Encounter sex rate (Lutnesky, 1994) may be traced as the inductor of sex change in experimental harems, the sex ratio, more precisely the availability of male(s) per female appears to be (i) the prime inductor of sex change, (ii) the important factor for regulation of the transitional load and (iii) the period of life span during which transitionals persist in natural populations of fishes.

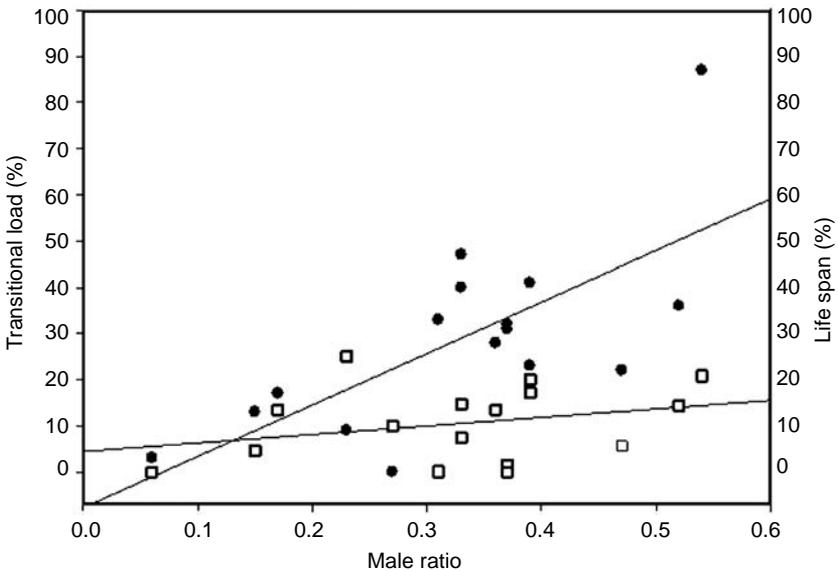


Fig. 23 Relationships between male ratio and transitional load ( $\square$ ) carried during the life spawn ( $\bullet$ ) of some protogynous hermaphrodites (data are drawn from Table 20).

## 8.2 Classifications

Sexual dichromatism occurs very commonly among the labrids (wrasses) and scarids (parrotfishes). The differences between the colour phases of the same species are often radical enough to have created confusion as to their taxonomic status (e.g. Rosenblatt and Hobson, 1969). "The sparisomatines show a bewildering array of colour patterns, often within a single species" (Robertson and Warner, 1978). The degree of sexual dimorphism and dichromatism displayed by some species has been used to recognize the patterns of sexual dichromatism in some tropical labrids (Robertson and Hoffman, 1977). In monochromatic species, females are capable of developing the same colour patterns as males (e.g. *Labroides dimidiatus*). In dichromatic species, females can not do so. There is a considerable range of variations in the degree and form, in which species are dichromatic. Two categories of dichromatism are distinguished on the basis of whether the chromatism covers the entire body or only partially. Both categories of dichromatic species may further be divided into two groups depending on the permanence of the terminal colouration: in temporarily dichromatic species, terminal phase fish can revert to the initial colour pattern, whereas permanently dichromatic species can not do so. Some haremnic pomacanthids, which may serve as good examples for temporary and permanent dichromatism are listed by Thresher (1982); for instance, *Centropyge heraldi*, *C. interruptus*, *C. shepardi*, *C. tibicen*, *Holacanthus ciliaris* and *Pomacanthus imperator* are permanently dichromatic haremnic pomacanthids, while *Centropyge argi*, *C. bicolor*, *C. flavissimus*, *C. nox* and *C. potteri* are temporarily dichromatic pomacanthids.

Among the permanently dichromatic species, the same elements are common to both phases but some are much more strongly developed (e.g. *Halichoeres bivittatus*, *Sparisoma rubripinne*) than in the others (e.g. *Thalassoma hebraicum*; *Halichoeres pictus*, ♂ only). The haremnic labrids are known to have the largest number of species displaying five different combinations of morphism and chromatism; The following are examples for them: 1. Fully dichromatic, dimorphic haremnic labrids: *Anampses caeruleopunctatus*, *Cheilus unifasciatus*, *Ebibulus insidiator*, *Halichoeres marginatus*, *H. melanurus*, *Labrichthys unilineatus* and *Macropharyngodon meleagris*, 2. Partially dichromatic, dimorphic haremnic labrids: *Anampses twistii*, *Cheilinus oxycephalus*, *C. trilobatus* and *Halichoeres hortulanus*, 3. Monochromatic, dimorphic haremnic labrid: *Pseudocheilinus hexataemia*, 4. Monochromatic monomorphic haremnic labrids: *Labroides bicolor* and *L. dimidiatus*, and 5. Partially dichromatic monomorphic haremnic labrid: *Helichoeres bicellatus* (Colin and Bell, 1991).

Two genotypic males have been reported from a number of labrids (Warner and Robertson, 1978) and scarids (Robertson and Warner, 1978)

and the phenomenon is referred to as diandry (Reinboth, 1967). The secondary males are derived from sex changed females; their testes are clefted or triangular in shape and possess the remnants of the ovarian cavity, its wall and vas deferens (Fig. 24); the sperm duct of the secondary male arises secondarily along the periphery of the gonad and surrounds the persisting central ovarian lumen (Kobayashi and Suzuki, 1990). Primary males are those that are hatched out as male without prior existence of a female phase. They have large lobate testes (Lo Nostro and Guerrero, 1996), and a simple sperm duct with no trace of any previous occurrence of ovarian duct or its remnants. Many protogynous species possess only secondary males and are called monandric protogynous hermaphrodites (Reinboth, 1967). It should be mentioned that among the scarids, primary males are absent in the subfamily Scarinae but are present in the Sparisomatinae. Prematurational sex change is not uncommon among the Sparisomatinae. Prematurely sex changed males are analogous to primary males but are not homologous to them, as their testes are definitely derived from the ovaries (Robertson and Warner, 1978). Secondly, primary males are also absent among the harem-forming labroids, except in such species, in which a major fraction of the populations is harem, while others are group spawners (see de Girolamo et al., 1999) and in such species, which may be harem in one habitat but not in others (e.g. *Halichoeres maculipinna*, Robertson, 1981).

### 8.3 Pathways and duration of sex change

Basically, there are two ontogenetic pathways through which protogynous hermaphroditic gonad may develop into one or the other sex. In the regular model, the initial immature ovary differentiates into a female, which after one or more spawnings, changes sex to become a secondary male, after passing through a short or long transitory hermaphroditic phase (Fig. 25). However, in the '*Pagrus*' model, the immature ovary may opt to pass through one of the three alternate pathways: a) It may mature into a female and continue as female until death, b) The mature female, after one or more spawnings, may change sex and become a secondary male, after a short or longer (e.g. *Pagrus*) transitory hermaphroditic period and c) The immature ovary may undergo prematurational sex change and still become a secondary male. Many sparids and serranids follow the *Pagrus* model. Sex change in the red porgy *Pagrus pagrus* might be a prolonged process that it is not necessarily completed during one reproductive cycle (Kokokris et al., 1999). However, maintenance of fish under high temperature and unlimited food supply may accelerate gonadal development and precocious sex change (Zohar et al., 1978). Notably, feeding post-larvae of the protandrous shrimp *Hippolyte inermis* on a diet containing benthic

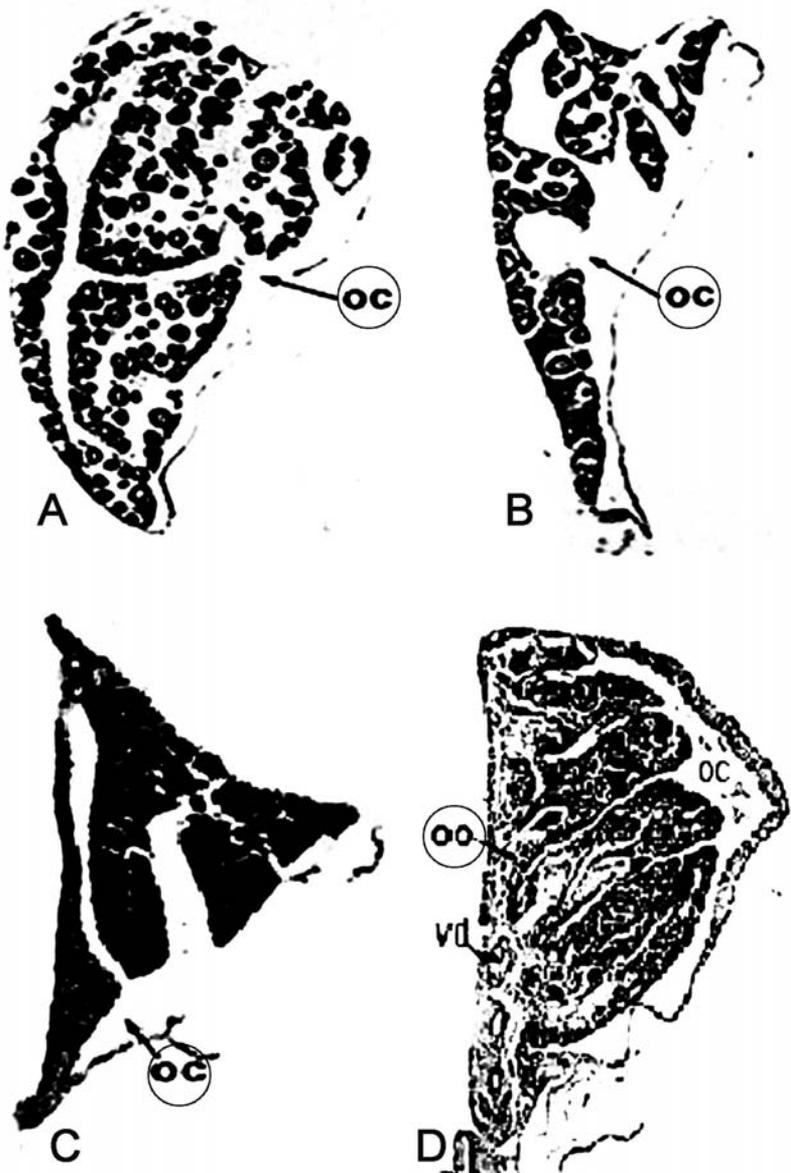


Fig. 24 *Cirrhilabrus temmincki*: A→B→C sex changing gonad from ovary to testis. Note the change in the shape of the gonad OC= ovarian cavity. D. Early secondary testis. Note the (vd) vas deferens (from Kobayashi and Suzuki, 1990).

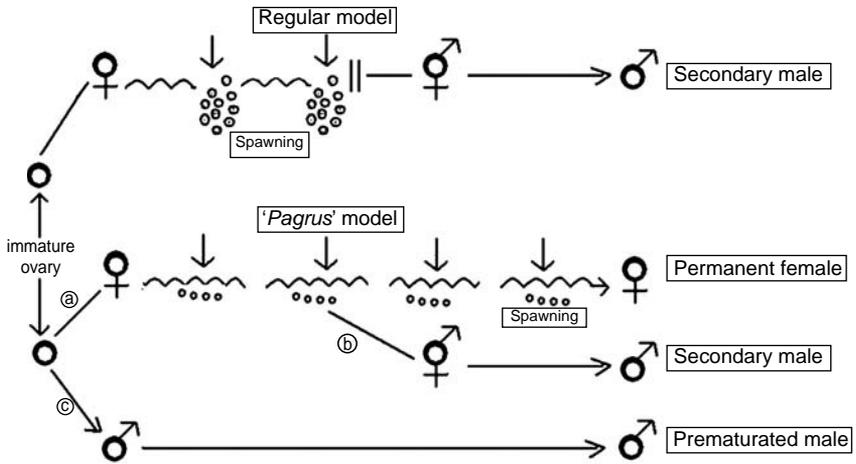


Fig. 25 Ontogenetic pathways of sexual development in protogynous hermaphrodites.

diatom *Cocconeis neothumensis* resulted in the production of 61% females, instead of the normal 5% females (Zupo, 2000).

With experimental removal of the territorial male, the sex changing female may initiate behavioural and gonadal changes associated with sex change. In harem species, the need for a harem master from a sex changing female is perhaps more urgent for continued maintenance of the harem. Apparently, the potential female begins the courtship display within hours after the disappearance of the territorial male, although the potential females of *Bodianus rufus*, *Halichoeres melanurus* and *Centropyge vroliki* require a period of rest prior to initiation of such courtship display (Sakai et al., 2003). In the harem and non-harem protogynous species, a minimum period of 7–25 days is required to initiate and complete the sex change (Table 21). This short period of 7–25 days is required for an individual under experimental conditions, when a male is absent. The period required is longer than 2 years for 50% of females to complete the sex change in a natural population of *Mycteroperca bonaci*, in which a quick sex change is known to occur (Crabtree and Bullock, 1998).

When the removed territorial male of *Labroides dimidiatus* was returned to the same harem after the potential female has already spawned with its other female members, there is no agnostic interaction between the former and the latter. Evidently, the potential female, which had not yet undergone gonadal sex change, becomes submissive to her original master (Nakashima et al., 2000). In *Centropyge vroliki*, the potential female commences spawning even on the very first day after the removal of the male, but can fertilize the eggs only 10–16 days after removal of the male (Sakai et al., 2003).

**Table 21** Time required for completion of gonadal sex change in protogynous fishes.

Species	Gonadal sex change (days)	Heramic	Reference
<b>Labridae</b>			
<i>Bodianus rufus</i>	7–10	Yes	Hoffman <i>et al.</i> (1985)
<i>Labroides dimidiatus</i>	17	Yes	Nakashima <i>et al.</i> (2000)
<i>Halichoeres melanurus</i>	14–21	Yes	Sakai <i>et al.</i> (2002)
<b>Scaridae</b>			
<i>Sparisoma radians</i>	12–18	Yes	Munoz and Warner (2003)
<b>Pomacanthidae</b>			
<i>Genicanthus watanabei</i>	25	Yes	Hioki <i>et al.</i> (1995)
<i>Apolemichthys trimaculatus</i>	25	Yes	Hioki and Susuki (1995)
<i>Centropyge vroliki</i>	10–16	Yes	Sakai <i>et al.</i> (2003)
<i>C. fisheri</i>	6	Yes	Hioki and Susuki (1996)
<i>C. acanthrops</i>	8	Yes	Aldenhoven (1984)
<i>C. bicolor</i>	<20	Yes	Sakai <i>et al.</i> (2003)
<b>Gobiidae</b>			
<i>Coryphopterus personatus</i>	20	No	Cole and Robertson (1988)
<i>C. glaucofraenum</i>	20	No	Cole and Shapiro (1992)
<i>Lythrypnus zebra</i>	14	No	St Mary (1996)

The chronological sequence of events during the post-male removal period of the bucktooth parrotfish *Sparisoma radians* has been precisely described by Munoz and Warner (2003). Following the removal of the male, the transitional colouration appeared on the 9th day, spawning began from the 10th day, patrolling of harem territory commenced from the 7–14th days and eggs were successfully fertilized from the 12–18th days. Fertilization success gradually improved to 80% on the 18th day; the sperm count of the nascent male was 19 million per spawning. Although the original harem master achieved 80% fertilization success with just 8 million sperm per spawning, the nascent harem master was unable to achieve even 50% fertilization success with the same 8 million sperm/spawning, as he has not yet 'learned' the art of coordinating the harem and spawning. Evidently, the nascent male may require a longer period to consolidate the harem and achieve regular effective spawning with the female members of his harem.

## 8.4 Sex ratio

Due to natural mortality, predation and fishing, female-biased sex ratio is to be expected among the protogynous hermaphrodites. The delay or resistance to sex change by females in some protogynous species may skew the ratio more in favour of females. The protogynous species are tentatively divided into five groups to understand the impact of dichromatism, diandry and social mating systems on sex ratio.

a) *Monandric monochromatic protogynous hermaphroditism*: Table 22 lists available data on the ontogenetic increase in male ratio. At the age or size, at which males begin to appear, due to sex changing females, the male ratio ranges from 0.01 to 0.33. In general, this value remains high for the serranids (mean 0.18), whereas in other families, it remains low (0.03), except for the labrid *Thalassoma cupido*. With increasing age/size, the number of sex changers progressively increased (Fig. 25). Consequently, the final male ratio increases to 0.06–0.54. The mean male ratio for the 15–18 species belonging to 6 families is 0.31, i.e. for every male; there are 2.23 females among the monandric monochromatic protogynous hermaphrodites. Hence, this type of hermaphroditism appears to have depressed the male ratio from 0.5 in gonochores to 0.31. Incidentally, the very high initial male ratio (0.18) observed for the serranids reflects the intense fishing pressure on these large, long living fishes.

**Table 22** Ontogenetic increase of male ratio in monochromatic monandric protogynous hermaphroditic fishes.

<i>Species</i>	Increase in ♂ ratio		Reference
	from	to	
<b>Serranidae</b>			
<i>Epinephelus flavolimbatus</i>	0.20	0.37	Bullock <i>et al.</i> (1996)
<i>E. akaara</i>	0.18	0.33	Okumara (2001)
<i>Mycteroperca interstitialis</i>	0.13	0.37	Bullock and Murphy (1994)
<i>M. bonaci</i>		0.06	Crabtree and Bullock (1998)
<i>Hypoplectrodes maccullochi</i>	0.20	0.54	Webb and Kingsford (1992)
<i>Serranus baldwini</i> *	0.05	0.30	Petersen and Fischer (1986)
<b>Labridae</b>			
<i>Choerodon schoenleinii</i>	0.01	0.15	Ebisawa <i>et al.</i> (1995)
<i>Clepticus parrae</i>	0.04	0.30	Warner and Robertson (1978)
<i>Cirrhilabrus temmincki</i>	0.01	0.39	Kobayashi and Suzuki (1990)
<i>Thalassoma cupido</i>	0.33	0.38	Kobayashi (1999)
<b>Sparidae</b>			
<i>Chrysoblephus puniceus</i>	0.01	0.36	Garratt (1986)
<i>Pagrus pagrus</i>	0.04	0.25	Kokokris <i>et al.</i> (1999)
<i>P. auriga</i>	0.02	0.11	Pajuelo <i>et al.</i> (2006b)
<b>Gobiidae</b>			
<i>Gobiosoma multifasciatum</i>	0.02	0.47	Cole and Justines (1982)
<i>Coryphopterus personatus</i>	0.11	0.39	Cole and Justines (1982)
<i>C. glaucofraenatum</i>		0.27	Cole and Shapiro (1992)
<b>Lethrinidae</b>			
<i>Lethrinus rubrioperculatus</i>	0.02	0.31	Ebisawa (1997)
<b>Pomacentridae</b>			
<i>Dascyllus reticulatus</i>	–	0.23	Schwarz and Smith (1990)
Mean	0.09	0.31	

\* hermaphrodite to male

Incidentally, Young and Martin (1982) reported basic data on the number of males and females of five lethrinid species collected from the North West Shelf of Australia and Gulf of Carpentaria, from which the estimated mean male ratio increases from 0.17 to 0.20 in *Lethrinus nematocanthus*, *L.choerorhynchus*, and *L. variegatus* sample during May-June 1979 and *L.lentjan* and *L.fraenatus* collected during December 1980. However, the ratio for samples collected for the first four species during other seasons was around 0.49. No reason is suggested for the observed wide variations. Similarly, the male ratio of Nassau grouper *Epinephelus striatus* is shown to increase from 0.31 with in the body size of 42–59 cm to 0.61 in the larger groupers of 51–55 cm. The grouper is considered essentially as a gonochore with potential for sex change (Sadovy and Colin, 1995).

*b) Monandric dichromatic protogynous hermaphroditism:* According to Colin and Bell (1991), 17 of 45 labroids inhabiting Enewetak Atoll are dichromatic with a harem mating system. Hence dichromatism appears to be prevalent among monandric and harem protogynous species. Dichromatism, especially conspicuous colouration of the terminal phase (TP) males has further depressed the male ratio to 0.30 (Table 23). Hence for every male, there are 2.57 females. However, the initial phase (IP) males of many scarids are known to have 2–5 times higher gonado-somatic index than their respective TP males (Robertson and Warner, 1978); the higher GSI of IP males may partially compensate the reduction in the number of males. The ratio between the IP: TP males remained as 0.80 : 0.20 for the monandric dichromatic progynous hermaphrodites (Table 23).

The male ratio increases with increasing age or size of labroid fishes: 1. No larger females appear after the age of 18 years in *Mycteroperca interstitialis*, although its life span lasts upto the age of 28 years (Fig. 26); a similar situation is observed in others, except in *Sparisoma rubripinne*, in which females persist almost upto the terminal end. In *M. interstitialis* and *Scarus croicensis*, males begin to appear almost immediately following the

**Table 23** Male ratio and ratio between Initial (IP) and Terminal (TP) colour phases of some dichromatic and monandric protogynous hermaphrodites.

