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Sex Change Strategies and Group Structure of Damselfishes

Akihisa Hattori[1,*] and *Margarida Casadevall*[2]

Introduction

The evolution of sex change strategies in fishes is closely related to mating systems (Warner 1984, 1988a,b). Field studies have revealed that fish species utilize a variety of strategies, including sex change, in order to increase their reproductive potential within the social context of each species (Warner 1988a,b, Munday et al. 2006). Although almost all species of damselfishes are non-sex changers, some species exhibit sex changes. For example, species in the genera *Amphiprion* and *Premnas* include protandrous (male-to-female) sex changers and those within the genus *Dascyllus* include protogynous (female-to-male) sex changers (Table 1). These genera have unique mating systems that are quite different from those of other species, although their reproductive and parental care behavior is similar to that of other damselfishes. Thus, they offer excellent models for examining the evolution of mating systems and sex change in fishes. Sex-changing species inhabit particular refuges (i.e., giant sea anemones for *Amphiprion* and *Premnas*, and staghorn branching corals for *Dascyllus*), usually forming small social groups with a size-ordered dominance hierarchy. In contrast, non-sex changing species are solitary, aggregational (or colonial) or gregarious (see below), and they usually utilize ubiquitous shelters such as narrow gaps within

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[1] Faculty of Liberal Arts and Education, Shiga University, 2-5-1 Hiratsu, Otsu, Shiga 520-0862, Japan.

[2] Environmental Sciences Department, Sciences Faculty, Girona University, 17071 Girona, Spain.
Email: margarida.casadevall@udg.edu

[*] Corresponding author: hattori@edu.shiga-u.ac.jp

Table 1. Definitions of some important terms on sex change and mating systems in this chapter (see also Thresher 1984, Warner 1984, 1988a,b).

Protandrous sex changers	Individuals that have undergone sex change from *functional* male to *functional* female
Protogynous sex changers	Individuals that have undergone sex change from *functional* female to *functional* male
Pre-maturational sex changers	Functional males that have not been functionally female in protogynous species, or functional females that have not been functionally male in protandrous species
Primary males	Functional males that have not been functionally female in protogynous species (They are considered to be genetically determined pure males)
Diandric species	Protogynous species that have both primary males and secondary males that have been functionally female unlike primary males
Non-functional hermaphroditism	A proportion of individuals exhibit both testicular and ovarian tissues but only reproduce as either male or female during their lives
Functional hermaphroditism	A proportion of individuals function as both sexes at some time during their lives
Functional gonochorism	Functional males and females have never experienced sex change even if an individual has both ovary and testis in the gonads
Monogamy	A functional male and a functional female keep a long term pair-bond to reproduce
Polygyny	A functional male reproduces with more than one or more females
Harems (Resource defense polygyny)	A functional male defends an all-purpose territory within which more than one functional female has home range and reproduces with the male
Lek-like spawning aggregations	During the reproductive period, functional males temporally defend mating and nesting territories at a spawning site which functional females visit. If a female spawns only once at a nest of one male, this system can be recognized as polygyny
Promiscuity (polygynandry, i.e., multi-male-multi-female polygamy)	Functional males and females defend territories, where each male courts any females that pass by and each female spawns with several males during the reproductive period (It is hard to confirm whether their mating is random or not)
Multi-male polygyny (a transitional state from harem polygyny to promiscuity)	Several functional males live in a large habitat keeping their own harems but their control of females is not strict so that some females can undergo sex change
Random mating systems (random pair formation or large group spawning)	Functional males and females pair randomly to reproduce (i.e., random pair formation), or they temporally aggregate at a site to release eggs and sperms at the same time (i.e., large group spawning)
Asynchronous development of the follicles	Oocytes at all the stages of maturation present
Atretic oocytes	Degenerating oocytes
Postovulatory Follicles (POF)	Follicular layers that remain in the ovary after the release of the ovum during spawning
Ambisexual gonads	Coexistence of ovaric and testicular tissue in the same gonad (ovotestis)
Rudimentary gonads	Without histological sex differentiation

rocks or rubble, and cracks and coral heads on reefs. In this chapter, we first explain the key concepts underlying sex change strategies. Next, we classify damselfish species into three categories (i.e., solitary or aggregational; gregarious or schooling; social) before summarizing the relationship between these categories and sex change. Since non-sex changers are common in some sex-changing species, we describe the flexible sex determination mechanisms in these species. Finally, the relationship between growth, inter-group movement and sex change strategies among damselfishes will be discussed in relation to shelter use patterns.