Species	♂ Ratio	Ratio IP : TP	Reference
Labridae			
<i>Pseudolabrus celidotus</i>	0.30	0.83 : 0.17	Jones (1980)
Scaridae			
<i>Sparisoma chrysopternum</i>	0.26	0.80 : 0.20	Robertson and Warner (1978)
<i>S. viride</i>	0.25	0.80 : 0.20	Robertson and Warner (1978)
<i>S. rubripinne</i>	0.33	0.75 : 0.25	Robertson and Warner (1978)
Mean	0.30	0.80 : 0.20	

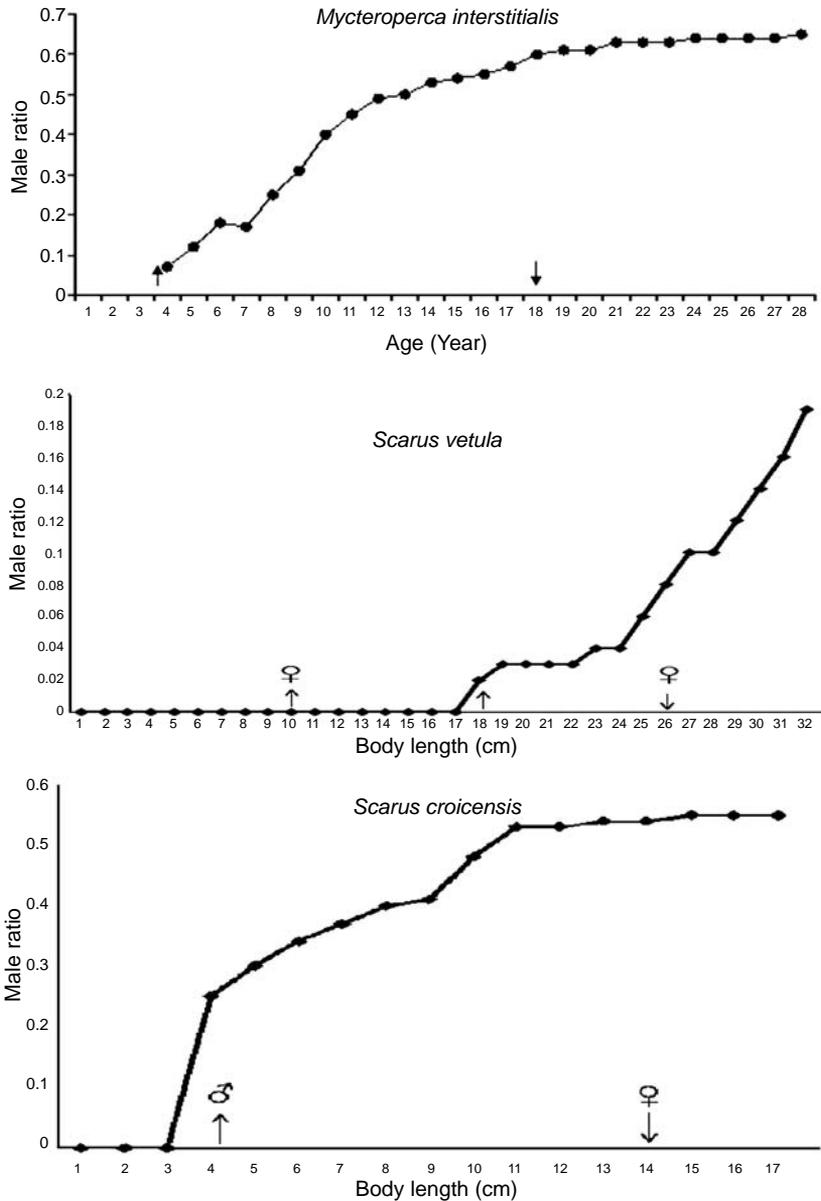


Fig. 26 Ontogenetic increase in male ratio with increasing age/body size in selected fishes. The upward arrows indicate the appearance of males and the downward arrows indicate the non-appearance of larger females beyond that age/size (Bullock and Murphy, 1994; Robertson and Warner, 1978).

age or size, at which mature females are found, indicating the commencement of earlier sex change. But in *S. vetula* males begin to appear at the size of 18 cm body length, while mature females are found from body size of 10 cm. Not only is there a delayed sex change in *S. vetula*, but also its male ratio does not exceed 0.2 even at the largest body size of 32 cm. However the ratio is more than 0.5 in the monandric *M. interstitialis* and diandric *S. croicensis*. With diandry, the male ratio rapidly increases to 0.5 even at the relatively smaller body size of 11 cm.

*c) Diandric dichromatic protogynous hermaphroditism:* Diandric protogynism appears to be not so common among labrids and scarids. For instance, 500 species belonging to 57 genera are recognized in Labridae (Donaldson, 1995a). In 1985, Nemtsov indicated that the number of diandric protogynous labrid species was only 10. Very high ratios are noted for the IP (0.26) and TP (0.27) males of *Scarus croicensis*. They have in turn, reduced the female ratio to an unusual value of less than 0.5. The mean ratio for IP males of the other 9 species belonging to Labridae (7 species) and Scaridae (2 species) doubles from 0.05 during the initial stage, when males began to appear, to 0.08 at the final stage (Table 24). Clearly, there is continuous generation of IP (primary) males. Figure 27 shows the occurrence and ontogenetic increases in primary and secondary males of selected diandric protogynous hermaphrodites. Secondly, the TP male ratio increases from 0.04 to 0.15, i.e. a 4-folded increase, as more and more females change sex, which reduces the ♀ ratio from 0.92 during the initial stage to 0.77

**Table 24** Ontogenetic increase in male ratio in diandric protogynous hermaphroditic fishes. Early stage: size at which IP male began to appear; Final stage: a size beyond which no larger female is found. IP: Initial phase ♂; TP: Terminal phase ♂.

Species	Early stage				Final stage			
	♀	IP	TP ♂	IP + TP	♀	IP	TP ♂	IP + TP
<b>Scaridae</b>								
<i>Scarus croicensis</i> <sup>4</sup>	0.77	0.22	0.01	0.23	0.47	0.26	0.27	0.53
<i>S. vetula</i> <sup>4</sup>	0.98	0.02	–	0.02	0.75	0.14	0.11	0.25
<i>Calomotus spinidens</i> <sup>5</sup>	0.88	0.09	0.03	0.12	0.80	0.06	0.14	0.20
<b>Labridae</b>								
<i>Suezichthys gracilis</i> <sup>2</sup>	0.97	0.01	0.02	0.03	0.83	0.05	0.12	0.17
<i>Thalassoma bifasciatum</i> <sup>5</sup>	0.94	0.05	0.01	0.06	0.82	0.06	0.12	0.18
<i>Halichoeres marginatus</i> <sup>1</sup>	0.96	0.04	–	0.04	0.70	0.03	0.27	0.30
<i>H. pictus</i> <sup>3</sup>	0.92	0.07	0.01	0.08	0.76	0.12	0.12	0.24
<i>H. poeyi</i> <sup>3</sup>	0.79	0.07	0.13	0.20	0.77	0.09	0.14	0.23
<i>H. maculipinna</i> <sup>3</sup>	0.87	0.08	0.05	0.13	0.68	0.11	0.21	0.33
<i>H. bivittatus</i> <sup>3</sup>	0.98	0.01	0.01	0.02	0.83	0.05	0.12	0.17
Mean	0.92	0.05	0.04	0.09	0.77	0.08	0.15	0.23

1. Shibuno *et al.* (1993b); 2. Kobayashi (1999), 3. Warner and Robertson (1978), 4. Robertson and Warner (1978); 5. Robertson *et al.* (1982)

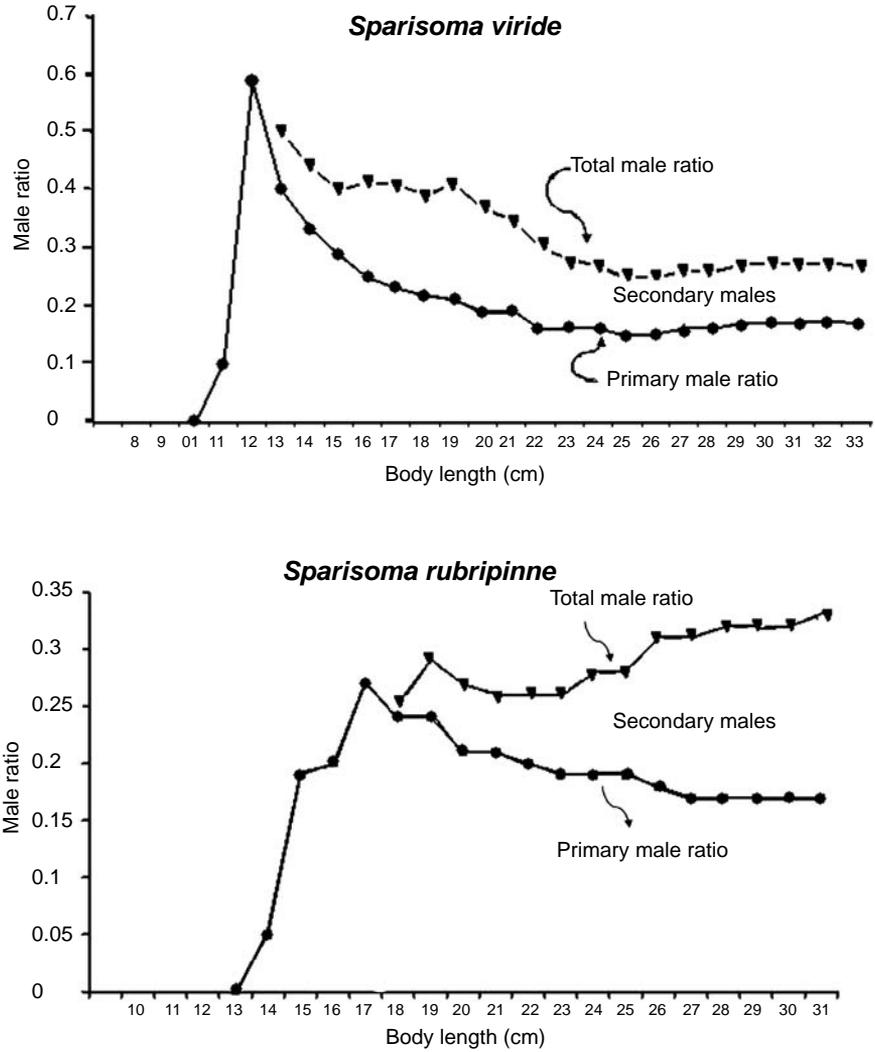


Fig. 27 Ontogenetic increase in male ratio in selected diandric sequential hermaphrodites (from Robertson and Warner, 1978).

during the final stage. Hence diandric dichromatic protogynous hermaphroditism has further depressed the male ratio to 0.23, i.e. for every male there are 3.34 females. At the final stage, the IP: TP male ratio is 0.35:0.65 and it may be compared to that (0.80:0.20) for the monandric dichromatic protogynous hermaphrodites.

Notably, the diandric hermaphrodites like the rock wrasse *Halichoeres semicinctus* with a dualistic pair and group spawning strategy has a sex ratio of  $0.36\text{♀} : 0.64\text{♂}$  and IP : TP male ratio of  $0.61 : 0.39$  (Adreani and Allen, 2008), which are quite opposite to those arrived from limited data available for 9 species, i.e. sex ratio  $0.77\text{♀} : 0.23\text{♂}$  and IP  $0.35 : TP 0.65$ . However, histological examination of the rock wrasse suggested a sex ratio of  $0.5 : 0.5$ . Although Adreani and Allen state that the majority of rock wrasse were group-spawners, they have not quantified them. Secondly they even suggest that the rock wrasse may represent an intermediate between gonochore and protogynous hermaphrodite. From their observations on 269 spawnings of the Japanese wrasse *H. marginatus* (see Table 24), Shibuno et al. (1993b) found that 5% of the wrasse alone opted for group spawning. Understandably, among the diandric hermaphrodites characterized by the dualistic mating system, sex ratio appears to be linked to the percentage of population opting for the pair, group spawning or dualistic mating system. Incidentally, the IP:TP male ratio reported by Robertson and Warner (1978) for the monandric dichromatic scarids *Sparisoma viride*, *S. rubripinne*, *S. chrysopternum* and *S. aurofrenatum* is  $0.78 : 0.22$ , a value closer to that reported in Table 24. But the IP : TP ratio reported by them for *S. radians* is  $0.6 : 0.4$ ; however, they have also noted that of 62 spawnings, 44% were by group-spawners; apparently, the increase in percentage of group-spawners raises the TP ratio to  $0.4\text{--}0.5$ .

d) *Social organizations and bachelors*: Many non-haremic sequential hermaphrodites, especially the scarids, cirrhitids, and pomacanthids display a very diverse array of social organizations and reproductive patterns, often within a single species. For instance, the mating system of the coral-dwelling damselfish *Dascyllus marginalis* varies from solitary males to pairs, harems and promiscuous groups, depending on the coral colony size (Fricke, 1980). Many species of hawkfish live in a kind of loose social organization. In *Cirrhitichthys falco*, groups of (i) one male with 5–7 females and (ii) one male, one sneaker male, two juveniles and seven females have been described. One male of *C. aprinus* is known to dwell with two females in Nyake-jima, Japan, four females in Papua New Guinea and seven females at Great Barrier Reef (Donaldson, 1987). When threatened of its territory, the male *C. falco* responded aggressively herding the females towards a particular spawning site. The social group of *Dascyllus reticulatus* was comprised of 1–2 resident males and 3–5 females (Godwin, 1995). Moyer (1990) lists the social organization and sexual dichromatism in selected pomacanthid angelfishes (Table 25).

The adults of *Scarus iserti* and *Sparisoma* live either as multi-male groups or in one-male groups, indicating considerable flexibility in sex ratio (de Girolamo et al., 1999). Parrotfishes display a very diverse array of

**Table 25** Social organization, sexual dichromatism among the protogynous hermaphroditic dimorphic pomacanthids (from Moyer, 1990).

Species	Social organization		Sexual chromatism	
	♂ + ♀♀	♂♂ + ♀♀	Permanent	Temporary
<i>Centropyge bicolor</i>	+		no	yes
<i>C. ferrugatus</i>	+		yes	
<i>C. interruptus</i>	+		yes	no
<i>Holacanthus tricolor</i>	+		yes	no
<i>C. bispinosus</i>	+		yes	
<i>C. multispinis</i>	+		yes	
<i>C. shepardii</i>	+		yes	
<i>C. tibicen</i>	+		yes	
<i>C. vroliki</i>	+		no	yes
<i>Genicanthus caudovittatus</i>		+	yes	no
<i>G. lamarck</i>		+	yes	no
<i>G. melanospilos</i>		+	yes	no
<i>G. personatus</i>	?	?	yes	no
<i>G. semifasciatus</i>		+	yes	no

social organizations and reproductive patterns. In *S. viride*, the described two social units have marked differences in vertical distribution. Within each of these groups, three behavioural classes have been recognized (van Rooij et al., 1996).

In these social organizations, home range and territoriality are not major constraints among the colonial members co-inhabiting with more than one species in the same coral. For instance, the goby *Gobiodon histrio* is known to occur as a small discrete colony consisting of a pair of juveniles or a pair of heterosexuals with a juvenile in the corals of *Acropora nasuta* at the Lizard Island on the northern Great Barrier Reef. In a coral, a heterosexual pair occupies a space of about 20 cm (arrived by dividing the [length + width + height of the coral] by 3) diameter and in general co-inhabits with other gobiids (Munday et al., 1998). Out of 11 *Acropora* species used by *Gobiodon* spp, each species occupies 1 or 2 species of *Acropora* and the occupation frequency is about 75% of the available corals (Munday et al., 1997).

Growth rate of territorial males in *Sparisoma viride* is negatively correlated with their spawning frequency (van Rooij et al., 1995). The sexually inactive 'Bachelor' (leaves the harem; e.g. *Centropyge ferrugatus*, Sakai, 1997), trades growth against current reproduction and thereby enhances its chances to acquire the status of TP. The IP males of *S. croicensis*, for instance, adopt this 'bachelor strategy' in the hope of eventually acquiring TP status and occupying a territory (Warner and Robertson, 1978). Non-harem social organizations and bachelor males may also considerably alter the sex ratio of the protogynous hermaphrodites. This brief shows the need for more research in this area and quantitative information.

e) *Protogynous hermaphroditic harems*: The size advantage model predicts that protogyny will selectively be favoured in species with harem mating system, where males monopolize mating success and sex change is under social control (Fishelson, 1970, Warner and Swearer, 1991). In harem species, the male defends his all-purpose-territory against other males, and females, lives and mates with the females in his territory. Hence the size of the male's territory may have a positive correlation with the number of females held by the male in his harem. For instance, the pomacanthid *Chaetodontoplus mesoleucus*, a territorial male holds only one female in his harem, but two females, when his territory area exceeds 375 m<sup>2</sup> (Moyer, 1990).

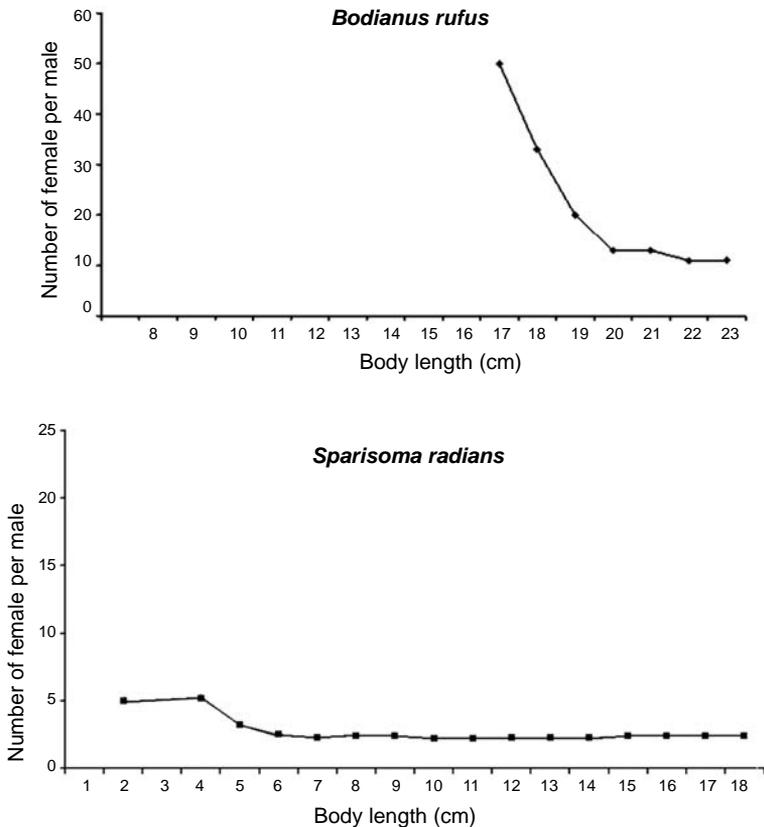
In general, the males may hold mostly 3–4 females but may also hold upto 10 females in his harem (Table 26). Consequently, the male ratio is

**Table 26** Number of females and male sex ratio in protogynous hermaphroditic harem fishes.

Species	Male sex ratio	Females (no/harem)	References
<b>Labridae</b>			
<i>Labroides dimidiatus</i>	0.26	2.9	Sakai and Kohda (2001)
<i>L. dimidiatus</i>	0.14	6	Sakai <i>et al.</i> (2001)
<i>Xyrichtys martiniensis</i>	0.22	3–6	Biard (1988)
<i>X. pentadactylus</i>	0.20	1–7	Nemtsov (1985)
<b>Scaridae</b>			
<i>Scarus vetula</i>	0.15		Robertson and Warner (1978)
<i>Sparisoma cretense</i>	0.25	3	de Girolamo <i>et al.</i> (1999)
<i>S. aurofrenatum</i>	0.20		Robertson and Warner (1978)
<i>S. atomarium</i>	0.27		Robertson and Warner (1978)
<i>S. radians</i> <sup>2</sup>	0.23		Robertson and Warner (1978)
<i>Cryptotomus roseus</i> <sup>2</sup>	0.27		Robertson and Warner (1978)
<b>Serranidae</b>			
<i>Cephalopholis spilopare</i>	0.22	1–6	Donaldson (1995b)
<i>Serranus fasciatus</i>	0.18	1–8 <sup>+</sup>	Petersen (1987)
<i>S. baldwini</i>	0.20	1–7 <sup>+</sup>	Petersen and Fischer (1986)
<b>Pomacanthidae</b>			
<i>Chaetodontoplus mesoleucus</i>	0.40	1–2	Moyer (1990)
<i>Centropyge ferrugatus</i>	0.25	3	Sakai and Kohda (1997)
<i>Pomacanthus imperator</i>	0.22	2–5	Thresher (1982)
<b>Chaetontidae</b>			
<i>Chaetodon trifasciatus</i>	0.29	2–3	Yabuta and Kawashima (1997)
<i>C. fromblii</i>	0.20	4	Hourigan (1986)
<i>Pyglopites diacanthus</i>	0.17	5	Gronell and Colin (1985)
<b>Parapercidae</b>			
<i>Parapercis snyderi</i>	0.13	3–10	Kobayashi <i>et al</i> (1993)
Mean	0.22	3.55	

+ Hermaphrodites; 2: Limited to a segment of population during breeding

reduced to 0.22 in 19 harem species belonging 6 families; i.e. every harem master may effectively hold 4–5 females in his harem. The origin of the harem mating system has reduced the male ratio to almost the lowest level. Incidentally, gonochoristic males are known to be at their maximum reproductive fitness with 2–3 females. Apparently, a harem with a larger (than 3–5) number of females may become a ‘permeable’ (Ross, 1990) social unit. Figure 28 shows that with protogynous hermaphroditism, the number of females available per male decreases with increasing body size both in labrids and scarids. Incidence of extraharem spawning by harem females has been reported (e.g. *Centropyge vroliki*, Sakai et al., 2003). It ranges from 2.6% to 10.0% of the total spawning of the females of the cleaner wrasse *Labroides dimidiatus* (Sakai et al., 2001). In such cases, the harem master may even penalize the female’s infidelity



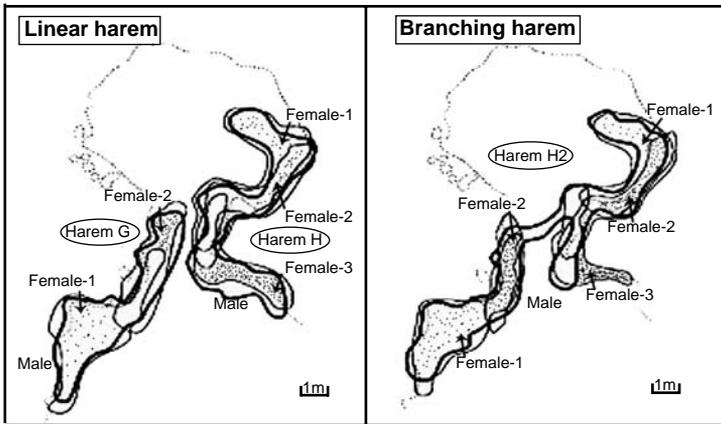
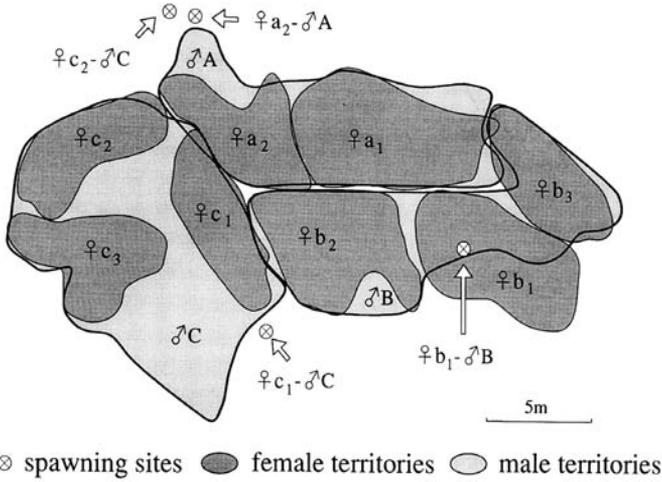
**Fig. 28** Decreased availability of females per male with increasing body size in a labrid and scarid (calculated from Warner and Robertson, 1978; Robertson and Warner, 1978).

by reducing her chances of changing sex and by achieving maximum spawning success with her; the expelled female from one harem may not readily be accepted in another (Robertson and Hoffman, 1977). Sakai et al. (2001) reported that 50% of extraharem visiting females of *Labroides dimidiatus* may choose to stay with a selected new harem, but on being accepted, their spawning frequency is reduced to 75% and fecundity to 69% for the first 57 days.