Size Advantage as an Explanation of Flexible Sex Change Strategies

Many teleost fish species utilize sex change strategies. If expected fertility (the number of viable offspring) is related to body size, an individual who can change sex at the proper size can have more offspring than one remaining exclusively male or female (Ghiselin 1969). This size-advantage hypothesis (SAH) predicts that the direction of sex change in a species depends on its mating system. Sex change from female to male (protogyny) is found in species with polygyny because larger males monopolize mating opportunities. In this strategy, the expected reproductive success of males will expand rapidly with increasing body size (Charnov 1982, Warner 1984, 1988a,b, Kuwamura and Nakashima 1998). Sex change from male to female (protandry) is expected in species with mating systems in which the expected reproductive success of females increases with increasing body size, whereas that of males is less sensitive to body size (Ghiselin 1969, Warner 1975, 1988a,b, Charnov 1982) because they would be unable to monopolize mating opportunities (Warner 1984).

At intraspecific levels, the SAH predicts the optimal size at which sex change occurs in the population of a species having sex reversal ability. However, the timing of sex change does not always correspond to predictions based on expected fertility because many fish species have more complex life-history pathways in relation to sex change (Warner 1984, Munday et al. 2006). In protogynous species with harem polygyny, for instance, the disappearance of the male from a harem usually causes the remaining largest female to undergo a sex change, but some small individuals may become solitary in order to take over the dominant status in a harem after changing into bachelor males growing faster than females (Warner 1984). The wide variations in the timing of sex change are related to different factors describing reproductive value (Warner 1988a, Munday et al. 2006). This reproductive value is defined as the expected reproductive success of an individual, which takes into account growth rate, mortality and fertility. Growth and mortality, as well as fertility, of individuals are largely influenced by the social context (Munday et al. 2006). Accordingly, SAH based on sex-specific reproductive values indicates that an individual should change sex when the reproductive value of the other sex exceeds that of its current sexual status. Thus, individuals from the same population are expected to change sex flexibly (at different sizes or ages) due to the trade-offs between sex-specific growth, mortality and fertility of each individual (Munday et al. 2006).

Group Types, Mating Systems and Sex Change

Feeding around ubiquitous shelters or a particular refuge

Damselfishes are mainly grazers, plankton feeders or they feed on a variety of small invertebrates (see Chapter VII). Being typically small fishes, seldom exceeding 10–15 cm in length, they feed close to shelters such as narrow gaps, cracks, or coral heads on a reef, to which they can escape from predators (Lowe-McConnell 1987). On Ishigaki Island, for example (Table 2), 24 species of damselfishes were found on 84 very small patch reefs (involving coral heads and outcrops; total area = 166 m^2) of

Table 2. Relationship among group types, sex change strategies and other characteristics of 24 damselfish species found on 84 very small patch reefs of the 0.28-hectare study site of Shiraho Reef, Ishigaki Island, Okinawa, Japan (Hattori and Shibuno 2010).

Species name	**Group type**	**Sex change**	**TL (cm)**	**No.**	**Shelter or refuge**	**Feeding and spawning sites for adults**
*Dascyllus aruanus**	Social[t]	♀→♂	5	545	Staghorn coral head	Almost same
*Amphiprion frenatus**	Social[t]	♂→♀	10	32	Giant sea anemone	Almost same
Amphiprion ocellaris	Social	♂→♀	6	3	Giant sea anemone	Almost same
*Chromis viridis**	Large school	None	6	321	*Acropora* coral head	Apparently different
*Chrysiptera cyanea**	Large shoal	None	4	716	Gaps and cracks	Almost same?
*Pomacentrus moluccensis**	Small shoal	None	5	151	Gaps and cracks	?
*Pomacentrus amboinensis**	Small shoal	None	7	19	Gaps and cracks	?
*Amblyglyphidodon curacao**	Small shoal	None	8	150	Gaps and cracks	Apparently different
*Abudefduf sexfasciatus**	Small school	None	8	27	Gaps and cracks	Apparently different
Abudefduf vaigiensis	Small school	None	10	1	Gaps and cracks	Apparently different
Amblyglyphidodon leucogaster	Small school	None	1.5[+]	7	Gaps and cracks	?
*Stegastes nigricans**	Solitary[t,a]	None	10	134	Gaps and cracks	Same (male); Different (female)
*Stegastes lividus**	Solitary[t,a]	None	14	71	Gaps and cracks	Same (male); Different (female)
*Cheiloprion labiatus**	Solitary[t,a]	None	4	41	*Acropora* coral head	Almost same
*Hemiglyphidodon plagiometopon**	Solitary[t] (single)	None	15	10	Gaps near reef base	Same (male); Different (female)?

Table 2. contd....

Table 2. contd.

Species name	**Group type**	**Sex change**	**TL (cm)**	**No.**	**Shelter or refuge**	**Feeding and spawning sites for adults**
*Dischistodus prosopotaenia**	Solitary[t] (single)	None	14	19	Gaps within rocks	Same (male); Different (female)
Chrysiptera biocellata	Solitary[t] (single)	None	6	1	Gaps and cracks	Same (male); Different (female)
Neoglyphidodon melas	Solitary (single)	None	13	3	Soft coral	Apparently different
Neoglyphidodon nigroris	Solitary (single)	None	4+	3	Gaps and cracks	?
*Pomacentrus chrysurus**	Solitary[t] (single)	None	4	10	Gaps and cracks	Same (male); Different (female)
*Pomacentrus adelus**	Solitary (single)	None	7	264	Gaps and cracks	Same (male); Different (female)
Pomacentrus bankanensis	Solitary (single)	None	4+	1	Gaps and cracks	?
Chrysiptera rex	Solitary (single)	None	4+	2	Gaps and cracks	?
Plectroglyphidodon dickii	Solitary (single)	None	4+	1	*Pocillopora; Acropora*	?

*: Core species on the 84 patch reefs (the others are casual species). TL: maximum total length (cm) of individuals found at the site, +: only juveniles were found, No.: average number of individuals found in six censuses on the patch reefs (see Hattori and Shibuno 2010), t: territorial, a: aggregational. Information on sex change is based on Fish Base. Other information is based on only personal observations (AH) on fish behavior at the study site.

the shallow back reef of a fringing coral reef (< 2.5 m in depth, Hattori and Shibuno 2010, 2013). While many of these species live around ubiquitous shelters (e.g., narrow gaps within rocks or rubble or cracks or coral heads on the reef) and have similar food sources (e.g., filamentous algae, zooplankton or benthic invertebrates), several species have particular food resources (e.g., polyps of *Acropora* coral) and/or particular refuges (e.g., staghorn coral heads or giant sea anemones).

Group types and shelter use

Damselfishes can be classified into three categories based on group structure (Lowe-McConnell 1987): (1) solitary (single or aggregational), (2) gregarious (school or shoal making) and (3) the intermediate (social species). Individuals from solitary species, such as for example *Stegastes nigricans* and *S. lividus* (Table 2), maintain single permanent territories (i.e., all-purpose territories for males and feeding territories for females), protecting their shelter and/or food sources against conspecifics and other competitors (Hattori and Shibuno 2013). Although solitary species often form conspecific aggregations with each individual maintaining adjacent territories, they never form social groups that involve a hierarchy. In this chapter, we do not call the aggregation of individuals with contiguous territories a “colony” because these aggregations do not seem to have an obvious function or constitute a social unit as a whole, unlike nest colonies. While many solitary species living in ubiquitous shelters mainly feed on filamentous algae, some species can also use other living organisms for both food sources and shelter. For example, *Cheiloprion labiatus* uses *Acropora* branching coral and *Neoglyphidodon melas* uses soft coral (Allen 1991, Myers 1991).