The size of the male's territory may impose a spatial constraint on the composition of the social group; consequently, it may have implications to the number of females in the harem and hence on sex ratio and/or on the spatial relationship among the females. The mean size of the 'exclusive' (Yabuta and Kawashima, 1997) territories of the male *Chaetodon trifasciatus* is 73 m<sup>2</sup> and each of the 3 females within the harem has a home range of 21 m<sup>2</sup>. Hence there is no overlap of home ranges of the females within the overall territory of the male (Fig. 29). When there is an overlap among the home ranges of the female members of the harem, the home ranges of similar sized females do not overlap with each other. However, home ranges of those of different sized females may overlap. Equal sized females are territorial towards each other. But the smaller ones submissively tolerate the overlap of their respective home ranges by the home ranges of larger ones. Hence the size-dependent relationship among the females within the overall male's territory is based on linear dominance.

Based on the spatial and dominance relationship among the female members, two types of harem structure may be recognized: 1. Linear harem with linear dominance order according to size with considerably overlapping home ranges of female members (Fig. 29) and 2. Branching harem composed of two linear harems occupied by two sub-groups of females dividing the male's territory. Home ranges of females of different linear harems do not overlap but within the same linear group home ranges overlap widely. The two largest females in the two linear harems are aggressive towards each other at the border of their respective home ranges.

Not much is known about the early stages of harem formation. However, Sakai (1997) has described alternate strategies of harem formation in a model fish *Centropyge ferrugatus*. In isolated harems comprising 2–3 females each, strict social control by the dominant male results in the female adopting a 'take over sex change' strategy, only after the male disappears. In harems adjacent to each other, however, take over sex change strategy is not adopted, even when one of the males disappears. Instead, a large harem including more than four females is formed by 'fusion' of two adjacent territories. In large harems, the dominant male is unable to socially prevent the largest female from changing sex and subsequently acquiring a portion of the harem. Adopting a 'bachelor strategy' the females at the



**Fig. 29** Upper panel: Territories and spawning sites of *Chaetodon trifasciatus*. Letters (with a numeral) indicate territory owners (from Yabuta and Kawashima, 1997). Lower panel: Examples of linear and branching harems of *Centropyge ferrugatus*. Thick and thin lines indicate male territories and female home ranges (from Sakai, Y and Kohda, M, 1997. Harem structure of the protogynous angelfish *Centropyge ferrugatus* (Pomacanthidae). *Env Biol Fish*, 49: 333–339 with kind permission from Springer Science and Business Media).

periphery of adjacent harems grow faster instead of spawning frequently and await for an opportunity to gain territorial status over its similar sized neighbours, within central home range. The ‘take over strategy’ results in higher fitness than the harem ‘fusion strategy’.

Besides the possible inherent genetic basis for the female-biased sex ratio in protogynous hermaphrodites, persisting females in some protogynic species and overfishing of large, long-living males (e.g. Serranids, Devries, 2007) may have also further tilted the scale in favour of the female ratio. Genetic studies are needed to understand the mechanisms of sex determination in these four types of sex changing fishes (Table 27). Research on the genetic basis by which the male ratio is progressively depressed in these identified patterns of protogynous hermaphroditism may prove rewarding.

**Table 27** Male ratio and number of females per male in protogynous hermaphroditic fishes.

Protogynous hermaphrodites	♂ ratio	♀♀/♂
Monandric monochromatic	0.31	2.23
Monandric dichromatic	0.30	2.57
Diandric dichromatic	0.23	3.34
Haremic	0.22	3.55

Incidentally, a low male ratio is not uncommon among the gonochoristic fishes. For instance, the viviparous poeciliids have male ratios below 0.4 (e.g. *Poecilia sphenops*, George and Pandian, 1997). Among the oviparous gonochores too, species with dimorphism have a low male sex ratio. Several acanthurids are known for this; for instance, the body weight of the male *Ctenochaetus striatus* is 25% larger than that of the female, when the sex ratio is 1♂: 4♀, i.e. there are 4 females for every male (Robertson et al., 1979). Likewise dichromatism is also not uncommon among the gonochores. Dichromatism is known among the cichlids but quantitative information is available only for the gonochoristic scarid *Leptoscarus vaigiensis*; its females and primary males are not as brightly coloured as the terminal males (Robertson et al., 1982). With the presence of dichromatism, *L. vaigiensis* appears to have reduced the cumulative male ratio to 0.29, a value closer to that (0.31) of monandric of dichromatic protogynous hermaphrodites (Table 22). In a Darwinian sense, the conspicuous colouration may help the TP males to attract the females but the same makes them more vulnerable to predators (Karino et al., 2000). In terms of energy allocation, IP males allocate relatively more energy for sperm production but the same may be partitioned between sperm production and production of colour pigment(s) in TP males.

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## Protandrous Hermaphroditism

The occurrence of protandry is not as prevalent as protogyny; it is limited to 21 genera belonging to Platycephalidae, Centropomidae, Sparidae, Pomacentridae, Gonostomatidae, Muraenidae (Sadovy de Mitcheson and Liu, 2008). Among the protandrous hermaphrodites, monogyny is common but digyny is limited to only a few species like *Lates calcarifer*. Correspondingly, literature available on protandry is relatively very limited. Yet, being candidate species for aquaculture, the black porgy *Acanthopagrus schlegeli* (e.g. Chang et al., 1991), *L.calcarifer* (e. g. Guigen et al., 1993) and *Amphiprion* spp. (e.g. Godwin and Thomas, 1993) have received much attention to induce sex reversal through administration of one or another steroid.

### 9.1 Gonadal organization

The bisexual gonads of sparids, known as the delimited type, differ from those of other hermaphrodites. In sparids, the gonads are paired ovotestes, each with an ovarian lobe in the mid-dorsal region of the body cavity, and testicular lobe as a band along the ventrolateral wall in the more posterior region of the gonad (Reinboth, 1974). The two heterosexual regions are separated by a connective tissue. However, alternate regression and growth of ovarian and testicular tissues during the first and second milting periods of the black porgy *Acanthopagrus schlegeli* suggest the existence of a strong interaction between ovarian and testicular tissues. Testicular tissue may play an inhibitory role in the development and growth of ovarian tissue and vitellogenic oocytes (see Lee et al., 2008).

The ovotestes of others like *Amphiprion melanopus* are comparable to those of the simultaneous hermaphrodites like *Rivulus* (Harrington, 1975) and *Serranus* (Reinboth, 1980). In all of them, there is no distinct boundary between the testicular and ovarian tissues and both are in the same cavity. However, there is a certain degree of topographical segregation of heterologous germinal tissues but without a morphologically discernible boundary (Reinboth, 1980). Ultra-structural studies have shown the

absence of a basal lamina both along the semeniferous tubules and around the previtellogenic oocytes; hence male and female germ cells are separated by their respective somatic, i.e. Sertoli and follicle cells. In *Gonostoma gracile*, the gonadal structures are uniquely dissimilar to the paired pattern of male and female zones and a serial alternation of male and female zones is found in the cord-like germinal ridge (Kawaguchi and Marumo, 1967).

Lee et al. (2008) have accurately described the histological events in the black porgy *Acanthopagrus schlegeli*. During the initial 2–3 years, the porgy is a functional male but subsequently it becomes a female. The chronological sequence of gonadal differentiation is schematically illustrated in Fig. 30. 1. Until the age of 5 months, the gonad remains undifferentiated (UDG). 2. At the age of 5 months, the gonad is differentiated into an inner putative ovary (POT) surrounding the central cavity (CC), and the

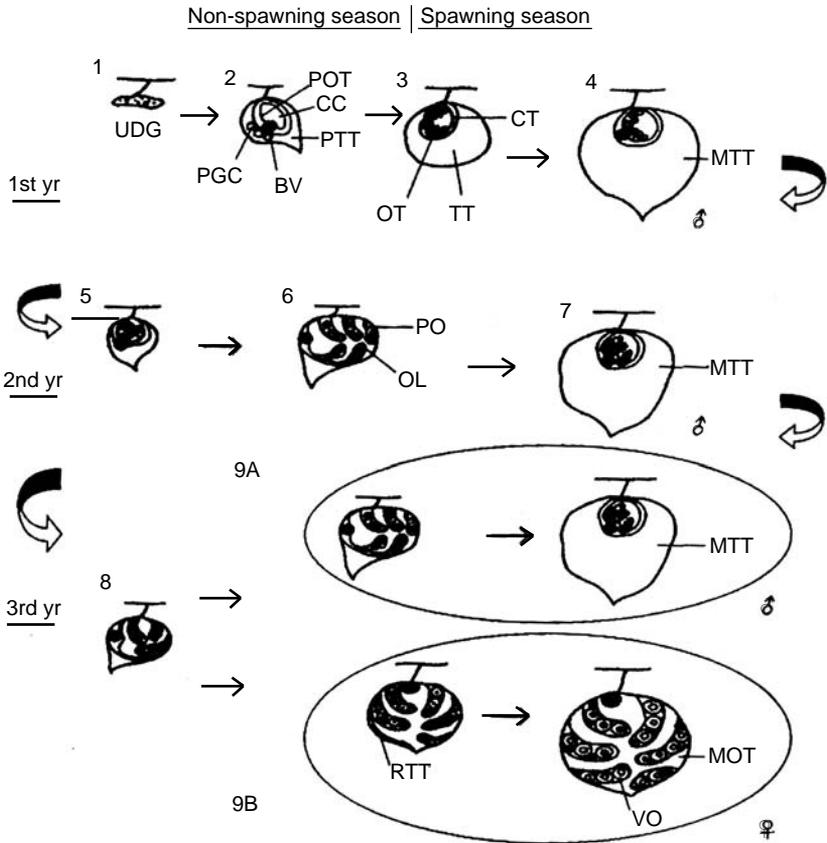


Fig. 30 Profiles of gonadal development in protandrous 0+ - 3-yr old black porgy *Acanthopagrus schlegeli*. For explanation see text (from Lee et al., 2008).

putative testicular tissue (PTT) occupies the distal end of the gonad and is far from the central cavity. 3. When the fish enters the pre-milting period, the ovarian tissue (OT) is limited as cell nests (CT) inside the central cavity and the testicular tissue (TT) rapidly proliferates. 4. During the first milting season, the central cavity is compressed by maturing testicular tissues (MTT). 5. At age two, the testicular tissues degenerate and regress, while the ovarian lamellae (OV) are budded towards the central cavity during the post-milting period. Oogenesis generates oocytes. 6. During the intersex period, an ovarian tissue proliferates but does not go beyond the primary oocytic stage. 7. Testicular tissues proliferate again, and the ovarian tissue is regressed, when the fish is a functional male. 8. The testicular tissue is regressed again and the ovarian tissue grows after the second milting period. 9a. The testicular tissue grows and the fish again becomes a functional male for the third milting period and 9b. In the process of natural sex change, testicular tissue is regressed (RTT) and remains as a remnant. The primary oocytes undergo vitellogenesis (VO = Vitellogenic oocytes). The fish undergoes sex change and becomes a functional female.

## 9.2 Sex ratio

Two models of ontogenetic increase in female ratio are apparent. In model 1, the female ratio steadily increases with increasing body size and may even attain a ratio of 1.0 (Fig. 31). For instance, the female ratio commences with 0.07 at the body size of 40–45 mm in the clown anemone fish *Amphiprion percula* and the ratio reaches 1.00 level at the size of 75–80 mm and remains at the same level until the fish attains the maximum size of 90–95 mm (Madhu and Madhu, 2007). A similar trend is also apparent for the salema *Sarpa salpa* (Villamil et al., 2002). In the salema, the estimated transition load is 6% and it lasts for 27% of its life span. Likewise, the estimated transition load for the pandora *Pagellus erythrinus* is 7.4% and it lasts for 40% of its life time (Pajuelo and Lorenzo, 1998, cf Table 20). For these three protandrous hermaphrodites, the female ratio averages to 0.31; this is exactly the reverse of that observed for protogynous fishes (cf Table 22). Clearly, protandrous hermaphroditism alters the ratio in the reverse direction.

The fact that the presence of males of the robalo *Elginops maclovinus* is limited to 45 cm size (2–7 years old) only and that the presence of larger females (24–58 cm size, 3–8 years old) in the southern Chilean coast clearly shows that the robalo belongs to model 1, although the robalo may be tending to evolve into model 2 (Licandeo et al., 2006).

In model 2, the female ratio remains at around 0.5 almost throughout the life (Fig. 31). For instance, the trend obtained from the data kindly provided by Pajuelo et al. (2006a) for *Diplodus vulgaris* shows that mature

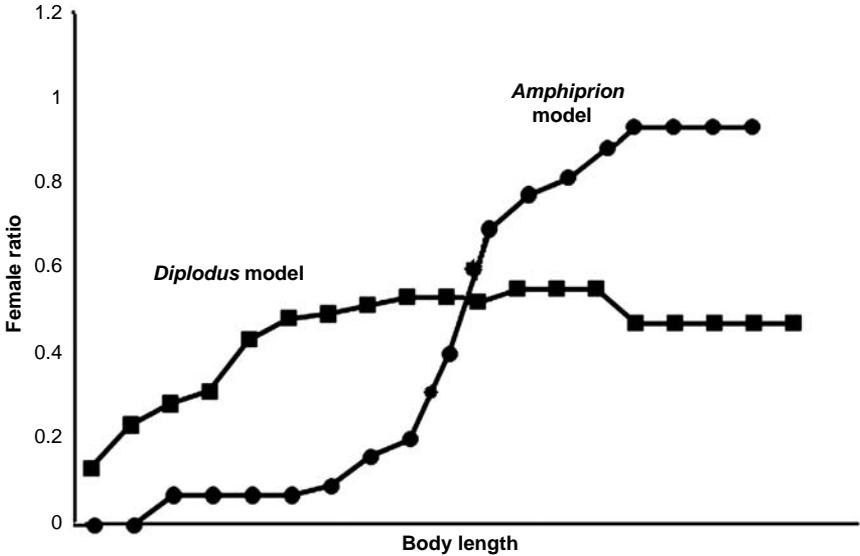


Fig. 31 Models of ontogenetic progression in female ratio with increasing body size. Note that the female ratio remains at around 0.5 until the end of life time in Diplodus model, while the ratio reaches 1.0 at the end in Amphiprion model.

males and females are present from the size of 14 mm and are there in equal ratio from 21 mm size to the end of 36 mm size. Clearly *D. vulgaris* belongs to model 2.

The very presence of 7% (26.7% see Micale and Perdichizzi, 1994) hermaphroditic transitionals clearly shows that *D. vulgaris* is a sequential hermaphrodite. The presence of males at 13 mm size and the subsequent appearance of females from 14 mm (with increasing numbers upto 18 mm), when transitionals began to appear suggest that the fish is a protandrous hermaphrodite. However, the presence of females at 14 mm size immediately following the appearance of males and the presence of males until the last raise some questions. 1. Are these young females (14–18 mm) representing prematurational sex change or are the smaller ones (13 mm size) representing primary females? 2. Do these persisting (until the end) males resist sex change and remain as males until death, as some females remain as females until death in some protogynic species? The sex ratios reported for the deep sea fish *Gonostoma bathyphilum* suggest the presence of primary females and precociously sex changing males (Badcock, 1986). The sex ratio (0.80♂:0.20♀) of the Australian sea perch *Lates calcarifer* suggests that for every female, there are 3.8 males, as some males have not undergone sex change (Moore, 1979). In the Gulf of Carpentaria, males precociously mature from the age of 1–2 years and consequently sex

change also occurs earlier resulting in the sex ratio of 0.5♀:0.5♂ at the age of 3 years instead of 7 years (Davis, 1984).

### 9.3 Pathways of sex change

Hattori and Yamagisawa (1991) have described the complicated pathways of ontogenetic sex change in the anemonefish *Amphiprion clarkii*. On the basis of fin colouration, six colour phases are distinguished and these phases correspond with six gonadal differentiation phases. With body growth and the attendant colour changes, the following pathways are identified: 1. Sub-adult ♂ → sub-adult → ♀ → adult ♀, i.e. females without passing through a functional male phase, 2. Sub-adult ♂ → adult ♂ → adult ♀ and 3. Sub-adult ♂ → adult ♂, i.e. males that are permanent males, as some females remain females until death in protogynous hermaphrodites. The difference in timing among individuals in the development of ovarian tissues of the hermaphroditic gonads involving the atrophy of testicular tissues causes the individuals to choose one of the three alternate pathways. The second pathway is regarded as the most common among the tropical anemonefishes.

From a long term study in Eilat, Isreal, Fricke and Fricke (1977) described the social structure of two anemonefishes *Amphiprion bicinctus* and *A. alkallopsis*. Typically, a social unit of *A. bicinctus* consists of a large female, a smaller male and varying number (upto 10) of non-breeding sub-adults and juveniles, none of which is the offspring of the adult pair. The unit size, however, is restricted by the size of the anemone and to no more than two adults. Thus, the permanent pair formation is favoured and the pair remains for even three years without changing partners.

In *A. alkallopsis*, the largest and oldest individual of a unit is always an  $\alpha$ -female; she dominates the male, sub-adults and juveniles. The  $\beta$ -male aggressively dominates all others, (but not the  $\alpha$ -female), especially the sexually competing  $\gamma$ -male. On removal of the  $\alpha$ -female in an aquarium, the  $\beta$ -male assumes the  $\alpha$ -female's position and changes sex in less than 63 days. However, in 18 of 24 units in the field, the  $\beta$ -males changed sex in 26 days and were joined by sub-adults of unknown origin and spawned fertilizable eggs; the males in six other units remained solitary. The  $\beta$ -males always acquired the unit; the reverse (serial) sex change from female to male could not be induced experimentally; this is understandable, as the testicular tissue is regressed and remains only as a 'relic' in the sex changed female.

From their 2-year long investigation at Murote Beach, Shikok Island, Japan, Hattori and Yanagisawa (1991) reported the formation and social structure of the anemonefish *Amphiprion clarkii* on the sea anemone *Parasicyonis maxima*. Mean density of the anemone was 5.7 individuals

per 100 m<sup>2</sup> and each sea anemone spread over an area of 1.03 m<sup>2</sup>. Pair breeding fish held territories covering 1–10 sea anemones. Hence more than 59–86% of sea anemone area was controlled by the breeding fishes. The mean home range of non-breeding sub-adult was 730 cm<sup>2</sup>, which did not overlap with those of other sub-adults.

Females of *A. clarkii* were usually larger than the males but in some cases, the males were larger than the females. A female produced 1–9 clutches in a breeding season and occupied a mean area of 545 cm<sup>2</sup> for spawning. During the period of investigation, 14 pairs separated or disappeared but 25 pairs were newly formed; of these 7 pairs were formed between breeders, i.e. matured males and females, 8 pairs between breeders and sub-adults, i.e. matured males or females and maturing sub-adult males and females, and 10 pairs between sub-adults, i.e. between newly maturing sub-adult males and sub-adult females. Unlike in *A. alkallopsis*, the harem mistress need not necessarily be the largest female in *A. clarkii*; even a sub-adult, whose gonad is not yet developed, can establish a social unit and subsequently develop the gonad within a span of 2–42 days from the formation of the unit and thus hold the unit successfully (see Hattori and Yanagisawa, 1991). Hence the potency of establishing a unit appears to differ from species to species within the genus *Amphiprion*.

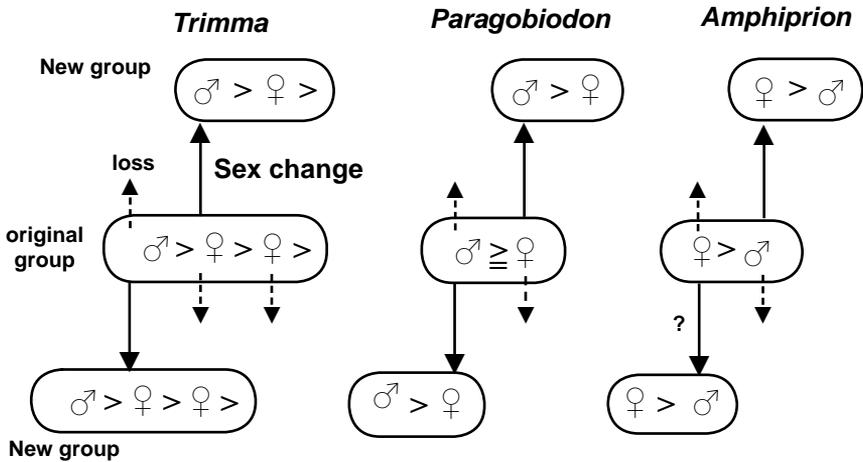
Thus, the protandry in the monogamous anemonefishes can be explained by the size-advantage model not only because the per capita production of fertilized eggs is higher, when the larger individual of a monogamous pair is the female (Warner, 1984) but also because the monogamous pair will start with random pairing since juveniles settle from the planktonic life randomly into each sea anemone.

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## Serial Hermaphroditism

Sequential hermaphroditism is a common reproductive strategy that has evolved many times. Sex change from female to male or male to female is favoured, when sex specific reproductive success is differentially distributed with size/age. However, serial sex change characterized by changing between sexes more than once during the life of a fish may be expected with maximum sex specific reproductive success (Nakashima et al., 1995). It is to the credit of Kuwamura et al. (1994), who first described the serial hermaphroditism or the ability to change sex more than once and in both directions from female to male and subsequently from male to female in the monogamous coral goby *Paragobiodon echinocephalus*; since then, serial hermaphroditism has also been confirmed to occur in other monogamous gobies *Gobiodon micropus*, *G. aculolineatus*, *G. quinquestrigatus* and *G. rivulatus rivulatus*, inhabiting *Acropora* corals from field and aquarium experiments at Sesoko Island, Japan (Nakashima et al., 1996) and in *Trimma okinamae* (Sunobe and Nakazono, 1993). It is also known to occur in a cirrhitid hawkfish *Cirrhitichthys aureus* (Kobayashi and Suzuki, 1992) a pomocanthid *Centropyge fisheri* (Hioki and Suzuki, 1996) and in the serranid *Epinephelus akaara* (Okumura, 2001). Controlling social conditions in captivity, Ohta et al. (2003) successfully induced bi-directional sex change in the Japanese wrasse *Pseudolabrus sieboldi*. The tendency and potency for serial hermaphroditism have also been shown to be in the gobies *Lythrypnus* spp (St. Mary, 1994, 1996), *Gobiodon okinawae* (Cole and Hoese, 2001) and humbug damselfish *Dascyllus aruanus* (Cole, 2002).

In consonance with the size advantage model, males of many gonochores and protogynous hermaphrodites, and females of protandrous hermaphrodites attain a larger body size at the time of sexual maturity (Fig. 32). The difference in body size of the latter is more apparent, as they are sex changed individuals. Considering the socially controlled 2-way sex changing gobies and comparing them with the anemonefishes in the context of the size advantage model, Kuwamura and Nakashima (1998) developed three models for sex change. In the monogamous *Paragobiodon* model, there is no size advantage, as breeding pairs are formed by a male



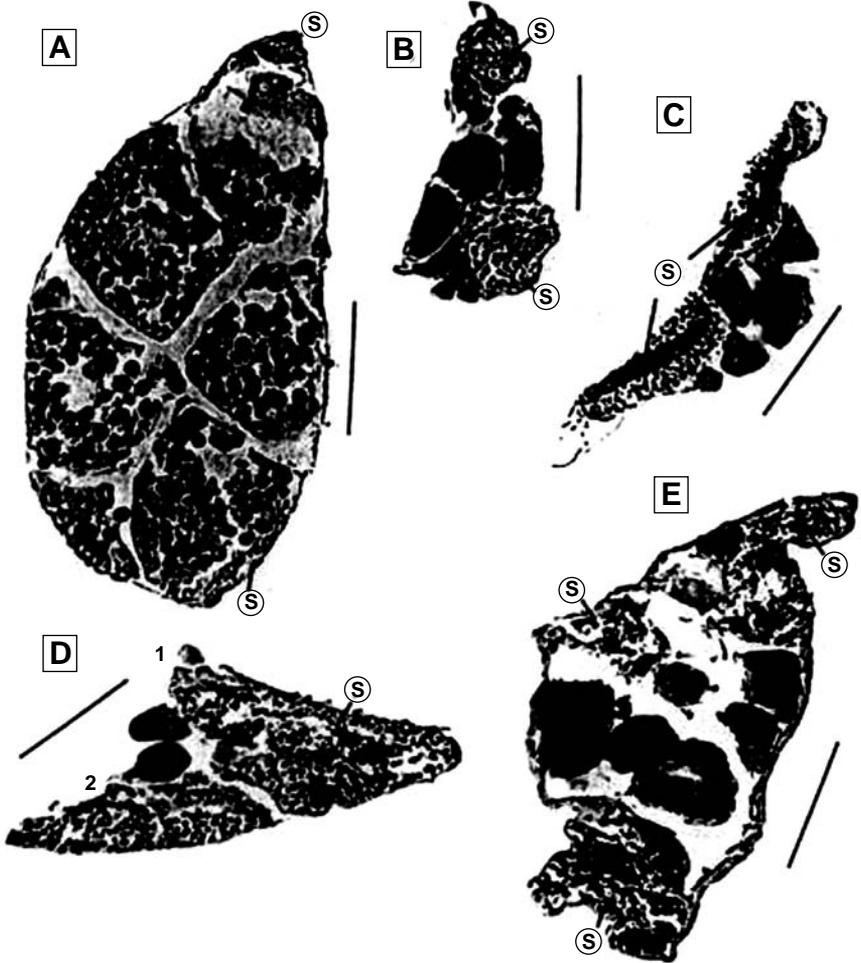
**Fig. 32** Social dominance and control of 2-way sex change in polygynous *Trimma* and monogamous *Paragobiodon* and *Amphiprion*. Sex change may occur in different directions after loss (shown by dashed arrows) of the dominant mate (top) or after loss of the subordinate mate (bottom). In newly formed pairs of *Paragobiodon* males are initially larger than their mates but later become the same size due to the growth rate advantage of females (from Kuwamura, T and Nakashima, Y, 1998 New aspects of sex change among the reef fishes: recent studies in Japan. *Env Biol Fish*, 52: 125–135 with kind permission by Springer Science and Business Media).

and female of equal size. Hence the female's ability to produce more eggs and the male's ability to guard them increase equally with increasing body size. Consequently, an isolated/widowed male or female goby grows faster and then may change sex to female or male, depending upon the sex of the new mate. In the polygamous *Trimma* larger body size for a male is an important requirement. Hence an isolated/widowed male changes into a female, only when the new mate is larger than 'him'; the isolated female changes into a male, when the new mate is smaller than 'her'. In the protandrous hermaphrodite *Amphiprion*, the female has to be larger; hence an isolated widowed female may change into a male, provided the new mate is larger than 'her' and the isolated male may change into a female, when 'his' partner is a smaller male.

### 10.1 Marian hermaphroditism

St Mary (1993, 1994) described a kind of mixed or preferably called Marian hermaphroditism in the goby *Lythrypnus dalli* as the first known example of a fish simultaneously maintaining both ovarian and testicular tissues. On the basis of histological examination of the gonad and assessing the shape of the genital papillae of *Lythrypnus* spp, St Mary reported that the proportion of gonadal tissue allocated to each sexual function ranges

from 100% female to nearly 100% male. For example, in *L.dalli*, there are pure females, strongly female-biased (5% allocation to testicular tissue) hermaphrodites, strongly male-biased (5% allocation to ovarian tissue) hermaphrodites and pure males (Fig. 33), which are in a way comparable to *Serranus fasciatus* (Hastings and Peterson, 1986; Petersen, 1987, 1990). Through a series of publications, St. Mary (1993, 1994, 1996, 1997, 1998, 2000) has brought a new dimension to the sex allocation theory. There appears to be no direct reproductive benefit associated with simultaneous



**Fig. 33** Gonadal structure of some gobiids that vary in allocation to male function. A. *Lythrypnus nesiotes*, allocation to male tissue = 1 %, B. *L. phorellus*, allocation to male tissue = 30 %, C. *L. nesiotes*, allocation to male tissue = 74 %, D. *L. nesiotes*, allocation to male tissue = 90 %, E. *L. spilus*, allocation to male tissue = 36 %, S = Spermatogonium. (from St Mary, 1998).

allocation to male and female function. However, fitness benefits appear to accrue through reduction in costs (time and energy) associated with sex change or maintenance of sexual flexibility (St. Mary, 1997).

Females and female-biased hermaphrodites are smaller than males and male-biased individuals. Using the values reported for the size-dependent sex frequency distribution of females (females and female-biased hermaphrodites) and males (males and male-biased individuals) kindly provided by St. Mary (2000), two generalized models of ontogenetic progression in sex ratios for the five species are drawn. Sex ratios in the field, size dimorphism and experimental evidences suggest that the males are derived from females of *L. dalli*, *L. nesiotis* and *L. spilus* (Fig. 34); hence they are more like the protogynous hermaphrodites and belong to the *Dalli* model.

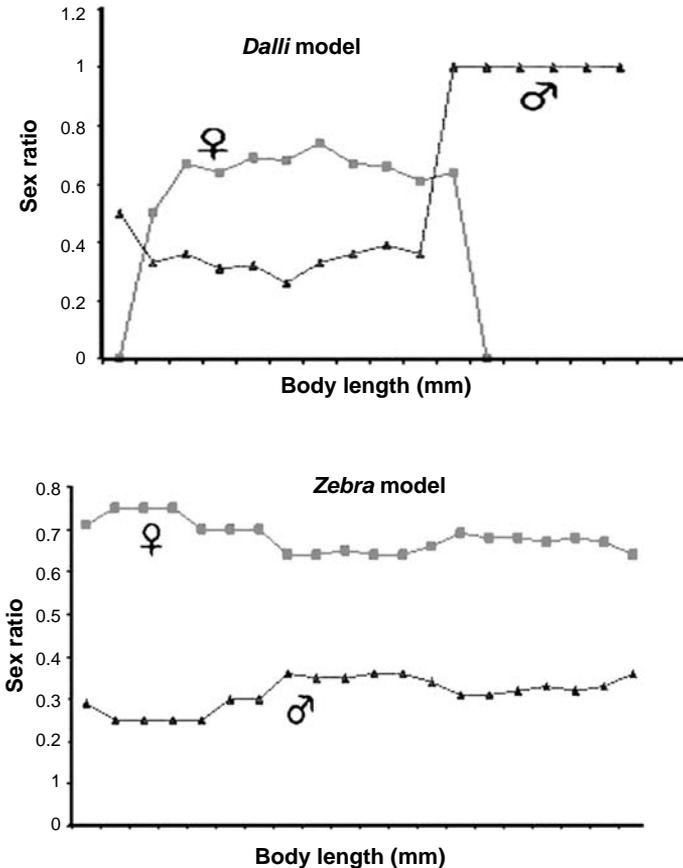


Fig. 34 Dalli and Zebra models of ontogenetic changes in sex ratio with increasing body size of the gobiids *Lythrypnus* spp.

In the strongly female-biased hermaphrodites, oocytes as well as tailed sperm are present, as in *L. zebra* and the strongly male-biased hermaphrodites have tailed sperm but not developed oocytes, as in *L. phorellus*. Understandably the sex ratio is female-biased in *L. zebra* but male-biased in *L. phorellus*. Most individuals of *L. zebra* and *L. phorellus* belonging to *Zebra* model are simultaneous hermaphrodites, i.e. have active female and male gonadal tissues. Yet, they do not reproduce simultaneously as males and females, an important feature that clearly distinguishes Marian hermaphroditism from simultaneous hermaphroditism.

There is also a striking similarity in sexual patterns of all the five species of *Lythrypnus*. Each species exhibits Marian hermaphroditism with a high degree of variability in allocation to testicular tissue among individuals. Among them, three distinct sexual patterns are identified: 1. *L. dalli* consists of (i) primarily pure females, (ii) strongly female-biased hermaphrodites and (iii) pure males, 2. *L. nesiotetes* has also (i) primarily pure females, (ii) strongly female-biased hermaphrodites and (iii) strongly male-biased individuals and 3. *L. zebra*, *L. spilus* and *L. phorellus* are characterized by the absence of pure males. The pattern of allocation in each species also suggests that sex change in both directions may be possible. *L. zebra* males have greater ability to reallocate and function as females in 2 weeks (St Mary, 1994, 1996). The greater flexibility of *L. zebra* may also relate to its more intermediate ( $\approx 75\%$ ) allocation; of the goby with pointed papillae in *L. dalli*, 42% are pure males, whereas none of *L. zebra* with pointed papillae are pure males. Hence intermediate allocation may be important in facilitating rapid shifts in allocation from male to female function.

## 10.2 Okinawan hermaphroditism

Cole and Hoese (2001) have described the presence of hermaphroditism in the goby *Gobiodon okinawae*, and the hermaphroditism is more complicated than the Marian hermaphroditism. Based on size ranges for fish having different gonadal morphologies, the gobies develop first as females; then they mature and function solely as females or become hermaphroditic. Females having vitellogenic oocytes are active females and others are inactive females. A hermaphrodite with ovotestis containing spermatozoa and earlier oocytic phases is called male-active hermaphrodites; the other with vitellogenic oocytes is a female-active hermaphrodite, while the former is a functional male, the latter is an inactive hermaphrodite. Both functional females and male active hermaphrodites possess the accessory gland system (AGS). No individual has mature gametes of both sexes simultaneously. Hence the 'Okinawan' model hermaphroditism differs from simultaneous hermaphroditism. It is partially related to

Marian hermaphroditism, as both Marian and Okinawan hermaphroditisms have pure females, and hermaphrodites, who function as males (Fig. 35). However, Okinawan hermaphrodites are colonial, whereas Marian hermaphrodites are solitary.

A total of 43 individuals of *G.okinawae* colonies were collected from the Lizard Island; their female: hermaphrodite ratio was 0.7:0.3. Among the 30 females, only 6 (5%) were active functional females. Of the remaining 13 hermaphrodites, 6 were male-active and 7 were female-active hermaphrodites. In each colony with 2–10 individuals, there were one or two active females and one male-active hermaphrodite and all others were inactive females and hermaphrodites. Findings based on experimentally maintained individuals suggest that adult inactive hermaphrodites can change sexual functions in either direction.

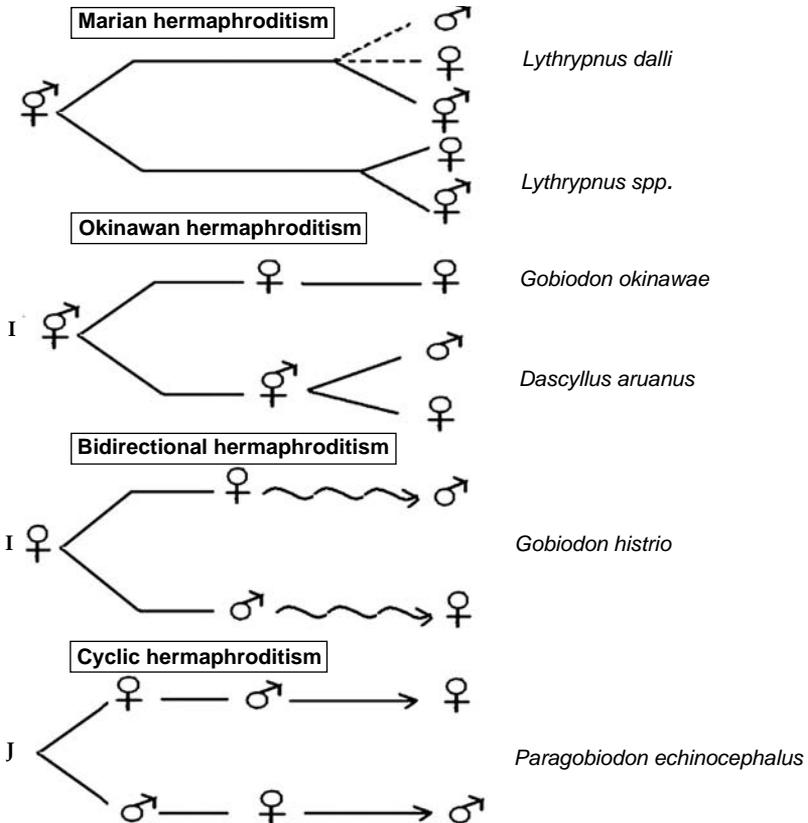


Fig. 35 Pathways of sex changes among serial hermaphrodites. I = immature, J = Juvenile. Strong and dotted lines indicate the major and alternate routes. Wavy lines indicate induced sex changing routes. *P.echinocephalus* juveniles mature first into either females or males but the immature *G. histrio* mature first into females only.

The sexual pattern of the protogynous hermaphroditic humbug damselfish *Dascyllus aruanus* is the same described for the goby *Gobiodon okinawae*. According to Cole (2002), the size range of individuals within the gonadal classes indicates that all the damselfish first develop an ovariform gonad. Some individuals then undergo ovarian maturation to become adult females, while others develop testicular tissue to form an ovotestis and become male-active hermaphrodites (Fig. 35). Subsequently, progressive loss of the ovarian tissue results in the development of a secondary testis from an ovotestis (instead of an ovary). Hence the damselfish may be regarded to exhibit a modified diandric protogyny.

Of the total 160 *aruanus* belonging to 13 colonies collected from Papua New Guinea, 16 (10%) were immature. Of the remaining another 16 (10%) were females with completely ovarian gonad laden with vitellogenic oocytes; another 41 (26%) were sexually-inactive hermaphrodites having ovotestis with no vitellogenic oocytes. Another 5 were males with only testicular gonad laden with spermatozoa; another 7 were intermediate but had spermatozoa and 5 possessed ovarian cum testicular tissues. But all 17 (11%) were regarded as males. All the remaining were hermaphrodites with inactive ovotestis. Overall, the sex ratio was 1♂:1♀ and histology of the gonad suggests that these hermaphrodites are capable of bidirectional sex change (Cole, 2002).

Each colony was composed of one male or 1–4 male-active hermaphrodites, one female or 0–2 female-active hermaphrodites, 5–10 inactive hermaphrodites and 1–5 juveniles; there were five ‘young’ colonies without male or male-active hermaphrodites.

### 10.3 Cyclic hermaphroditism

Marian and Okinawan hermaphrodites are indeed potential serial sex changers. However, among serial sex changers, in which serial sex change occurs within an individual of a species, two types may be recognized: 1. Cyclic sex change, in which the same individual changes sex *twice* or more, say from female to male and then to female and so on, and 2. Bidirectional sex change, in which sex change occurs only *once* in an individual either from female to male or twice from female to male and then to female. Secondly, all the serial sex changes have been recorded only from sequential protogynous hermaphrodites, including in *Gobiodon okinawae*, in which immature females may change to become hermaphrodites.

Cyclic sex changers: The simple size advantage model has successfully explained the relation between sex change and mating system in fishes. As the female’s fecundity and male’s ability to afford paternal care increase with body size in a similar way, no size advantage may exist especially among monogamous gobies. *Paragobiodon echinocephalus* is monogamous

with paternal egg care and lives on branching coral *Stylophora pistillata*. However, the breeding pairs of this goby are more or less of equal size with no size-fecundity advantage. In such gobies, the simple size advantage model predicts gonochorism. However, histological studies have suggested protogynic hermaphroditism. With this background, Kuwamura et al. (1994) undertook an investigation in *P. echinocephalus* on the coral reef of Okinawa to know the type of sex change that takes place in the goby. Of 360 identified gobies, 166 were juveniles, 120 females and 74 males. The juveniles differentiated into females ( $n = 54$ ) more frequently than as males ( $n = 16$ ). Females (32/120) of the first marked group changed sex more frequently than males (5/74). Three individuals changed sex twice: the first two from ♀→♂→♀ and the third from ♂→♀→♂ (see Fig. 1 of Kuwamura et al., 1994).

Based on the change of the shape of urogenital papilla, the total number of protogynic and protandrous sex change observed was 48 and 10 cases, respectively. The protandrous sex change occurred during July–August in a breeding male (19.8 mm) to a breeding female (21.4 mm) and required a period of 1–2 months. Protogynous sex change took place both during and after the breeding season and required a period of only 3–4 weeks, as observed in the aquarium.

After mate loss, 35 of 36 females and 41 of 46 males acquired new mates within a few months, either remaining on the original coral or moving to other corals. When they remained on the same coral, unmated females changed sex more frequently (74%) than the males (3%). When they moved to other corals, females changed sex less often (38%) than when remaining in the same coral but males changed more often (33%). Hence the frequencies did not differ between the sexes. New mates were usually smaller than unmated females; they were derived from juveniles or females. Mostly unmated females and very rarely unmated males changed sex.

In the Japanese hawkfish *Cirrhitichthys aureus* too, a single individual changed sex twice, i.e. from female to male and subsequently from male to female (Kobayashi and Suzuki, 1992).

#### 10.4 Bidirectional hermaphroditism

The monogamous goby *Gobiodon histrio* inhabits on the Actinacian coral *Acropora nasuta*. It is suggested to be a protogynous hermaphrodite. To study its social structure and bidirectional sex changing ability, Munday et al. (1998) marked 13 female-female pairs and 12 male-male pairs on the Lizard Island at the Great Barrier Reef and recorded the number of successful sex changers after 28 days. Of six pairs of female-female pairs recovered, protogynous sex change occurred with the largest females changing to males. Of two male-male pairs recovered, evidence for successful change from male to female was obtained. Thus sex change

from female to male in this protogynous goby readily occurred; however, protandrous sex change did occur but not frequently. It may be noted that whereas Kuwamura et al. (1994) reported that cyclical sex changes in *Paragobiodon echinocephalus* occur in the same individuals either from ♀→♂→♀ or from ♂→♀→♂, the observation by Munday et al. (1998) is limited to bidirectional sex change in *Gobiodon histrio* either from ♀→♂ or ♂→♀ in different individuals (Fig. 35). Yet, *G. histrio* has the ability to change sex in either direction.