Gregarious species form shoals or schools. Shoals are defined as aggregations of individuals that can swim in any direction; individual territories are not maintained, and these species have no dominance hierarchy. For example, *Amblyglyphidodon curacao* and *Chrysiptera cyanea* form shoals. Schooling species form groups that stay in a home range within and around ubiquitous shelters, such as cracks and coral heads on reefs. These fish hover and swim synchronously (Lowe-McConnell 1987). These schools do not show a dominance hierarchy. This characteristic allows the number of individuals within a school to grow, as shown, for example, by *Chromis viridis* which often form very large schools (Lecchini et al. 2006).Thus, gregarious species live around ubiquitous shelters usually feeding on zooplankton (Allen 1991). In gregarious species, group size is not determined by shelter size. Large group size may function as security against predators.

The intermediate species often form small groups with a size-ordered dominance hierarchy, when they use spatially discrete particular refuges such as branched coral heads or giant sea anemones. These social species sometimes defend their group territories against other conspecifics, when they use continuous refuges (see below). When group size becomes very large, the size-ordered hierarchy disappears. For example, *Dascyllus aruanus* and *Amphiprion frenatus* are social species inhabiting a particular refuge, with group size dependent on refuge size.

Group types, mating systems and sex change strategies

Spawning and parental care behavior is similar among species of damselfishes (Allen 1991, see Chapter III). During the reproductive period, adult males defend nesting sites on substrata. Simultaneously, they can also perform specific courtship displays to adult females. Adult females lay demersal eggs on the nests of the selected mates who fertilize, protect and ventilate the egg masses. Exceptionally, however, the species of a monotypic genus, *Acanthochromis polyacanthus*, show prolonged parental care behavior after hatching, defending the fly against predators (Robertson 1973).

Mating systems of damselfishes can be classified into four categories (Thresher 1984): (1) monogamy, (2) harems (resource defense polygyny), (3) lek-like spawning aggregations and (4) promiscuity (Table 1). Because it is rather difficult to follow the reproductive behavior of individually recognized fish, only a few studies have confirmed whether their mating is polygynous, random, or size-assortative in the last two systems.

Gregarious species basically form lek-like spawning aggregations during the reproductive period (Thresher 1984), in which each adult male temporally defends a mating and nesting site around the original shelter or on a different substratum, and adult females visit them to *select* mates. Consequently, females usually reproduce with two or more males, and single males usually fertilize the eggs of two or more females (Ochi 1986). In lek-like spawning aggregations, larger males and females seem to enjoy higher reproductive success because larger females have higher fecundity and they do not prefer to small males' nesting sites (Ochi 1985). Accordingly, sex change strategies have never been found in these species, as predicted by the SAH.

Promiscuous mating systems are present in solitary species, including those with aggregations such as *Stegastes nigricans* and those without aggregations such as *Dischistodus prosopotaenia*. Each male has an all-purpose territory and courts any female that temporally goes out of her territory and passes by his territory and each female spawns with several males. Even if their territories are contiguous to each other being confined to a very small patch reef, they do not show harem polygyny (Karino and Nakazono 1993). Because many solitary species use algae as food sources, each individual (irrespective of sex) may need a single non-overlapped large feeding territory, preventing individuals from forming harems. Thus, dominance hierarchies have not been found in these species. Accordingly, growth of individuals is likely not influenced by social contexts. Food availability or productivity within feeding territory may be an important determinant of an individual's reproductive value. Furthermore, surprisingly, female mate choice in "promiscuous" damselfishes has often been observed. Because low survival rates of the demersal eggs guarded by males would largely decrease the reproductive success of females and males (Knapp and Kovach 1991), females usually prefer males with a large body size (e.g., Schmale 1981) or vigorous courting behavior that indicate high ability of male parental care (e.g., Knapp and Warner 1991). In addition, larger females have higher fecundity. Therefore, sex changers have never been found in these species, as predicted by the SAH. The term "promiscuity" should be replaced by "polygynandry" (with non-random mating systems). Promiscuity must be characterized by random mating systems, in which protandrous sex change is usually favored according to SAH (Ghiselin 1969, Warner 1975, 1988a,b, Charnov 1982). Because protogynous and protandrous sex-changing damselfishes are not found in lek-like spawning aggregations and promiscuous mating systems, respectively, size-assortative mating would be observed more frequently than expected in these systems.