The destiny of those experimentally marked pairs, which could not be recovered is suggested to have opted for migration to other corals in search of a suitable mate rather than to undertake the costly sex change. Kuwamura et al. (1994) too have shown that a high percentage of 'widowed' *P. echinocephalus* males and females acquired new mates from the same or other corals. According to Munday et al. (1998), sex change may occur only where 1. Coral gobies occur at low densities, 2. Movement among corals is risky due to large predators and 3. Social status is likely to change in the newly formed pair.

Table 28 summarizes the different types of hermaphroditism reported among teleostean fishes. The described attributes and examples distinguish the different types of hermaphroditism. Protogynous hermaphroditism has been the source, from which four major types of hermaphroditism seem to have originated.

### 10.5 Cascades and causes of sex change

Gonadotropins have been implicated in the proximate control of socially induced sex change in fishes. Leuteinizing Hormone Releasing Hormone (LHRH) known to regulate gonadotropins, is distributed throughout the brain but more densely at the preoptic area, a brain region involved both in sexual development and control of sexual functioning and behaviour in adults (Grober and Bass, 1991). Arginine vasotoxin (AVT) is a hypothalamic peptide that regulates reproductive behaviour in fishes and the size of AVT cells changes during sex change. For instance, the size of AVT immuno-reactive magno-cellular preoptic cell (AVT-ir) in the *Trimma okinawae* female, regardless of its prior sexual status, is significantly larger (22  $\mu\text{m}^2$ ) than that of the male; the mean cross sectional area of AVT-ir cell is larger following male to female change and the reverse is true with female to male transition (Grober and Sunobe, 1996). Contrastingly, the AVT-ir cell of the male *Lythrypnus dalli* measures 135  $\mu\text{m}^2$  but in the female 96  $\mu\text{m}^2$  only. With transition from female to male, the AVT-ir increases to 137  $\mu\text{m}^2$ . Yet, AVT-ir cells provide the signal for release of arginine vasotoxin, which regulates the reproductive behaviour (Reavis and Grober, 1999). However, the neuroendocrine changes resulting in the release of vasotoxin/vasopressin peptides are the ones responsible for initiation and completion of

**Table 28** Types of hermaphroditism recorded among teleostean fishes and their attributes.

Type of hermaphroditism	Attributes and examples
1. Simultaneous	
i) Self-fertilizers	Internally self-fertilizers, <i>Rivulus marmoratus</i>
ii) Gamete-exchangers	Mutual exchange of gametes between pairing hermaphrodites, <i>Serranus nigricans</i>
2. Sequential	
2a Protogynous	
i) Monandrous	All secondary males arise from females only, <i>Choerodon schoenleinii</i>
ii) Diandrous	Besides secondary males, primary males are also generated, <i>Synbranchus marmoratus</i>
2b Protandrous	
i) Monogyny	All secondary females arise from males only, <i>Amphiprion akallopsis</i>
ii) Digyny	Besides secondary females, primary females are also generated, <i>Lates calcarifer</i>
3. Serial	
i) Marian	Solidary. Males and females simultaneously maintain different proportions (0 to 100 %) of ovary and testis. Protogynous hermaphrodites. Unlike simultaneous hermaphrodites they function only as male or female at a given time. Pure males rarely present, <i>Lythrypnus dalli</i> . Equal allocation of ovary and testis may facilitate sex change in either direction, <i>L. zebra</i> .
ii) Okinawan	Colonial. Pure males, pure females, and male-active and female-active hermaphrodites as well as male-inactive and female-inactive hermaphrodites. Some colonies have only male-active hermaphrodites and others. Inactive hermaphrodites can change sexual functions in either direction, <i>Gobiodon okinawae</i> , <i>Dascyllus aruanus</i>
iii) Bidirectional	Protogynous hermaphrodites. On experimental induction ♀ changes (once) to ♂ and previously sex changed ♂ changes (twice) to ♀, <i>Gobiodon histrio</i>
iv) Cyclical	Protogynous hermaphrodites. Both males and females change sex more than once in natural habitat ( <i>Paragobiodon echinocephalus</i> ) and on experimental induction ( <i>Trimma okinawae</i> )

the gonadal sex change. Accordingly, the chronological sequence of events is in the following order: neuroendocrine changes resulting in the release of vasotoxin/vasopressin peptides → gonads → steroids → morphological change like the papilla → spawning (Reavis and Grober, 1999).

The typical pattern of sex changed behaviour displayed by the gonadectomized coral reef fish clearly indicates that the gonad does not initiate sex change (Godwin et al., 1996). Hence Reavis and Grober (1999) undertook experiments in the laboratory on the Marian hermaphrodite *Lythrypnus dalli* to study not only the effects of social factors on the

duration of sex change, but also on the neuroendocrine and behavioural changes. The two social factors considered by them were: 1. Inhibition by males and 2. Social stimulation by other females. Accordingly, in the Male-Removal (MR) type, the dominant male was removed and thereby disinhibited the 3–5 females in the social unit, in which one female was bigger than all other females (MR-B type) or all females were of equal size (MR-nb type). In the second, No Male (NM) type, the females were not under dominance and inhibition, as there were no males in their social unit; however, the unit consisted of all equal sized females (NM-nb) or it had one larger female and other smaller females (NM-B). In the third type (MO), the males, who have already changed sex (from females to) males, and undertaken broodcare once or more, were kept together to induce sex change for the second time.

The duration required for sex change was significantly affected by the type of sex changes and also by season. Sex change by the largest female occurred most rapidly in (5.7 days) MR-B fish. Sex changers in other NM-B and MR-nb groups required longer but almost equal durations of 8.6 and 9.2 days, respectively. Hence both the absence of larger females and dominance by the male prolonged the sex changing duration. Sex change during summer required shorter duration of 5.5 days than during other seasons (9 days); apparently temperature accelerates the rate of sex change. In the MR-B group, one large female changed sex in the presence of a male; consequently, the original male became a female and spawned on the 14th day. Apparently, the Marian hermaphrodite *L. dalli* is capable of bidirectional sex change, i.e. from ♀→♂ and from ♂→♀. However, different results were obtained, when the sex changed males were kept together to induce sex change for the second time. Three of them changed sex for the second time and became females on the 32nd, 46th and 53rd days, but one of them died, the second one spawned infertile eggs on the 46th, 68th and 93rd days after the second sex change and of the 8 clutches laid by the third, one batch alone developed upto the eye stage. Hence, the Marian hermaphroditic *L. dalli* is capable of bidirectional sex change but is not capable of true cyclical sex change, i.e. ♀→♂→♀.

Larger sex changers in MR-B group displaced the rank of other females, immediately following the male removal. Sex changers in the other groups did not show that sort of rapid displacement. This early establishment of dominance accounts for the overall difference in the durations required for sex change. These differences indicate that the changes in the neuroendocrine cells and enzyme production are the first to initiate behavioural sex change in *L. dalli* and possibly in other fishes (Reavis and Grober, 1999).

In the absence of adequate information on neuroendocrine pathways and purely from ecological observations and experiments, many hypotheses have been proposed on the mechanism of sex change. These proposals

along with their accountabilities and limitations are briefly summarized in Table 29. Based on distribution and predictability of resources including mates, Ross (1990) identified five distinct mechanisms of sex change; the causes and attributes of them are also summarized by him. However, the mechanisms and related hypotheses of sex change may be brought under two major groups. The first one includes the hypotheses of Bullough (1947) and Robertson (1972), in which members of the social units are aggressively dominated and inhibited from sex change by a male (*Protogynous suppression*) or by a female (*Protandrous suppression*). In the absence of the suppressing inhibitor male or female, the sex change is initiated and completed. This sort of suppression occurs mostly in non-permeable social units, which are small, limited by certain numbers and is characterized by rigid dominance hierarchies. Their food resources are fixed and present at predictable locations that limit the unit size. For instance, the anemonefishes are obligate commensals on small corals that occur at

**Table 29** Summary some hypotheses, their proposals, limitations and accountabilities.

Hypotheses & proposals	Proposal	Objections/remarks
Size (Bullough, 1947)	♀ changes sex on reaching critical size	(i) small & large ♀♀ change sex in <i>Thalassoma duperrey</i> (ii) ♀ changes sex in the presence of ♂ in <i>T. lucasanum</i> (Warner, 1982) (iii) ♀♀ change sex at different sizes in 2 populations of <i>Anthia squamipinnis</i> (Shapiro, 1981)
Inhibition (Robertson 1972)	Territorial ♂ inhibits sex change by others	(i) Sex change occurs in the presence of territorial ♂ (ii) Multiple sex change also occurs (Shapiro, 1981)
Size ratio induction (Shapiro and Lubbock 1980)	Sex change induced by size composition reaching a threshold size ratio	} Account for the occurrence of sex change in the presence of ♂
Sex ratio induction (Ross et al., 1983)	Sex change induced by sex ratio composition reaching a threshold size ratio	
Priming (Shapiro, 1979)	A behavioural profile 'primes' a limited number of ♀♀ to change sex	
Absolute encounter rate (Lutnesky, 1994)	Facultative sex change occurs, when $\alpha$ -♀ receives threshold level stimulation encountering smaller ♀♀ and is below threshold level of inhibition from encountering the ♂	It could incorporate size-ratio or sex ratio hypotheses (Lutnesky, 1994)

low densities; these features of anemone limit the size of social unit and mobility of individual members of the unit and thereby cause the formation of non-permeable social units.

The second major group includes most other hypotheses described in Table 29. In them sex change is induced by specific stimuli like the presence of small females in protogynous sex change, and social characteristics like the size or sex ratio or behavioural events such as encounter rates. Sex ratio induction refers to the sex change caused by a change in sex ratio of a social unit due to recruitment or mortality. In size ratio induction, the relative size ratio of a unit is the cue for sex change. These occur mostly in permeable social units, which are large (>12 members) and are associated with resources of varying location and unpredictable distribution. Species, with such large and permeable social units, lack stable dominance relationship. Hence sex change in them must be induced by the presence of other members of the unit or specific behavioural changes in them. For example, the protogynous seabass *Anthias squamipinnis* feeds on plankton, which are variable in occurrence in space and time but by virtue of their abundance, they may not limit the size of the social unit. Median sub-group size of the fishes is about 19 members, which freely move from one sub-group to the other. Such social groups are highly permeable.

Describing the causes and social attributes of sex changing mechanism, Ross (1990) indicated that the mechanisms of protandrous and protogynous suppression are characterized by small social units, stable hierarchy and low agility of females. On the other hand, with the mechanisms of sex ratio and size ratio induction, the social unit is large and unstable with highly agile females.

Apart from these social factors, the environmental factor like availability of space/nest may also serve as a mechanism of sex change. The Mediterranean wrasse *Coris julis* is a diandric, permanently dichromatic sequential hermaphrodite. Sex change is accompanied by change from initial phase (IP) to terminal phase (TP) colouration. At Corsica, its sex ratio is 0.72♀ : 0.16 IP♂ : 0.12 TP♂. TP males are territorial. During breeding season, a territory is occupied by a TP and a few IP. The spawning frequency is 3.9 times/hour for the TP males but 0.31 times for the IP male. Such high spawning frequency appears to be adaptive, as the breeding season is limited to a span of 4 months only. This is quite a contrast to the tropical wrasse, which breeds almost throughout the year.

Lejeune (1987) undertook experiments in shallow water (A) and deep water (B) stocks. The territories were installed over highly variable substrates and were often contiguous, for want of space. The density and territory size of stock A were 41 individuals per 1000 m<sup>2</sup> and 423 m<sup>2</sup>, but they were 164 individuals per 1000 m<sup>2</sup> and 50 m<sup>2</sup> for the stock B. The territory size is often the result of an equilibrium between the cost of defense

and benefits. A high density decreased the territory size of the TP in the stock B, and resulted in earlier sex change at the age of 5–6 years with an increased proportion of TP males. The percentage of TP was 5.4% in stock A but it was as high as 12.1% in stock B. However, the proportion of TP males in these stocks appears to be modulated by opportunity to operate as TP male, i.e. the availability of territory was an important cue for sex change. Hence more than social factors like size or sex ratio or encounter, it is the environmental factor namely the availability of space for the territory that seems to determine timing of sex change.

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## Mating Systems

The mating systems of the teleosts range from promiscuity to polygamy and to monogamy. Available information on this theme has been reviewed from time to time; however, they are limited to monogamy (Barlow, 1984, 1986, 1988) or to one group of fishes (e.g. coral-inhabiting hermaphrodites, Moyer et al., 1983; Colin and Bell, 1991; cichlids, Kuwamura, 1986). A comprehensive account for the group teleosts is still wanting.

Females produce relatively a limited number of large, poorly-mobile gametes but most of which are likely to be fertilized. In contrast, a male produces a large number of mobile gametes and has the potential to fertilize eggs of many females. Hence the number of eggs that a male can fertilize is determined by the number of female's gametes available to him. Consequently, the males compete among themselves for females. On the other hand, females select a male, who is strong among the contenders; the selection may often be based on size, quality of the territory, nest and so on. This process of competition and selection is a one-time affair among monogamous species, as the pairs intend to make sure the availability of his/her partner. However, sex ratio and availability of resources may lead to one or another form of mating system. For instance, male-biased sex ratio may lead to polyandry (e.g. *Julidochromis marlieri*, Yamagishi and Kohda, 1996) and female-biased sex ratio may result in polygyny (e.g. *Pomacanthus imperator*, Thresher, 1982). Resource limitation may impose monogamy; small size of the solitary morph of the anemone *Entacmaea quadricolour* may impose monogamy in *Premnas biaculeatus* (Srinivasan et al., 1999). Likewise space limitation seems to have imposed monogamy in *Signigobius biocellatus* (see Barlow, 1984).

### 11.1 Monogamy

*Typhlogobius californiensis* is the earliest known example of monogamy among gobies (MacGnitte, 1939). Since then reports on the existence of monogamous fishes have been accumulated. Following the system of considering monogamy under different types in mammals and birds,

Barlow (1984) was perhaps the first to recognize and apply the same to monogamous fishes. During the 1980's a number of hypotheses were proposed to trace the causative factors for the formation of monogamy among fishes. The 1990s witnessed the period of experimental testing of these hypotheses.

In general monogamous species share common features of site-fidelity and territoriality. Based on genetic consequences, monogamy may be divided into two types: 1. Exclusive monogamy, in which a pair of male and female confines most of their spawning (copulations) to the same partner and 2. Biparental monogamy, in which a pair of male and female remains together as partners after fertilization until the offspring no longer require their care. These definitions are not meant to be absolutes. In fact, monogamy lies on a continuum from monogamy to polygyny/polyandry and even to promiscuity (Wickler and Seibt, 1983).

For teleostean fishes, monogamy was viewed a little differently by Barlow (1984) under the types: 1. Mutual monogamy, in which reciprocal benefits render each partner to be fidelous to the other. Since formation, the pair moves and, spawns together repeatedly and has an enduring relationship. 2. Manipulated monogamy, in which, one partner aggressively and unilaterally monopolizes the other and the relation may lead to protogyny/protandry. One mate forcefully excludes other members of the same sex so that his/her mate has no opportunity to fertilize or to be fertilized by the intruder. By restricting the time of spawning to tidal/lunar cycle and so on, the female may also restrict the male to invest time in her at the cost of finding other females (Fischer, 1981). However, exclusive and biparental monogamies are chosen for further description.

Exclusive monogamy is common among the coral reef fish species belonging to Solenostomidae (Ghost pipefishes), Syngnathidae, (Seahorses and pipefishes), Serranidae (Seabasses), Branchiostegidae (Tilefishes), Chaetodontidae (Butterflyfishes), Pomacanthidae (Angelfishes), Pomacentridae (Damsel-fishes), Gobiidae (Gobies), Acanthuridae (Surgeonfishes), Balistidae (Trigglerfishes), Monacanthidae (Filefishes), and Canthogasteridae (Sharpnose puffers). Notably, these coral fishes display no parental care of eggs and fry. A single exception is the planktivorous damselfish *Acanthochromis polyacanthus* with biparental care (Robertson, 1973). As groups, the solenostomids and syngnathids undertake uniparental care of eggs, which are gestated by males. Among syngnathids, polygamy occurs in species such as *Syngnathus abaster*, *S. acus*, *S. floridae*, *S. rostellatus*, *S. schlegeli*, *S. scovelli*, *S. typhle* and *Entelurus acquareus*, which are characterized by the reverse role of competition between female and female. However, with male-male competition, monogyny takes place in *Hippocampus fuscus*, *H. subelongatus*, *H. whitei*, *H. zosterae*, *H. denise*, *Hippichthys penicillus*, *Stigmatopora nigra*, *S. argus*, *Corythoichthys*

*intestinalis*, *C.haemotosterus*, *Dunckerocampus dactyliophorus*, *Doryrhamphus excises*, *D.japonicus* and *Phyllopteryx taeniolatus* (Wilson et al., 2003). These coral fishes tend to be small, though the balistids are large. They spawn repetitively over long periods of time, often daily. Spawning may occur within a territory, as in damselfishes, or the pairs may ascend out of the territory, as in butterflyfishes. For most species, dispersal is restricted to larval stages in the plankton (Barlow, 1986). Incidentally, when a male of exclusive monogamous pair was removed, the territory shrunk, and some females lost the territory too. Removing the females had no such effects; the males successfully maintained the territories at a little additional cost (e.g. *Chaetodon multicinctus*, see Barlow, 1986).

Typically, the biparental monogamy occurs in freshwater fish species belonging to Osteoglossidae (bonytongues), Ictaluridae (American catfishes), Heteropneustidae (Asian catfishes), Aphredoderidae (Pirate perches), Cichlidae and Channidae (Snakeheads). They are characterized as 1. Biparental caretakers of eggs and fry, 2. Territorial for only breeding, 3. Having demersal eggs, 4. Large fishes, 5. Seasonal breeders and 6. Capable of dispersal at any age. Distinct seasonality and flooding of aquatic systems by monsoon make it more profitable for a large female to remain with the male and assist in caretaking. Incidentally, in a large country like India many rivers flood during different calendar months and the snakeheads like *Channa striatus* are claimed to breed non-seasonally and have seedling available to make their aquaculture almost a year-round activity. But in reality, *C.striatus* is indeed a seasonal breeder and its breeding is limited to a few calendar months in different parts of the country (Fig. 35).

Among marine fishes too, biparental care is limited to the egg stage only and is exhibited by species belonging to Gobiidae, Pholidae (Gunnels), Anarhichadidae (Wolffishes) and Gobiesocidae (Clingfishes). Many of them are eel-like fishes and lay a clutch of adhesive eggs, which are fixed to the substratum.

Hypotheses and experiments: Biparental care needs a precursor male care of eggs. Then "males are favoured as site-constant individuals because males are able to produce new batches of fertile gametes and re-mate at shorter intervals than females. A male monopolizing a site will leave more descendants than a female occupying that site for the same duration" (Bayliss, 1978). However, males typically invest less in their offspring relative to females; hence the parental investment theory predicts that males should behave polygynously, and monogamy should be uncommon (Trivers, 1972).

Monogamy has generally been explained by the need for biparental care or the uniform distribution of limiting resources. With the absence of biparental care and population limitation by recruitment rather than resources, monogamy in coral-reef fishes indeed poses a challenge to

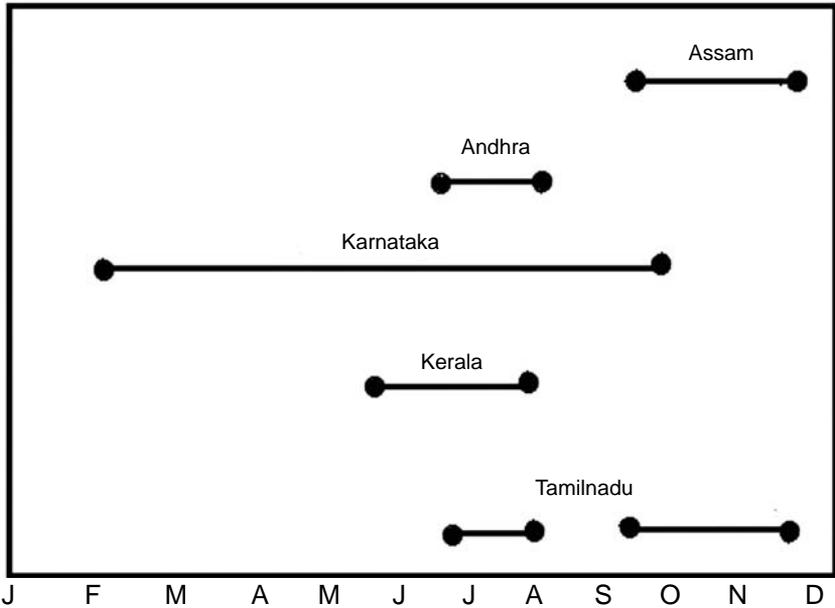


Fig. 36 Spawning season of the striped murrel in *Channa striatus* in Indian states (from M.A. Haniffa, personal communication).

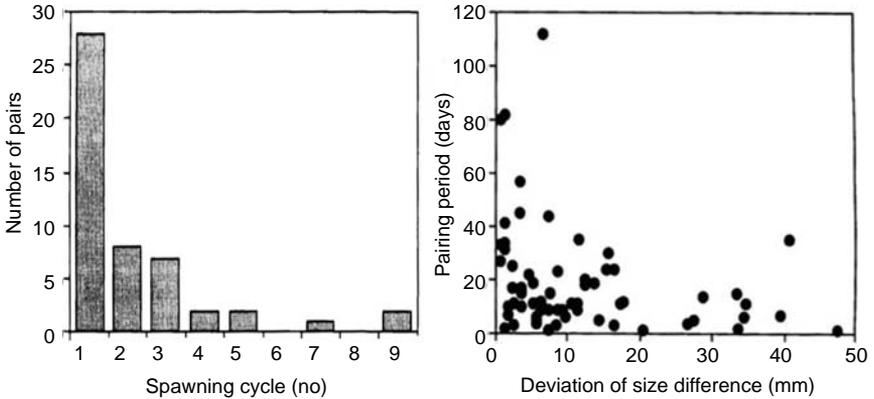
these hypotheses. Hence Barlow (1986) hypothesized that an abundance of resource (nest sites etc) allows all males to breed and given a one-to-one sex ratio, females would be able to guard a monogamous mate. The hypothesis suggests that guarding the sex gains a selective advantage from monopolizing a mate. Males can assure their paternity by sequestering females and several benefits have been suggested for females that mate a guard.

Reavis and Barlow (1998) tested the available hypotheses in the coral-reef goby *Valenciennesa strigata*. The female goby spawn every 13 days throughout the year and at all times of the lunar cycle; males care for eggs in the nest for 1–4 days and can spawn with a second female within 6 days. Thus females limit male reproductive success (Reavis, 1997). However most pairs mate monogamously over multiple rounds of reproduction. Hence, the goby provides an ideal model to test the available hypothesis. These hypotheses are (i) limited and uniformly distributed resources (Emlen and Oring, 1977), (ii) limited resources and pairs remain together to defend territory (Hourigan, 1987), (iii) low population density, pairs remain together because mates are rare (Clark and Phole, 1992) and (iv) abundant resources, both sexes enforce monogamy, which provide benefits to both (Barlow, 1986). They may be brought under two heads: 1. Limited resource hypotheses, which include those of Emlen and Oring

(1997), Hourigan (1987), and Clark and Phole (1992), in which one or the other resource is limiting and 2. Abundant resource hypothesis of Barlow (1986). Testing these hypotheses Reavis and Barlow (1998) found no evidence for resource limitation; food densities within territories did not differ from that at the unused habitats adjacent to territories; potential nest sites were also available in these unused habitat. If resources limit the population, territory defense might require the co-ordinated efforts of a pair; however, widowed goby maintained their territories. All the widow(er)s remated and the time required to remate was equal between sexes and during different seasons. Widow(er)s spawned with their new mates 9 days after the mate removal. All males maintained a nest but both sexes enforced monogamy by mate-guarding and females benefited from guarding a high quality (large) mate and fed more than when they paired with small males. Hence abundant and uniformly distributed resources can lead to monogamy by lowering the cost of guarding a mate, when mate guarding provides benefits.

The findings of Reavis and Barlow (1998) that the widowed/deserted goby soon find a mate and rapidly remate was confirmed in another goby *Valenciennesa longipinnis*. The close relationship between the monogamous goby involves division of labour in burrowing and maintenance of the burrow, which is likely to be one of key benefits of pair formation (Takegaki and Nagazono, 1999b). In fact, the females burrow less frequently when paired with large males, as the burrowing ability of a large male with its big mouth is high. Takegaki and Nagazono (1999a) reported that in the Japanese goby too, mate desertion occurred, when larger potential mates, relative to the existing partner, became available. The number of solitary males was more than that of females, indicating that the female desertion was more often than males. The occurrence of size mis-matched pair and overlapping home ranges seem to facilitate mate desertion. Deserting males and females soon found respective new mates and commenced spawning within 2 days after desertion. However, the deserted gobies had to wait for 1–20 days to find a new mate and start remating. About 56% pairs separated after only one reproductive event. However, there were also fidelous pairs, which did not separate up to the ninth spawning (Fig. 37). Deviation of size difference appears to be the major cause for desertions. The larger the deviation of size difference, the shorter is the paired duration (Fig. 37).

With more than 1000 and odd species, Cichlidae is one of the most speciose families among fishes. Of them 29% are substrate brooders, 70% are mouthbrooders and 1% comprise intermediates between them (Keenleyside, 1979). More than 60% cichlids are maternal mouthbrooders (Kuwamura, 1986). Hence the cichlid females have to meet the costs of time and energy for production of gametes and protection of eggs and fry.



**Fig. 37** *Valencienna longipinnis*: Left panel: frequency distribution of the number of spawning repeated by the same pairs. Right panel: relationship between pairing period and deviation of size difference between mates in 70 pairs (from Takegaki and Nakazono, 1999a).

This is quite a contrast to all other fish groups, in which males meet the costs of egg brooding, while the females bear the costs of egg production. Through a simple but elegantly designed series of experiments, Balshine-Earn (1995) provided quantitative estimates for the two components of reproduction in a cichlid, namely the high costs of mouthbrooding and gamete production. St Peter's fish *Sarotherodon galilaeus* is a biparental mouthbrooding cichlid; the females spawn once in 13 days but the males are ready to milt even on the next day after mating, irrespective of mouthbrooding or not. By removing the mouthbrooded eggs/fry from females and males, the costs of mouthbrooding and egg production were assessed. Mouthbrooding costed the females heavily; the female prolonged the inter-spawning interval from the usual 13 days to 17 days; its subsequent brood size was reduced by 5.2 g, while that in a non-mouthbrooder was increased by 2.3 g. However, the egg size of 1.9 mm and 2.5 mm was maintained in the first and second broods by the mouthbrooding and non-mouthbrooding females, respectively. The mouthbrooding female did not lose body weight but the male lost weight by 0.1 g.

All types of parental care in cichlids are based on two cardinal elements: territoriality and orchestrated courtship behaviour of the territorial male and its female partner; these vary greatly in different mating systems from monogamy to polygyny. With emancipation from mouthbrooding, the cichlidan males are inclined to become polygynic (Fishelson and Hilzerman, 2002). Figure 38 shows the proposed pathways of evolution of parental care and mating systems of the Tanganyikan cichlids (Kuwamura, 1997) and suggests that these cannot be considered in isolation.

In cichlids, two main methods of parental care are recognized: guarding (substrate-brooders) and mouthbrooding. Some of them exhibit

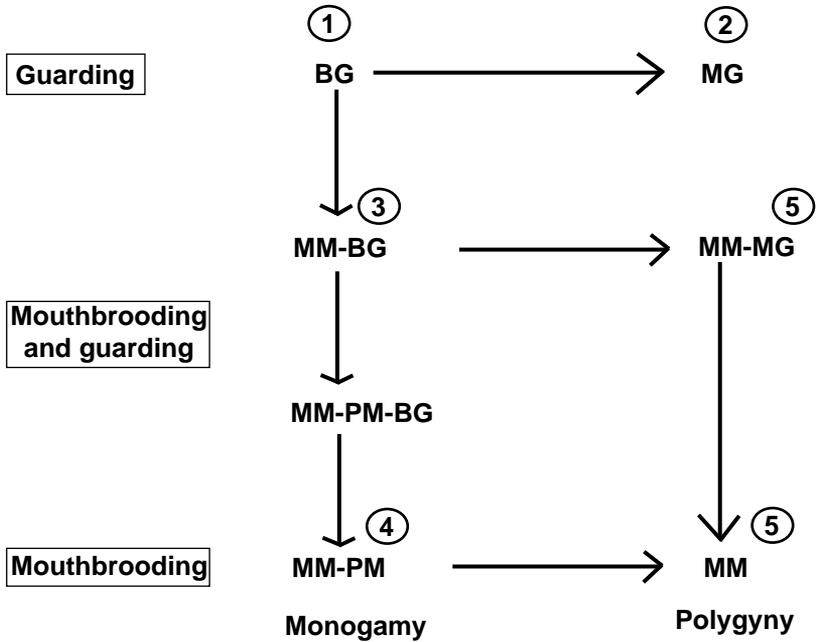


Fig. 38 Pathways of evolution of parental care and mating system in Cichlidae. MM = maternal mouthbrooding, BG = biparental guarding, MG = maternal guarding, PM = paternal mouthbrooding (from Kuwamura, 1997).

different combinations of both guarding and mouthbrooding, which may involve biparental or maternal care alone. Kuwamura (1997) recognized five methods of parental care among the Tanganyikan cichlids: 1. *Biparental guarding* cichlids spawn large numbers of (1,000–10,000) small (<2 mm) adhesive eggs, which on attachment to the rocks, are guarded by a monogamous pair, 2. In *maternal guarding*, only females take care of the offspring, 3. In *maternal mouthbrooding and biparental guarding*, females mouthbrood eggs and wrigglers (newly hatched and non free-swimming young), and fry (free swimming young) are guarded by both parents. 4. In *female to male shift of mouthbrooding*, the female's first mouthbrood eggs and wrigglers and then males take over the role. In *Chromidotilapia guntheri*, the male is the first to brood the deposited eggs and the wrigglers are mouthbrooded by both parents (Keenleyside, 1991) and 5. *Maternal mouthbrooding* involves only females in brooding and guarding (Fig. 39).

Among the polygynic cichlids, three types are recognized: 1. *Harem polygyny*: A male defends his territory, which includes more than one female staying atleast for a brooding cycle (Table 14). During the rainy season, 36% of the female members had male helpers to avoid predation of young ones but as the water level fell and the density of predators went

	Egg	Wiggler	Fry	Examples	Species (no)
1. Biparental guarding		BG		<i>Neolamprologus toae</i>	21
2. Maternal guarding		MG		<i>N. furcifer</i>	25
		MG		<i>Lamprologus lemairii</i>	
3. Maternal mouthbrooding and biparental guarding		MM	BG	<i>Perissodus microlepis</i>	10
4. Female to male shift of mouthbrooding	MM	PM	BG	<i>Xenotilapia flavipinnis</i>	11
	MM		PM	<i>Tanganicodus irsacae</i>	4
5. Maternal mouthbrooding		MM	MG	<i>Labochilotes labiatus</i>	8
		MM		<i>Cyathopharynx furcifer</i>	52

Fig. 39 Patterns of parental care in Tanganyikan cichlids: For abbreviations see Fig. 38 (from Kuwamura 1997, modified).

up, more than 55% of the female members had male helpers (see Barlow, 1984), 2. *Male territory-visiting (MTV) polygyny*: Males maintain territory, while females visit to spawn but no pair bond ensues, as the females leave the male territories after spawning and pick-up the fertilized eggs and 3. *Non-territorial (NT) polygyny*: Males maintain no territory but defend only the spawning site during the spawning season. No pair bond continues after spawning (Kuwamura, 1997).

Experimental studies on polygyny and fidelity of St Peter's tilapia *Sarotherodon galilaeus* recall similar observations made on the Japanese goby *Valenciennesa longipinnis* (Takegaki and Nakazona, 1999a). About 55% of the observed 111 reproductive cycles involving 15 marked St Peter's tilapia, the males were territorial, did not take part in egg brooding and deserted the females after one spawning-cycle; others stayed up to 5 cycles, showing a tendency towards monogamy. The presence of additional females greatly influenced the decision-making of the male, decreasing his fidelity to his mate. The remaining 45% non-territorial males tended to take part in brooding more than the territorial males. Thus, the rich variety of reproductive strategies of males and their ability to change one to another characterize the high flexibility and adaptability of these cichlids (Fishelson and Hilzerman, 2002).

Recognition and identification of one's own species, sex and offspring among others are of critical importance to the reproductive fitness and experimental studies in cichlids provide an insight into this domain. The sexes of the monogamous cichlids are typically isomorphic and they pair well before spawning is imminent. They require a relatively longer duration to weigh multiple source of information on species, sex and quality of mate. On the other hand, multichannel 'dichromatism' in polygynous

species facilitates quick recognition of species and sex. Secondly, anisogamy predicts that females are very selective, whereas males may not be. But in monogamous species, males and females are equally choosy, especially when parental care is obligatory for the survival of the offspring. Thus fitness of male and female is similar (Barlow, 1992).

The Midas cichlid *Cichlosoma citrinellum* failed to distinguish between its own species and *C. labiatum*, when viewing through a one way mirror (Holder, 1991). However, the Midas did it, when chemical cues were provided, indicating that species recognition is based on a simple sensory mechanism involving olfaction (see Barlow, 1992). The ability to recognize sex was greatly diminished, when the choice was through a one-way mirror, which precluded interactive feed-back (Holder, 1991). Bayliss (1976) suggested that species and sex are recognized through interactive dynamics of courtship, especially during pair formation.

It is in this context, that Barlow's (1992) publication on species and sex identification in the monogamous, polychromatic Midas cichlid *Cichlosoma citrenellum* is very interesting. The Midas distinguishes between its own and a highly similar heterospecific behind a one-way mirror, only when visual and chemical cues are matched. Likewise, recognition of sex is hindered, even in the presence of chemical cues, when interactive feed-back is precluded. By active choice of the colour of the mate, the Midas cichlids mate assertively by colour in nature (Dominey, 1984). Greyish green (N) Midas females, whose parents are N in colour, spawn mostly with N males and gold (G) Midas of G parents select G Midas to the same degree (Barlow and Rogers, 1978).

In substrate-brooders like the convict cichlid *Cichlosoma bifasciatum*, the main stimulus to recognize their brood is visual; for instance, when a brood is replaced by eggs from another species or artificial eggs, the parental fish continues to fan and guard them, only if the new ones look like the old ones (Myrberg, 1964). Weber (1970) showed that the cichlid females fan artificial eggs made of wax. In the absence of visual cues, such as at night, it was not clear whether tactile and/or chemical stimuli allow the fish to find their eggs, and properly guard and fan them. Using wax eggs or eggs in tea bags, Reeb and Colgan (1992) experimentally investigated the fanning and guarding activity of the female convict cichlid and concluded the following: 1. The fish did not fan wax eggs at night suggesting that the tactile cues are not adequate to induce the female to ventilate the eggs. It is likely that the wax eggs did not mimic some subtle tactile cues of importance to the fish. All the fishes fanned natural eggs in tea bags indicating that the tactile cues are not necessary for nocturnal fanning and the chemical cues emanating from the eggs in the tea bags were sufficient to induce the female to commence the fanning activity.

Incidentally, chemical cues have already been linked to the recognition of young ones by the parental cichlids (Lutnesky, 1989).

Within cichlid communities, brood mixing has been shown to occur under natural conditions, Nelson and Elwood (1997) experimentally showed that the biparental cichlid *Pelvichromis pulcher* discriminated between their own youngs and added conspecific young ones; however, the young ones were adopted into the brood, if they were of similar age/developmental stage to the parent's own young ones. If the added young ones were at a different developmental stage, they were consumed. Parents separated from broods up to 4 days accepted their young ones on reunion. But separation for longer than 4 days resulted in cannibalism. Chemical rather than visual stimuli seem to be involved in this relation. Both sexes appear to have equal information about their young ones.

## 11.2 Alternate mating strategies

Parker (1984) defined sperm competition as the contest between sperm from two or more males to fertilize ova. Males exhibit different morphological (e.g. jaw structure of hooknose *Oncorhynchus kisutch*; size: *Symphodus ocellatus*; body colouration: initial and terminal phase males of diandric protogyny), physiological (e.g. parasperm lump of the sculpin *Hemilepidotus gilberti*) and/or behavioural (e.g. cuckolder deceiving a male, as if he is a female) strategies to gain access to females. Time and energy investments by a terminal/territorial male on one or more of these strategies may allow him to allocate relatively less investment on gonad, whereas all other competitive conspecific males may allocate more on gonadal investments (Taborsky, 1994). There is also evidence that the Gonadosomatic Index (GSI) is higher, when there is a greater potential for sperm competition. Stockey et al. (1997) found a significant correlation between the intensity of sperm competition and relative testis size. As the time of milt ejaculation may considerably alter sperm competition, Kanoh (2000) regarded the timely sperm ejaculation as a measure of the male's investment rather than GSI.

Warner and Robertson (1978) distinguished the competitive role of streakers and sneakers. However, Taborsky (1998) put all of them together as 'parasites' and found evidence for the simultaneous competitive (rather than parasitic) spawning among 140 oviparous teleosts belonging to 28 families, indicating the prevalence of this alternate strategy. Preferring to retain the older term of Alternate Mating Strategy (AMS) and keeping in line with the definitions of stalker, sneaker, satellite and so on of Warner and Robertson (1978), Table 30 classifies some of these strategies; based on

**Table 30** Components of alternate mating strategies adopted by teleostean males.

## I. Genotypes

1. Primary vs secondary males  
e.g. Diandric protogynous hermaphrodites
2. Monochromatic vs dichromatic males  
e.g. Scarids
3. Harem vs pairing males with satellites  
e.g. *Pelvicachromis pulcher*
4. Territorial vs precocious males
  - a) Parentals vs cuckolders, e.g. *Lepomis macrochirus*
  - b) Jacks vs hooknoses, e.g. *Oncorhynchus kisutch*

## II. Phenotypes

1. Pair vs group-spawning  
e.g. *Rhodeus ocellatus*
2. Ontogenetic morphs
  - a) "initial vs terminal" phase males  
e.g. *Leptoscarus vaigiensis*
  - b) Territorial vs sneaker, satellite males  
e.g. *Symphodus ocellatus*
  - c) Territorial vs sneaker, satellite, piracy males  
e.g. *Telmatochromis vittatus*
  - d) Territorial vs territorial, sneaker, satellite males
  - e) Male vs hermaphroditic streaking  
e.g. *Serranus fasciatus*
  - f) Dominance based linear ranking  
Territorial vs sneaker cum cannibal  
e.g. *Zacco temmincki*

the components participating in the AMS. Broadly the competing conspecific male morphs are divided into I Genotypes and II Phenotypes. Of the genotypes, groups 1, 2 and 3 have already been described elsewhere.

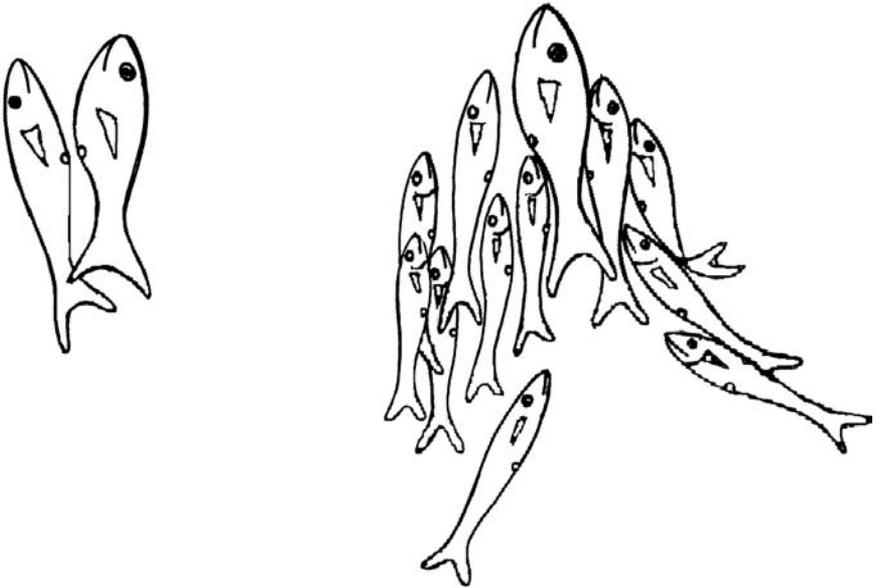
**Territorial vs precocious males:** In the bluegill sunfish *Lepomis macrochirus*, some males, designated as 'parentals', mature at the age of 7 years and use a territorial mating strategy, defending nests and courting females. The other males are called 'cuckolders'; among them young 'streakers' aged 2–3 years, dart into the nests during spawning to stealthily fertilize eggs; the other 'satellites' aged 4–5 years bear the female's colour and behaviour, and deceive the 'parental', as if they are females in the nests. Despite receiving equal feeding and sharing equal maternal genes, the offspring sired by the cuckolders precociously grow fast. However, the cuckolders die before they ever reach the size of mature parentals and there is no evidence that they ever become parentals themselves (Gross, 1982, 1984). Females do not discriminate cuckolders from parentals and spawn in the presence of parentals and cuckolders. Both the sneaking and streaking cuckolders are excellent sperm-competitors and fertilize about 80% of the eggs that the females spawn. Nest-tending parentals provide

less care to their 'suspected' progenies: they may partially cannibalize or abandon their brood (Neff, 2003a, b). Incidentally, microsatellite analyses to confirm the paternity of cannibalized or abandoned eggs may reveal whether the nest-tending parental had any cue to distinguish the progenies sired by him from those of the cuckolders (cf Jones et al., 1999). Surprisingly, the females of sunfish may also release up to three times more eggs in the presence of cuckolders than when they spawn in the presence of a parental only (Fu et al., 2001). Neff (2004) found that the progenies of cuckolders grew faster than those of the parentals. In the field, he found that the progenies of the cuckolders had three times higher survival than those of parentals, especially from the predation of *Hydra canensis*.

In the coho salmon *Oncorhynchus kisutch*, the jacks mature at the age of 1+ and survive up to the age of 2+, whereas the hooknoses mature and succumb at the age of 2+ and 3+, respectively. The hooknoses fight for a position close to the female, but the jacks stealthily sneak into the position. The life time fecundity of a jack is equal to that of a large hooknose. Large jacks are at a disadvantage for want of adequate hiding places and the young hooknoses for losing the fight to get the position (Gross, 1984, 1985).

Pair vs group-spawning: When pelagic-spawning marine fishes like the labrids, scarids and serranids release their gametes freely into the water, Fertilization Success (FS) (and not fertilization rate, see Robertson, 1996) can be influenced by factors such as water turbulence and the proximity and density of spawners, which affect sperm concentrations and hence the egg-sperm encounter probabilities (Levitan, 1991). In spite of the release of a large number of sperm by males, the rapid diffusion of eggs and sperm by water movement in the time required for the sperm to reach the eggs reduces the FS substantially. For instance, the physical separation of sexes by a distance as little as 20 cm at the moment of gamete release may dramatically reduce the FS far below 100% (Levitan, 1991; Lasker and Stewart, 1993).

Among pelagic-spawning coral fishes, as in *Thalassoma bifasciatum*, there are two types of males, one spawning in single, i.e. male-female pairs, and the other spawning primarily in groups. With relatively narrow bodies and the ability to swim rapidly, the pair can closely oppose their respective gonopores at the apex of 'spawning rush', when gametes are released. Consequently, the gametes are diluted to a lesser degree and thereby the pairs ensure higher FS. However, in group-spawning males, who are not agnostic to each other and are mostly primary males (see Warner and Robertson, 1978), close proximity between the female's gonopore with those of 5–20 males may not exist (Fig. 40); however, the simultaneous exposure of eggs to 6–50 times more sperm released by group-spawning males may provide some more opportunity for the sperm to meet the eggs



**Fig. 40** A generalized comparative picture showing the orientation of pair-spawning and group-spawning fishes at the apex of spawning rush. Note the proximity of vents between the pairing female and male in pair-spawners and the distances between the vents among group-spawners.