In contrast, mating systems of the social species differ considerably from those of other damselfish species. They live in unique refuges, such as *Dascyllus* in branched coral heads (with complicated hard structure) and *Amphiprion* and *Premnas* in giant sea anemones (without complicated hard structure). Because of this obligate anthozoan-dwelling nature, the survival rates of these species must be higher than those of other damselfish species (Karplus 2014). By inhabiting isolated refuges, these fish are confined to a narrow space, allowing the growth rates of subordinate individuals to be influenced by the dominant individuals. Thus, by dwelling within a small refuge, these fish can form small groups with a dominance hierarchy: feeding and breeding sites are restricted within narrow limits.

Many species living in small groups have the environmental potential for polygyny (EPP, Emlen and Oring 1977): the dominant individual in the group can monopolize mating opportunities. In a social species, such as *Dascyllus aruanus*, small groups show harem polygyny with protogynous sex change (Fig. 1), although temporal monogamy

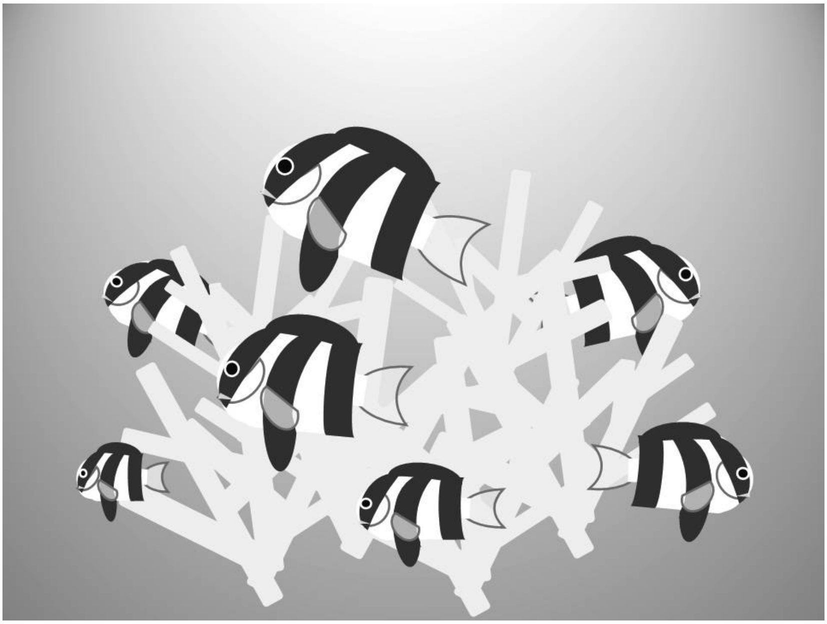

Fig. 1. Small social group of *Dascyllus aruanus*, which inhabit particular refuge (Staghorn coral head).

will be displayed in very small refuges (Fricke and Holzberg 1974, Fricke 1977). When living in larger groups, however, these same species have no EPP. *Dascyllus aruanus* shows multi-male-multi-female polygamy without sex change (see below). However, small groups of another social species, *Amphiprion clarkii* do not show polygyny; rather, they are basically monogamous and show protandrous sex change. While living in habitats containing a high density of host sea anemones, monogamous pairs of *A. clarkii* can sometimes aggregate with temporal polyandry and few sex changes can be observed (Yanagisawa and Ochi 1986, Ochi 1989a,b, Hattori and Yanagisawa 1991).