(Shapiro et al., 1994). Understandably, the primary (IP) males, who are known to opt for group spawning or streaking, (Warner and Robertson, 1978) show nearly four times greater GSI (Table 31) than the secondary males, who are known to more correctly assess the quantum and appropriate time of ejaculation (Munoz and Warner, 2003; Kanoh, 2000).

Many experimental investigations were undertaken to estimate the FS in pair and group spawners (e.g. Petersen et al., 1992). To estimate the FS of pair and group spawning *Thalassoma bifasciatum*, Marconato et al. (1997) designed net and bag sample collections at different intervals following the release of gametes and came to the following conclusions: 1. Eggs collected by bags 30 seconds after the gamete releases are probably the most accurate measure of the FS, 2. Minimum sperm count to ensure fertilization is  $10^5$  and at the count of  $10^6$ , the success is above 98%, 3. The FS of eggs sampled at 30 seconds after the gamete release was significantly lower for pair spawning (90–97%) than those (98.0–98.9%) obtained for group spawning and 4. In general, group spawns fertilize larger proportions of a female's egg than do pair spawns, although the magnitude of the difference may change from a non-significant 2.6% to significant one of 3.5%.

**Table 31** Gonado somatic index of some labrids and scarids PM = Primary male, SM = Secondary male (from Robertson and Warner, 1978; Warner and Robertson, 1978).

Species	Gonado somatic index	
	PM	SM
Labridae		
<i>Bodianus rufus</i>		0.09
<i>Halichoeres radiatus</i>		0.13
<i>Clepticus parrae</i>		0.12
Mean		0.11
Scaridae		
<i>Scarus vetula</i>		0.11
<i>S.taeniopterus</i>		0.05
Mean		0.08
Labridae		
<i>Halichoeres bivittatus</i>	0.35	0.59
<i>H. garnoti</i>	0.08	0.15
<i>H. maculipinna</i>	0.16	0.16
<i>H. pictus</i>	0.30	0.13
<i>Thalassoma bifasciatum</i>	2.71	0.22
Mean	0.72	0.30
Scaridae		
<i>Cryptotomus roseus</i>	1.15	0.41
<i>Sparisoma rubripinne</i>	1.57	0.12
<i>S. chrysopternum</i>	0.16	0.07
<i>S. viride</i>	0.33	0.06
<i>S. radians</i>	3.35	0.76
<i>S. aurofrenatum</i>	0.67	0.11
<i>S. atomarium</i>	0.27	0.20
Mean	1.07	0.25
<b>Mean of means</b>	<b>0.90</b>	<b>0.28</b>

Incidentally, mass-spawning refers to simultaneous and apparently synchronous spawning of the majority of a mating aggregation consisting of 500 to 2000 members. Coralfishes of at least nine families form large mating aggregations as part of their reproductive repertoire. The formation of these aggregations often entails extensive migration to the mating site (Domeier and Colin, 1997). In *Acanthurus nigrofuscus* (Kiflawi et al., 1998) and *Thalassoma bifasciatum* (Marconato et al., 1997), over 60% of group spawned eggs are fertilized within 5 seconds. Hence mass-spawning may make no significant contribution to the already high FS attained by group spawning (98.5%).

In pair-spawns, the male gauges its sperm output to the size of the female and hence to the number of eggs released in individual spawning episodes (Shapiro et al., 1994). Group-spawning involves sperm competition and the number of sperm ejaculated by males is much higher than in pair spawns. At least pair-spawning and harem males are capable of

controlling both in terms of timing (see p *Rhodeus ocellatus*, Kanoh, 2000) and quantum (*Sparisoma radians*, Munoz and Warner, 2003) of sperm ejaculation according to the need, a phenomenon possibly aimed to optimize a regime of sperm economy. A male may 'trade' a lower FS for a higher number of matings, if that allows him to maximize the total number of eggs fertilized, a strategy, which is contrary to the female's interest of having all her eggs fertilized.

To demonstrate the sperm economy and FS, Marconato et al. (1995) undertook an investigation in the polygynous Mediterranean wrasse *Xyrichtys novoacula* by collecting all sperm and eggs released in individual spawns from three adjacent male territories holding different numbers of females. A female spawns once daily and the territorial male courts successfully with all female members of his territory once a day. Males holding 4 or 6 females each release larger numbers of sperm per spawning than that of the male simultaneously holding 12 females in 2 territories each with 6 females. With increasing cumulative egg output by all the females, the number of sperm released by the territorial male increased from about  $5 \times 10^6$  sperm for  $0.5 \times 10^4$  eggs to  $15 \times 10^6$  sperm for  $1.6 \times 10^4$  eggs (see Fig.2 of Marconato et al., 1995) and thereby indicates that the territorial male does indeed gauge his sperm output according to the number of eggs released by the females. Although the FS decreased from 98% to 87%, the male with the largest harem consisting of 12 females fertilized the highest total number of (56,777) eggs every day in comparison to 24,482 and 36,864 eggs fertilized by males holding 4 and 6 females, respectively during the reproductive season lasting from May to October.

Sperm limitation is known to occur and reduces FS among group spawning species (Petersen, 1990; Shapiro et al., 1994) and has implications to the probability of egg-sperm encounters, especially where the egg size is also increased resulting in the reduction of fecundity and duration of floatation of eggs due to the possible increase in egg density. Considering the GSI of males and egg volume and density of 25 species belonging to the Labridae, Scaridae and Serranidae, Robertson (1996) came to the following conclusions: 1. There is no significant difference between volume and density of pair-spawning and group-spawning species; for instance, egg density of pair-spawning *Serranus tabacarius* and group-spawning *Halichoeres maculipinna* is equal, i.e.  $0.06 \mu\text{g}/\text{ml}$ , despite the volume of the former being nearly 3.5 times larger than the latter, 2. The male GSI of group-spawning male serranids and labrids is nearly 1.5 to 2.0 times greater than those of respective pair spawners. In serranids, the GSI of male leopard grouper *Mycteroperca rosacea* are equal (7.2) to other group-spawning groupers and is considerably higher than other pair spawning groupers like *M.phenax* *M.tigris* and *M.microlepiz* (GSI 0.6–3.0) (see Erisman et al., 2007), 3. Egg density does not decline with increasing

volume, and, 4. Hence sperm limitation seems unlikely to represent a significant problem for pair-spawning. Thus egg size/density relationships offer no clear support for the sperm limitation hypothesis.

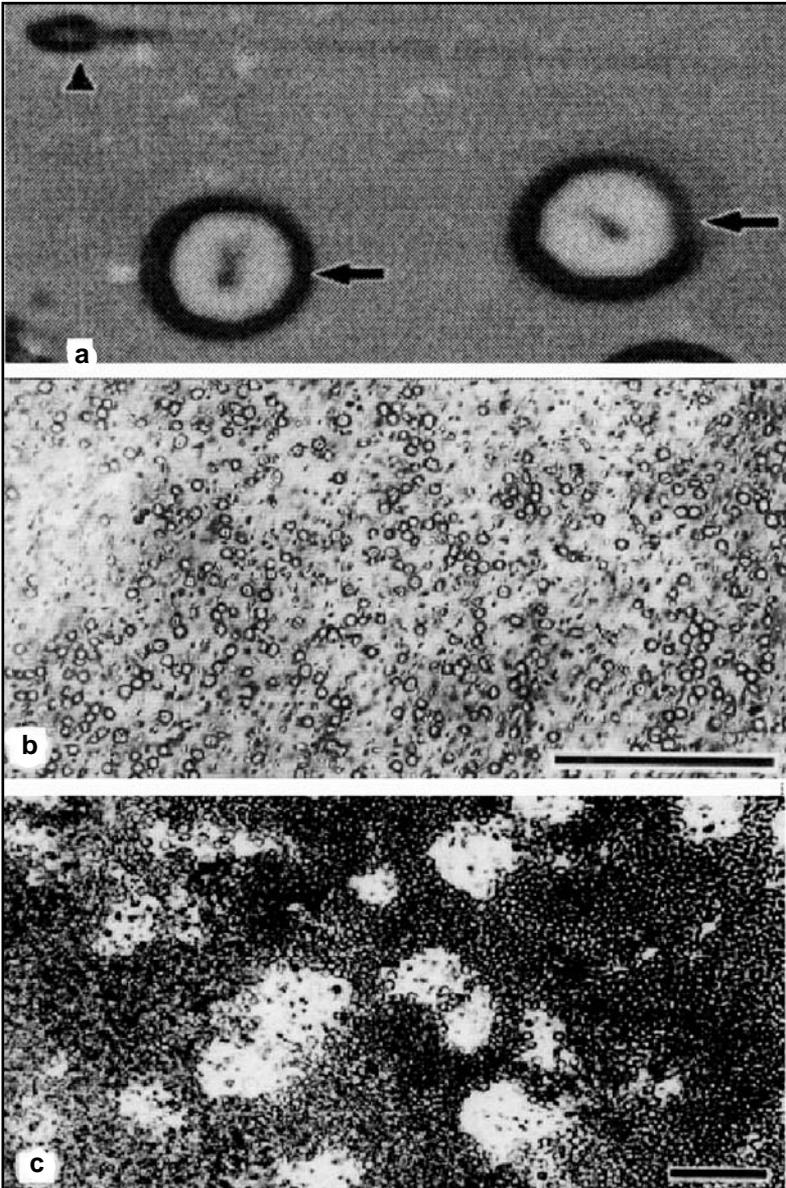
Kanoh (2000) provides quantitative data on the FS of pair spawning and group spawning from his experiments on the rose bitterling *Rhodeus ocellatus*. According to him, pair spawning involves one male and one female (OSR:1♀:1.1♂); on arrival of a female at the mating site namely the mussel *Anodonta woodiana* of a territorial male, courtship movements are commenced to oviposit eggs by one female and the male ejaculates successively. In group-spawning (OSR:1♀:4.9♂), no male or female performs courtship movements, but ejaculation occurs continuously. Pair spawning with sneaking (OSR:1♀:3.1♂) is distinguished from group-spawning according to whether courtship movements are commenced or not. Kanoh also grouped sneakers into smaller ('streaker' that seldom forms a territory) and medium-sized ('sneaker' that sometimes forms a temporary territory but intrudes into others) sneakers.

The frequency of the three mating strategies was 36% for pair-spawning, 46% for pair-spawning accompanied by one or more sneakers and 18% for group-spawning. In pair-spawning with interruption by sneakers, 18, 11, 10, 5 and 1% pair-spawnings were accompanied by 2, 3, 4, 5 and 7 sneakers, respectively. With increasing number of accompanying males in pair-spawning or group-spawning, the timing of ejaculation was inappropriate. Thus the frequency of pre-oviposition ejaculations made was over 60% for sneakers and 36% for territorial males, when intensely contested by sneakers. Early ejaculation especially prior to oviposition/egg spawning may considerably reduce the valuable shelf life of sperm for about 20 seconds (see Kiflawi et al.,1998). Consequently, the fertilization success, as determined by isozyme analyses of 'fathers' and progenies showed that reproductive success was 61, 31 and 11% for the pair spawning, pair spawning accompanied by sneaking and group spawning strategies, respectively. Incidentally, the use of genetic markers such as isozyme analysis of genetically polymorphic protein markers or the direct techniques of DNA fingerprinting provides a more reliable data on fertilization success rather than the visual observations on successful pair spawning and group spawning.

Besides bringing their vents as close as possible, say within 20 cm (Leviton, 1991), and ejaculation of semen at appropriated quantum (Munoz and Warner, 2003) and time (Kanoh, 2000), the pair-spawners may also adopt other tactics to increase their FS; a tactic may be to produce parasperm to block the sperm of sneakers and other contestants from reaching the eggs and thereby reduce sperm competition from sneakers and others. In many aquatic animals including fishes, sperm polymorphism has been reported (e.g. marine snails: Buckland-Nicks et al., 1982; Cottid fish, Hayakawa

et al., 2000) i.e. the males occasionally produce normal eusperm along with dimorphic parasperm. Eusperm have complete haploid genome, whereas the parasperm are binuclear, due to incomplete second meiotic division; and hence can not fertilize eggs. A parasperm is a large (nearly 10 times larger than the eusperm) erythrocyte-like cell (5–7  $\mu\text{m}$ ) without a flagellum (Fig. 41). The parasperm are released along with eusperm at mating. The role of the parasperm in the sculpin *Hemilepidotus gilberti*, whose reproduction is characterized by polyandrous oviposition involving sneaking by neighbouring territorial males, has been elucidated by Hayakawa et al. (2002). The released sperm move through the sea water and reach the eggs that are enclosed with viscous ovarian fluid (OF). Eusperm actively swim through the OF and fertilization takes place within the OF, where spawned eggs retain the ability to be fertilized (Hayakawa and Munehara, 1998). The OF covers the whole egg mass during egg spawning, although it gets dissolved in sea water within a few hours. It is not clear whether the presence of OF is responsible for the extended shelf life of eggs for about 1–3 hours (see Fig.8; Kiflawi et al., 1998). While the non-mobile parasperm are moved through sea water, which is in confluence with OF by colliding eusperm, the parasperm form lumps and obstruct the movements of late-arriving sperm and thereby minimize sperm competition from sneakers and others. Hayakawa et al. (2002) have proposed that eusperm of the territorial male reach the eggs first, while his parasperm lumps hinder the sperm of other males from reaching eggs. However, their proposal remains to be tested in other fishes, in which spawning by territorial is contested by sneakers.

Regarding the ontogenetic transformation of sneakers to satellites and to territorials, relatively more information is available for *Symphodus* spp. A summary of information on size ratio, GSI and some aspects of the mating system of four species belonging to the Mediterranean wrasse *Symphodus* suggests the following: 1. The smaller sneakers and satellites constitute 70–80% of the males, and thereby indicates that these phenotypes grow to attain the status of territorials, 2. Smaller males and females of all the 4 species have 1.5 to 4 times greater GSI values than those of the larger males and females; incidentally, Taborsky (1998) showed that the GSI sharply declined from about 10 in sneakers (2–4 g), 8–5 in satellites (4–6 g) and remained at around 2 among territorials (8–11 g); further, he found that the sneakers and satellites lost 0.44% of their initial body weight after the reproductive season, while the territorials lost only 0.17% of their body weight for the entire breeding season, 3. With elaboration of the nest, territorial defense and egg ventilation are accompanied. But with simplification of the nest or no nest, the frequency of interference of territorial spawning is decreased from 47% in *S.roissali* to 0.4% in *S.melanocercus* and 4. There is a tendency for smaller males to mate



**Fig. 41** *Hemilepidotus gilberti*: a) shows parasperm ← and eusperm ▲. b) shows parasperm and eusperm mixed in sea water. Granular materials like beads and the small dots between parasperm are the eusperm. c) shows the formation of lump of parasperm after 5 mins (from Y, Hayakawa et al., 2002 Obstructive role of the dimorphic sperm in a non-copulatory marine sculpin *Hemilepidotus gilberti* to prevent other male's eusperm from fertilization. *Env Biol Fish*, 64 : 419–427, with kind permission of Springer Science and Business Media).

smaller females and larger males to mate larger females; thus, 50 to 87% smaller males have successfully mated with smaller females; 13% males, which did not mate with smaller females in *S. melanocercus* interfere only with 0.4% matings between pair spawners, i.e. territorial male with a large female.

*Axoclinus nigricaudus* and *A. carminalis*, the Mexican blennoids show bimodal size distributions of males and females (Neat, 2001). The size bimodality is so clear that one may doubt whether smaller sneakers (small non-territorials SNT) and medium sized satellite (larger non-territorial LNT) males with different body colouration and territorials are genotypes. However the presence of territorial males in the lower modes, especially among 13 mm size classes indicates that these sneakers and satellites are only phenotypes. Yet it is not clear why the males of these blennoids do exhibit such a clear bimodal size distribution.

*A. nigricaudus* occupy relatively smaller territories (40 cm<sup>2</sup> each), with a larger aggregation (4–10) and has a male ratio (0.8♂) higher than those (territory: 60 cm<sup>2</sup>, aggregation: 1–7, male ratio: 0.66♂) of *A. carminalis*. The former includes larger number of smaller sneakers (small non-territorials SNT) and satellites (medium-sized non-territorials LNT) than the latter. Consequently, the number of mating success achieved by satellites and sneakers of *A. nigricaudus* is nearly twice (98%) higher than that (57%) of *A. carminalis*. A remarkable feature of *A. carminalis* is that the territorials of adjacent territories successfully mate with the females of alien territories that too in high frequencies (43%). *A. nigricaudus* has greater GSI, as it has a higher male ratio, encounters more intense contest from a larger number of sneakers, satellites and alien territorials, and more intense sperm competition. However both blennoids have a bigger accessory testicular gland. It is possible that the gland plays a role by differently determining the size, viability and motility of sperm of territorials and others (Neat, 2001).

Through his publications, Katano (1983, 1990a, 1992) has brought to light a different dimension of acute competition between pair-spawners and a large number of satellites contesting them. Using linear dominance as an index, he recognized territorial and satellite (or sneaker?) males of the dark chub *Zacco temminckii*. A paired male bury the released eggs in the redd by vibrating his anal fin, but the satellites prevent to cannibalize the eggs. Of 381 observations on spawning, the satellites stole 31% spawnings, cannibalized 61% of pair-spawned eggs and did both in 8% of spawnings. With the increasing number of spawning success by satellites the number of buried eggs decreased. Thus, the satellites caused a loss to paired males in sperm competition and egg cannibalism, indeed a rare phenomenon not thus far known in other pair-spawning species. Since body size and dominance by aggression mark the territorial male, it may be appropriate that, a reference is now made to the sexual dimorphism and sperm competition.

### 11.3 Morphism

The size advantage model (Ghiselin, 1969) has successfully explained the adaptive significance of larger male size in many dimorphic gonochores and protogynous hermaphrodites. Because males typically invest a little on their offspring relative to females, parental-investment theory predicts that the males should behave polygynously (Trivers, 1972) and male-male contests should result. In species, in which males compete for mates, territories and other ecological resources to attract females, the contest competition favours large males (Anderson, 1994). Large aggressively dominant males may have greater access to many females and tend to monopolize spawning with them. Investment on somatic growth to attain the large body size (Macroandry, Robertson, 1985) and accompanying dominant behaviour may allow only smaller investments on testis. Conversely, the males that mate randomly or in a group, and stalker, sneaker and satellite males, which do not invest in body growth (Microandry, Robertson, 1985) and colouration (cf. I P♂) can afford to have heavy gonadal investments and await intense sperm competition. Considering surgeonfishes as examples, the implications of male dimorphism on the spawning pattern, sperm competition and GSI are explained by Robertson (1985).

Among 58 minnow species of North America, Pyron (1996) found that sexual size dimorphism (SSD) varied greatly from species with microandry to species with macroandry but in most species females and males were isomorphic. SSD is largely a function of the mating system; males characterized by macroandry tend to mate with many females. Hence, Pyron considered three important variables of mating systems namely (i) probability of sperm competition, (ii) male territorial guarding and (iii) male-male contest. The probability of sperm competition was scored from 1 to 4; scores 1, 2, 3 and 4 were assigned to (a) pair spawners, (b) crevice-spawners, (c) pair-spawners but mostly group spawners and (d) group spawners. Male territorial guarding behaviour was given score 1 for no territorial guarding, 2 for guarding of territory and 3 for guarding territory and eggs. Male-male contest behaviour too was scored from 1 to 3 based on whether males have (1) No contests, (2) Contests present and (3) Frequent contests. Using these score systems, Pyron constructed many cladograms and found: 1. There was no significant correlation between SSD and sperm competition score, 2. The correlation between SSD and male-male contest score was also not significant, 3. But that with male territorial guarding score was positive and significant and 4. The male guarding score is a significant predictor of SSD. Parker (1992) found sperm competition and male guarding were significant predictors of SSD at the family level. But Pyron found that the relationships between mating system variables and SSD in North American minnows were significant

even at species and genus levels. Only male territorial guarding was a significant predictor of SSD at the species level and male-male contest was a significant predictor of SSD at the genus level. However, Pyron's study showed concordance with Parker's (1992) conclusions that males should be the same size (isomorphic) or smaller (but not dwarf; i.e. microandry) than females in species with high sperm competition. Indeed, males are usually 60–80% of the length of females among the viviparous guppies like the poeciliids and anablephids and 80–90% of the length of females among goodeids. In some of them competition for access to a female and hence sperm competition are very intense (Bisazza, 1997). As the evaluation of SSD appears to be strongly influenced by the mating systems, more investigations like that of Pyron are desired.

#### 11.4 Lekking and promiscuity

Lekking is the temporary aggregation of sexually active males for reproduction. The aggregation of males on the lek is visited by females singly or *en masse*, who select the males with whom they mate. Once mating is accomplished, the females leave the lek. The incidence of lekking by one or more species belonging to many families are listed by Loiselle and Barlow (1978); the following are important: Cichlidae, Sparidae, Labridae, Scaridae, Acanthuridae, Poeciliidae, Cyprinidae, Characidae and Centrarchidae. All lek fish are oviparous. Among cichlids, the egg-laden female leaves the lek, while the male remains awaiting for other mates. Lekking teleosts are characterized by polygamy. Evolution of lekking in teleosts depends upon suitable spawning sites. Lekking sites must provide adequate mobility to move between separate areas for breeding, feeding and performance of parental care (Loiselle and Barlow, 1978).

Although many publications indicate the occurrence of lekking in fishes, none has made detailed study. Lek-like courtship occurs in *Synodus dermatogenys*, as males aggregated at the Wong Beach Saipan, Marian Islands, especially when population numbers are high (Donaldson, 1990). Some lek-characteristics described by Loiselle and Barlow (1978) like no feeding at the lek, presence of more males, reduced aggression and dimorphism are described but not quantified.