We have to make a special reference to the unique damselfish, *Acanthochromis polyacanthus*. It seems to be a gregarious species but exceptionally does not form lek-like spawning aggregations during the reproductive period: adult males and females form breeding pairs defending small caves of dead corals for spawning and nesting sites in order to keep the pair territories, while they live in large groups during the non-reproductive period (Robertson 1973). This species shows neither social group nor sex change. This fact clearly indicates that monogamy is not directly related to sex change ability. Social groups with size-ordered hierarchy and the degree of body size differences between adjacently ranked group members are closely related to protogynous and protandrous sex change strategies, as shown below. Table 3 summarizes the relationship between group type, mating systems and sex change with reference to habitat type.

Table 3. Relationship between group type, mating systems and sex change in damselfishes. Habitat type (shelter or refuge) is also shown.

Group type	**Mating systems**	**Sex change**	**Habitat type**
Solitary or aggregational	Polygynandrous (with female mate choice), close to size-assortative mating	None	Ubiquitous shelter
Gregarious	Lek-like spawning aggregations (with female mate choice), close to size-assortative mating	None	Ubiquitous shelter; special sites for spawning and nesting
	Monogamy (only *Acanthochromis polyacanthus*)	None	Ubiquitous shelter; small caves for spawning and nesting sites
The intermediate (Social)	Harem polygyny	♀→♂	Unique refuge (with complicated hard structure)
	Monogamy	♂→♀	Unique refuge (without complicated hard structure)

Sex Change Strategies in Small Social Groups of *Dascyllus*, *Premnas* and *Amphiprion* spp.

The flexible protogynous strategy of Dascyllus *includes pre-maturational sex changers and non-sex changers*

The genus *Dascyllus* includes 10 mainly zooplanktivorous species, although it was recently shown that small species can also actively feed on small benthic crustaceans (Frédérich et al. 2010). Based on differences in morphology, biogeography and body coloration, they have been grouped into three species complexes: the aruanus, reticulatus and trimaculatus (Godwin 1995, Bernardi and Crane 1999, McCafferty et al. 2002). Individuals from the first two complexes are small-bodied (except for one large *D. flavicaudus* in the reticulatus complex), dwell in and around live branching coral colonies, and often form small social groups. Phylogenetic studies revealed that the ancestral *Dascyllus* species was small-bodied and closely associated with branched corals, and that the trimaculatus complex evolved recently, being less associated with corals (Bernardi and Crane 1999, McCafferty et al. 2002, Frédérich and Sheets 2010). The aruanus and reticulatus complexes contain protogynous species (Godwin 1995, McCafferty et al. 2002). For example, *Dascyllus aruanus*, the most studied species in the genus, shows a good correspondence between mating systems and sexuality pattern, as predicted by the SAH. In areas of scattered small branching corals, this species forms small, spatially discrete groups, each of which consists of one dominant adult male and several small adult females with a size-ordered hierarchy (Fricke 1977, Coates 1982). Sex change is socially controlled within group: disappearance of the male from the group causes the remaining largest female to undergo a sex change and become the dominant male (Fricke and Holzberg 1974, Coates 1982, Shpigel and Fishelson 1986). Juveniles may form pairs within very small corals, in which the larger individual is an adult male, not undergoing functional sex change, and the smaller becomes an adult female (Fricke and Holzberg 1974). When living in large branching coral colonies, in which a 1:1 functional sex ratio is found, this species often consists of primary males or pre-maturational sex changers (Table 1) and adult females (Cole 2002). The complicated branching structure of the coral may provide members with a type of screen, and dense coral cover maybe able to limit the amount of aggressive interactions from dominants to subordinates. In areas of continuous large coral colonies, *D. aruanus* shows *functional* gonochorism (Asoh 2003). Similar phenomena have also been observed in *D. melanurus*, which belongs to the aruanus complex (Asoh and Beaupre 2005) and in *D. flavicaudus*, which is the exceptionally large species in the reticulatus complex (Godwin 1995). The trimaculatus complex consists of large-bodied species that have higher mobility (Godwin 1995). As adults, each of these species often forms large feeding aggregates over ubiquitous shelters, like gregarious species, whereas the juveniles of these species always form groups within particular refuges (e.g., branching coral heads or giant anemones). This ontogenetic habitat shift occurs after they grow up (Booth 1995). All species of the complex are considered to be *functionally* gonochoristic (Godwin 1995, Bernardi and Crane 1999, McCafferty et al. 2002). In *D. albisella*, which belongs to the trimaculatus complex, monogamous and occasional polygynandrous mating systems (Table 1) without sex changers are described (Oliver and Lobel 2013).