There are reports on infidelity among monogamous pairs, in which the relation lasts for a single spawning period alone (Fig. 33). However, a rare genetic monogamy has been described in the monogamous channel catfish *Ictalurus punctatus*; employing seven microsatellite loci, Tatarenko et al. (2006) showed that the egg guarding male has mated with his partner only. Quite contrastingly, van Doornik et al. (2008) found that in the Pacific Ocean perch *Sebastes alutus*, 71% females had broods sired by multiple paternity. Elsewhere it was described that using cloned microsatellite loci,

Jones et al. (1999) described the 'bed-hedging' multiple maternity in the pipefish *Syngnathus typhle*. Microsatellite DNA analysis by Hutchings et al. (1999) showed that there is multiple paternity within a single spawn of the cod *Gadus morhua*; of 17 males that were encircling the spawning female, the dominant one fertilized 85% eggs, the second dominant one 11% eggs and the third fertilized the remaining 4% eggs.

The extent of promiscuity of some fishes readily crosses even the taxonomic border. For instance, in sympatric populations of the closely related sand goby *Pomatochistus minutus* and *P. lozanoi*, Wallis and Beardmore (1980) found three species diagnostic protein loci and the fourth one virtually so. In a sample of over 1,600 individuals, 5 were with a hybrid genotype at all 3 loci. Of these 5, one was very unique. Based on the distribution of these four loci in that 5th individual, Wallis and Beardmore concluded that the individual may be an  $F_2$  hybrid resulting from a cross between two  $F_1$  hybrids of *P. minutus* and *P. lozanoi*.

Perhaps one of the most interesting promiscuities is that between the monandric protogynous hermaphroditic wrasses *Halichoeres margaritaceus* and *H. miniatus* from the Lizard Island, Great Barrier Reef. Under stable social conditions, the male of each species guards many intraspecific females and mates exclusively with those females daily. However, when the male *H. miniatus* was removed, the male *H. margaritaceus* enlarged his territory area to incorporate both *H. margaritaceus* and *H. miniatus* females and *H. miniatus* females participated in the courting process, resulting in pair-spawning bouts. When eggs from such spawnings were collected, 72% were found to be fertilized and these embryos hatched and developed into larvae. Though it is not clear whether these larvae are hybrids or gynogens, the experiments were successfully repeated a couple of times (Walker and Ryen, 2007). Due to the behavioural interaction and spawning between the *H. margaritaceus* male and the *H. miniatus* female, *H. margaritaceus* males affected sex change progression in *H. miniatus* females.

### 11.5 Parental care

Most fishes do not show any post-fertilization care of the eggs. No parental care is found in 78% of the teleostean fishes (Sargent and Gross, 1986). Consequently, less than 1% of the eggs of broadcasters like the pelagic spawning fishes survive (e.g. Watnabe et al., 1996). Among the 22% teleosts, which do care for their eggs and young ones, 11% are taken care by males, 7% by females and 4% by biparental care (Sargent and Gross, 1986). One or another form of parental care is more prevalent in freshwater families (57%) than marine families (15%, Bayliss, 1981; see also Wootton, 1998), suggesting that the parental care has evolved in fishes inhabiting spatially and temporally unpredictable environment for survival of the

zygote and young. Parental care may include guarding eggs and larvae from predators, ventilation of eggs to provide a flow of oxygenated water and removal of water containing nitrogenous excretory products and accumulation of silt and dead eggs. It costs time and energy; for instance, the cost of brooding eggs in *Chromis hypsilepsis* results in 85% reduction in feeding (Gladstone, 2007).

Among those, who care for their eggs and young ones, the cichlids present a contrast between provision of external care, as in substrate brooders and internal care, as in mouthbrooders. Biparental substrate brooding (guarding) is associated with monogamy and maternal mouthbrooding mostly with polygyny (lekking). However, various intermediate or exceptional patterns are also known (Keenleyside, 1979; McKaye, 1984, Kuwamura, 1986). Of more than 700 cichlid species present in Lake Tanganyika, nearly 30% are biparental substrate brooders and 70% maternal mouthbrooders (Keenleyside, 1979). Mouthbrooding predominates among the African cichlids, while most of the American and Asian cichlids are biparental substrate brooders. Among the substrate brooders, the most studied *Lamprologus brichardi* guard eggs and fry in pairs or harems and often with helpers (Taborsky and Limberger, 1981). The average number of eggs/larvae held in the mouth ranges from 1 to 139 in 32 Tanganyikan cichlids (Kuwamura, 1986).

*Lamprologus furcifer* attaches her eggs to rocky substratum (Yanagisawa, 1987). Very fast embryonic and post-embryonic development is the characteristic of substrate brooders. For instance, the developmental stages of embryogenesis, swimming and disappearance of yolk sac are completed within 48, 120 and 160 hours after fertilization in a substrate brooder *Cichlasoma cynaguttatum*, against 84, 196 and 360 hours after fertilization in a mouthbrooding *Astatotilapia flavijosephi* (Fishelson, 2005). Histogenesis of the taste buds is commenced in these cichlids 75 and 160 hours after fertilization.

Of 9 broods censused in a substrate brooder *Lamprologus furcifer* by Yanagisawa (1987), 8 perished; of the 9th clutch containing 86 eggs, 27 and <10 survived upto the yolk sac larval stage and yolk-sac free larval stage, respectively. Hence a smaller portion of eggs produced (<1.1% of the 9 clutches) survived. In the biparental guarding by *Neolamprologus tetracanthus* pair, removal of one of the two guarding parents resulted in the quick loss of young ones by predation (Matsumoto and Kohda, 1998).

Interestingly, maternal mouthbrooding increased the survival of eggs and young ones; an estimate of the surviving young from the egg (73–90 eggs/clutch) stage is about 11% for *Ctenochromis horei* (Ochi, 1993). In a biparental mouthbrooder and guarder like *Haplotaxodon microlepis*, the estimated survival is as much as 47% ; the losses incurred by the female

and male were 26% and 27% respectively, suggesting that with increasing size of embryo/larval stage, the parents regularly lost a few embryos/larvae. Briefly, internal parental care by mouthbrooding appears to have ensured greater survival of young ones. Not surprisingly, over 70% of the cichlids have opted for mouthbrooding. Incidentally, a biparental substrate brooder may invest nearly two to eight times more on gonad (see Table 6) than a biparental mouthbrooder. However, mouthbrooding effectively reduces the feeding area and prevents feeding in the male and female *Xenotilapia flavipinnis* (Yanagisawa, 1986). Kuwamura (1986) provides more examples for the prevention or reduction of feeding in mouthbrooding cichlids. Clearly, there appears to be two alternate strategies of breeding for cichlids namely: 1. By investing more on gonad to produce fast-developing large number of smaller eggs or 2. By investing less on gonad to produce slow-developing small number of large eggs and to suffer less feeding or no feeding, while mouthbrooding. In this 'trade-off', most cichlids have chosen the mouthbrooding strategy.

(i) *Egg cannibalism*: Filial cannibalism and egg cannibalism may also reduce progeny survival among oviparous fishes. Mouthbrooding is perhaps a costlier mode of parental care than the others like substrate tending. A lengthy mouthbrooding period accompanied by a shorter interspawning interval prompt a male of the cardinal fish *Apogon niger* to partially cannibalize its own brood to compensate for the lack of food during the mouthbrooding period (Okuda, 1999). In *A. lineatus*, the male cannibalized 30% of its own brood (Kume et al., 2000). Thus filial cannibalism increases, when paternal energy reserves are decreased, which may be a key factor affecting cannibalism and progeny survival (Manica, 2002; Payne et al., 2002; Neff, 2003b). Considering the effect of mate availability on filial cannibalism, Kondoh and Okuda (2002) developed a model that predicts filial cannibalism under the following conditions: 1. Low energy reserve of the male, 2. High mate search efficiency, 3. High population density, 4. Female-biased sex ratio, 5. Lengthy paternal care period, and 6. Shorter refractory interspawning period of female.

However, it is difficult to account for the widespread occurrence of egg cannibalism among both pelagic and demersal spawners. For instance the Baltic sprat *Sparatus sparatus* regularly cannibalized about 15% of the eggs, abundant during peak spawning (Koster and Mollmann, 2000). In the Gulf of Alaska and Bering Sea, the walleye pollock *Theragra chalcogramma* form large aggregation with millions of individuals, and each female produces a half million free floating eggs in about 10 batches over a period a few weeks. Schabetsberger et al. (1999) recorded that male pollock cannibalized three times more than the female. It is not clear whether the eggs are cannibalized as a means of recouping energy spent on courting (Manica, 2002).

In the Japanese rivers, Katano and Maekawa (1995) observed that 97% eggs were cannibalized by male and female satellites of *Zacco temmincki* leaving only 3% eggs to be buried safely for incubation by a mating pair. Notably, the cannibals produced 1.5 times more eggs than the non-cannibals. Likewise, most egg-eating clingfish have a larger body size than individuals that mainly feed on crustaceans (Hirayama et al., 2005). Most uncharitably, the sneaky sharpfin *Telmatherina sarasinorum*, an endemic of Wallace's Dream ponds, the Malili Lakes, Sulawesi, Indonesia, has gone to the extent of enticing the heterospecific female of *T. antoniae* to quiver and spawn her eggs, only to be partially cannibalized by the enticer (Gray et al., 2008).

(ii) *Parasitic load*: By definition, a parasite lives on its host resources and the host compensates this loss either by limiting its activity like egg guarding or increases its food intake. Monogenean parasites like the dactylogyrid live on the gills of the host damsselfish *Stegastes planifrons*. The female damsselfish is not able to detect a parasitized one from the healthy males (see also Taylor et al., 1998). Sasal (2006) tested the relationship between the monogenean parasitic load and egg loss within the nest of the damsselfish and found a significant correlation between the total surface area of the clutches and egg loss during the guarding period. This finding suggests that the cost of the guarding male losing a proportion of its clutch is lower than the cost of trying to keep all the eggs in poor fanning condition. Sasal claims that his findings are the first of its kind.

(iii) *Brood parasitism* is the one, in which eggs are laid in the nests of another species and the young are reared by a host parent. Kramer and Smith (1960) described the utilization of nests of largemouth bass *Micropterus salmoides* by goldshiners *Notemigonus salmoides* (see also Hunter and Hasler, 1965). Nests of smallmouth bass *M. dolomieu* are used by longnose gar *Lepisosteus osseus* and the host is known to hatch out more eggs in the nest, in which the eggs of longnose gar were also incubated (Goff, 1984). These are examples of a mild form of 'brood parasitism', in which association has been beneficial to both the host and 'the parasite'.

Baba et al. (1990) have reported a true brood parasitism by the parasitic minnow *Pungtungia herzi* (Cyprinidae) on the nests of the freshwater perch *Siniperca kawamebari* (Serranidae) from the Ibo River, Hyogo Prefecture, Japan. The perch lays relatively large (2.5 cm) whitish eggs on the 2–3 stems, which make up the nest during the period from May to August. The parasitic minnow places her small (2.1 cm) whitish eggs among the eggs of the perch in the perch's nest from mid May. The incubation duration of the minnow's eggs is 12–16 days and falls well within the incubation period (16–20 days) of the perch. Over 76 and 6% of minnows eggs are placed at a time, when the eggs of the perch are just in stage 1 and 2, respectively. A number of reasons may be attributed to explain why the oviposition of *P.*

*herzi* is concentrated at stage 1 of the host's eggs: 1. It is easier to oviposit at stage 1, as the host guards his eggs less aggressively than those at the subsequent stages of 2–4. 2. The whitish or rosy eggs of the perch at stage 1 are more conspicuous to the parasitic minnow than at stage 2 and later. 3. The nest site has been cleaned by the host more recently. On removal of the host male, eggs of both the host and parasite were soon predated. Hence the host afforded protection to the eggs of the perch and minnow. However for reasons not described by Baba et al., the hatching success of the perch was significantly reduced to 17% in the parasitized nest from 26% in the non-parasitized nests.

*Ovoviviparity*: The term ovoviviparity refers to intermediate stages between oviparity and viviparity; but within oviparity three levels are distinguished. They are: 1. Ovuliparity refers to fertilization within the external environment; 2. Zygoparity indicates the oviparous reproductive mode, in which the fertilized ova are retained within the reproductive tract of the female (e.g. *Helicolenus dactylopterus dactylopterus*, Munoz et al., 2002) or hermaphrodite (e.g. *Rivulus marmoratus*, Harrington, 1967) for short periods and 3. Embryoparity is the pattern of oviparous reproduction, in which the embryo is formed and may develop to an advanced state prior to its release from the female's reproductive tract (Wourms et al., 1988).

Incidentally it is known, that primary and secondary males do occur in the internally self-fertilizing hermaphroditic killifish *Rivulus marmoratus* (Harrington, 1968, 1971). Besides, the occurrence of pure males has also been claimed from the laboratory population (Cole and Noakes, 1997). The presence of pure males and females, and the absence of any intromittent organ in the male to transmit the sperm to the female's reproductive tract indicate the possibility of existence of functional gonochorism within this species. Recent molecular analysis provides strong evidence for outcrossing between individuals of this species (Lubinski et al., 1995). Hence *R. marmoratus* may prove to be a useful fish to study the transition from ovuliparity to zygoparity (see Wourms et al., 1988).

*Viviparity* is recorded from about 500 species belonging to 13 families of teleosts (Wourms et al., 1988). However, the list is being continuously updated for instance, a new viviparous fish *Gremmonus nagaredai* (Bathitidae) is reported from the Hawaiian Islands (Randall and Hughes, 2009). Viviparity is usually facilitated by an intromittent organ like the gonopodium (e.g. Poeciliidae), andropodium (e.g. Hemiramphidae), pripium (e.g. Phallostethidae), penes (e.g. Cottidae), tubular genital papillae (e.g. Athernidae) or anal fins (e.g. *Corynopoma riisei* (Burns et al., 1995). As mentioned elsewhere, the introsperm of viviparous fishes has an elongated head containing an elongated nucleus (3.6 to 31.6  $\mu\text{m}$  in length, Burns et al., 1995) to ensure its passage through the more viscous fluids of the female reproductive tract. Spermatozoa are transferred in pockets

called spermatozeugma. Two different methods are adopted to produce the spermatozeugma. In the first one, the spermatozeugma are fully formed within the spermatocysts, prior to their release (e.g. *Xenurobrycon*). In the second one, the loose spermatozoa are released, which are subsequently clustered into spermatozeugma within the posterior storage areas of the testis (e.g. *Glandulocauda*).

Because of the inherent interest in viviparity of fishes and because of the small size and short life span, many poeciliids have received much attention. All but one of the 150 species of poeciliids known are to be viviparous. Brood size within the family ranges from a few to 315 young at a time (Krumholz, 1948). Once internal fertilization was evolved, various modifications in fecundity and modes of viviparity were possible. For example, *Poecilia reticulata* begins to brood at a relatively smaller size (21 mm ) and earlier age (48th day), is reproductively active for 440 days brooding 24 eggs per brood with the longest inter-brooding interval of 22 days and produces smaller eggs (ovum size = 1.4 mm ; 0.9 mg each). On the other hand, *P. monacha*, which produces the largest eggs (2.0 mm size, 2.1 mg each), matures at the age of 82 days and broods 12 eggs. *P. prolificia* matures at the age of 65 days, but has only 190 days reproductive life; it can brood only 4 eggs once every 7 days but has the smallest eggs of 0.8 mm and 0.2 mg each. Thus adjustments have been made in ova size, fecundity, and size of the young, amount of nutritional supplement from mother to embryo, degree of placental development, degree of superfetation, variations in the brood intervals and gestation periods and duration of the reproductive season and length of reproductive life. These factors usually are interlinked so that a modification in one may be adjusted in the other so that reproductive fitness is maintained (Thibault and Schultz, 1978).

Superfetation means the gestation of rows of developing multiple broods within a female. It is advantageous for two reasons: 1. It avoids the surge of nutrient load for providing a single clutch of large number of eggs, as in *P. reticulata*, and 2. Increases the space available for larger embryos. In general, with increasing number of superfetation, there must be a steady food supply; otherwise, embryos will die and a reproductive effort to that point is lost (Thibault and Schultz, 1978).

It is known that an egg of the ovoviviparous rockfish *Sebastes marinus* lose over 30% of its dry weight during development (Wourms, 1981). In truly viviparous poeciliids too, the loss ranges from 30% in *Poeciliopsis monacha* to 50% in *Poecilia reticulata* (Thibault and Schultz, 1978). But in others, who draw nutrients from their mothers, the increase ranges from 16% in *Poeciliopsis lucida* to 1,700% in *Poeciliopsis prolificia*, there are other poeciliids like *Heterandria formosa*, which gain as much as 3,900% (Wourms, 1981). Incidentally, estimates from the data reported for the syngnathids,

which gestate their embryos in the trunk pouch, suggest a loss of 1.236 J per embryo. This is equivalent to 26.5% of the energy content of the newly deposited zygote of the pipefish *Nerophis ophidon* (Berglund et al., 1986b). However, the corresponding value is as much as 85% of the egg mass for the seahorse *Hippocampus kudo* (Thangaraj and Lipton, 2009).

Temperature appears to alter the sex ratio of viviparous fishes. In *Poeciliopsis lucida*, Sullivan and Schultz (1986) reported that an exposure of the mother to a higher temperature (30°C) induced the production of all-male progenies but the lower one (24°C) led to female-biased sex ratio. The results may be compared with that of Harrington (1967), who obtained males, when eggs were incubated at temperatures lower than 20°C. Likewise, Schultz (2008) also found that the sex ratio of a brood of the dwarf perch *Micrometrus minimus* was male-biased (60% males), i.e. those females conceived in early January, when the prevailing temperature was 7°C less than during April, produced progenies with a female-biased sex ratio. However, those conceived subsequent to January, gave birth to progenies that were also male-biased but with 58–75% males. Hence higher temperatures appear to induce more male progenies in viviparous fishes.

Between chosen oviparous fishes, hybridization may not occur, when the sperm head is too large to enter the narrow micropylar opening in the egg. For example, the sperm of *Gymnocorymbus ternetzi* readily finds its way through the wide micropylar opening and short micropylar canal in the eggs of *Hemigrammus caudovittatus* (David and Pandian, 2006). However, the sperm of *H. caudovittatus* is unable to enter through the narrow micropylar opening and the long narrow micropylar canal (Fig. 7). Hybridizations among viviparous fishes have an additional hurdle of minimizing the disparity in equal ova size and/or gestation period. More than 70% attempts to hybridize *Poecilia sphenops* and *P. velifera* were successful (George and Pandian, 1997), but the success was limited to 5% in crosses between *Poeciliopsis monacha* and *P. lucida* (Schultz, 1973), perhaps due to disparity in the egg size.

Included in the *Poeciliopsis* are three diploid and three triploid unisexual forms. In an attempt to show that the first found trihybrid *Poeciliopsis cx* originated through hybridization and polyploidization, a large number of hybridization experiments were undertaken between closely related sympatric bisexual species like *P. viriosa* and *P. monacha*. Recording the total absence of hybrid embryos in all the attempts to hybridize, Vrijenhoek and Schulz (1974) indicated a strong reproductive incompatibility between *viriosa* and *lucida* that would make the origin of *P. cx* by hybridization between *P. viriosa* of *P. lucida* impossible. The most probable cause of reduced fertility in crosses of *P. cx* with males of *viriosa* or *monacha* is that the small ova of the unisexuals contain inadequate yolk

to allow the completion of embryogenesis of the large hybrids of *monacha* and *viriosa*, the two species with large ova and embryos.

An alternative route was the hybridization between *P. monacha* ♀ and *P. lucida* ♂. Of 57 matings attempted, only 5 were successful in the sense of producing fertile progenies that survived to reproduce. Difficulties with this cross were not due to genomic incompatibility but due to disparity in size of the hybrid embryo. *P. monacha* has large embryos nourished by ova of 2.2 mm whereas *P. lucida* has small embryos of 1.4 mm eggs. However, subsequent generations do not have surplus yolk because they develop in medium sized hybrid eggs (1.8 mm). When a synthesized *P. monacha-lucida*, heterozygous for  $E^I$  and  $E^{II}$ , was mated to an  $EE$  male of *P. lucida*, all eight progenies produced were  $E^I E$  and none were  $E^{II} E$ , a result indicating that the parental genome marked with the  $E^{II}$  allele is not transmitted to the eggs. It is an indication for the forthcoming hybridogenesis in *P. monacha lucida*. Thus, Schultz (1973) claimed that he repeated the hybridization that took place millions of years before between *P. monacha* and *P. lucida* and has indeed 'synthesized' unisexual species in his laboratory.

The zebrafish *Danio rerio* has become the choice for most experimental studies in molecular biology of fishes. By virtue of their small size (< 5g), short generation time (48–120 days), relatively long reproductive life (13–21 months) and short inter-spawning/parturition period (12–30 days), production of large number of eggs/progenies (11–150) and amenability to stripping of eggs and milt (despite their small size), many other fishes

**Table 32** Reproductive traits of selected fishes, which can serve as models for genetic experiments.

Species	Generation time (age in days)	Reproductive life (period in months)	Fecundity (no of eggs/progenies)	Inter-spawning/parturition period (days)
<b>Oviparous</b>				
<i>Danio rerio</i>	120	>12	100	7
<i>Gymnocorymbus ternetzi</i>	120	21	150	30
<i>Puntius conchonius</i>	90	21	80	15
<b>Viviparous</b>				
<i>Poecilia reticulata</i>	48	15	26	22
<i>Poeciliopsis monacha</i> *	82	13	11	12

\*Thibault and Schultz (1978)

may also serve as ideal animals for genetic experiments (Table 32). The scope for experimentation is further enhanced by the availability of differently coloured strains with different body forms and meristic characters and their amenability to intra- and interspecific hybridization, polyploidization and genome-inactivation by simple procedures (see Pandian and Koteeswaran, 1998; David and Pandian, 2006).

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