In haremic species, the male's reproductive value increases according to its capacity for defending resources and sheltering sites required by the females, whereas the female's reproductive value increases with body size (Munday et al. 2006). The size of a group would increase as the size of its refuge increases, if group members can coexist without large body size differences between adjacently ranked individuals. A switch to multi-male polygyny (Table 1), as in *D. marginatus* (Fricke 1980), may be due to the increased number of individuals in the refuge, since the refuge may be too large to be defended by a single male. Experiments on the basslet *Pseudanthias squamipinnis* under field conditions indicate that the females of protogynous species can be induced to change sex by recruitment of a threshold number of additional adult females into the group, even in the presence of the resident male (Shapiro and Lubbock 1980). Multi-male polygyny has also been induced in the saddleback wrasses *Thalassoma duperrey* by field experiments (Ross et al. 1990).

Flexible protandrous strategy of anemonefishes, including pre-maturational sex changers and non-sex changers

Species in the genera *Amphiprion* and *Premnas* are usually called anemonefish (Chapter XII). *Premnas* includes only one species (*Premnas biaculeatus*) and *Amphiprion* consists of 29 spp., which are zooplanktivorous or omnivorous species (Allen 1975, Fautin and Allen 1992, Allen et al. 2008, 2010, Chapter XII). All of them are symbiotically associated with giant sea anemones. All these species are confirmed or inferred to have protandry with a monogamous mating system (Fig. 2; Allen 1975, Fautin and Allen 1992). These fishes inhabit isolated single or aggregated host sea anemones, which are essential resources for their refuge. Their hosts comprise of only 10 species in the field. Although each of the 10 anemone species has a single form,

Fig. 2. Small social group of *Amphiprion ocellaris*, which inhabit particular refuges (giant sea anemone).

two of these host species, *Entacmaea quadricolor* and *Heteractis magnifica*, often appear in aggregational form (Allen 1975, Fautin and Allen 1992). A breeding pair of anemonefish needs a sufficiently large host, allowing the female to lay a clutch of eggs on the hard substratum near the edge of the oral disc of the anemone, and the male takes care of the demersal eggs (Fautin and Allen 1992, Arvedlund et al. 2000).

Within a single isolated host, small groups of anemonefish form a size-ordered hierarchy (Fig. 2; Allen 1975, Fautin and Allen 1992). Socially controlled sex change within an isolated small group is well studied in three species: *Amphiprion percula* living in *H. magnifica* (Buston 2003a,b, 2004a,b), *A. ocellaris* living in *Stichodactyla gigantea* (Mitchell 2003, 2005, Hattori 2012) and *A. frenatus* living in *E. quadricolor* (Hattori 1991, 2005). Each small social group consists of an adult female, an adult male (with the female being much larger than the male) and zero to four non-breeders (i.e., subadults and juveniles). The female disappearance induces the male to change sex and the third-ranked (subadult) fish becomes the breeder male. When the adult male disappears, the third-ranked fish inherits the male breeding position. Some non-breeders may form groups on a very small host; when the host grows sufficiently large, the largest fish becomes female (i.e., without sex change) and the second member becomes male. Similar patterns of socially controlled sex change in small groups have been described in *Amphiprion bicinctus* (Fricke and Fricke 1977) and *Premnas biaculeatus* (Wood 1981, 1987), although information on host distributions is unavailable, and also in case of *A. melanopus* in isolated small aggregations of *E. quadricolor* (Ross 1978a,b), although no information on group isolation is available.

According to the SAH, protandrous sex changers are expected to occur in species having mating systems in which the expected reproductive value of males is less sensitive to their body size, whereas that of females increases with increasing body size (Ghiselin 1969, Charnov 1982, Warner 1988a,b). In random mating systems, pairing would occur randomly with respect to body size, so large males would not have a mating advantage. Pair formation in anemonefishes was often thought to occur randomly with respect to body size (Fricke and Fricke 1977, Kuwamura and Nakashima 1998). Larval settlement on a sparsely distributed host anemone was regarded as occurring randomly, with post-settlement movements between isolated single hosts thought to be very difficult (Allen 1975, Fricke and Fricke 1977). Moreover, due to the small carrying capacity of the anemone, only two adults were able to inhabit a single host (Fricke and Fricke 1977, Fautin and Allen 1992, Hattori and Yamamura 1995, Kuwamura and Nakashima 1998). However, recent studies have demonstrated that larval settlement onto a single host is not random (Elliott et al. 1995, Schmitt and Holbrook 1999, Elliott and Mariscal 2001, Buston 2003b, Hattori 2005, Mitchell 2005) and individuals often move between hosts (>40 m) in order to obtain larger mates or hosts if the opportunity arises (Hattori 1994, 2005, Hirose 1995). It was suggested that protandry in anemonefishes secures mates without the risk of adult movement between sparse hosts and ensures that the larger member of a breeding pair is always female, thus enhancing the fecundity of both the members of a pair (Fricke and Fricke 1977, Kuwamura and Nakashima 1998, Munday et al. 2006). However, this does not explain why anemonefishes are monogamous, since species living in small groups usually have EPP and in anemonefishes third-ranked fish within a group are sometimes larger than the smallest breeders in other groups (Hattori 1995, Buston 2004a,b, Mitchell 2005).

The summed body lengths (or total biomass) of a group of anemonefish are well known to be determined by the size of the host anemone (Fig. 3; Allen 1975, Ross 1978a, Hattori 1991, 2005, Mitchell and Dill 2005, Buston 2003a). Recent studies have revealed that large body size differences (or ratios) between group members adjacent in rank are constant within a population, which is necessary for subordinates to avoid *fatal eviction* from the refuge when single hosts are isolated (Buston 2003a,b, Hattori 2012). Accordingly, given the summed body lengths, a constant body size difference (or ratio) would determine the body size composition of the group members (Hattori 2012). In other words, the body size difference between the pair members would be

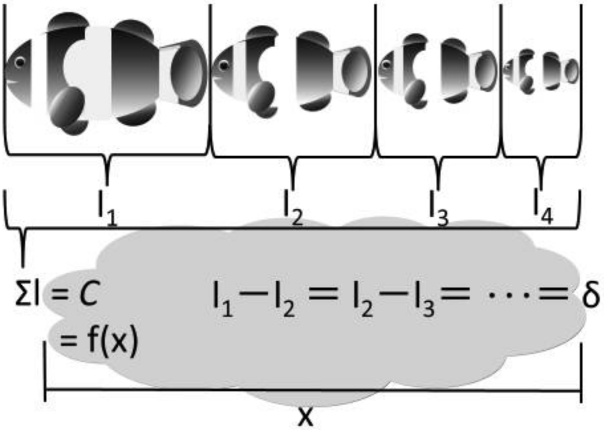

Fig. 3. Summed body length of group members in *Amphiprion* can be considered to be a function of host anemone size (x). Thus, $\sum l$ is the constant for a given host size (x). In addition, body size differences between group members adjacent in rank are considered constant within a population.

predictable where the anemonefish live in an isolated single host, of which the size was given. This suggests that pair formation does not occur randomly with respect to body size.

In the limited shelter space of a host anemone, the growth of the dominant fish retards the growth of subordinate fishes (Allen 1975, Hattori 1991, 1995, Buston 2003b). Indeed, the dominant fish can use most of the resources, such as food and space (Allen 1975, Buston 2003b). In a social system where limited shelter space determines the summed body length of group members, the expected reproductive success via male function will not increase with increasing body size because the growth of a male retards the growth of females and the reproductive success of the male depends on the summed body length of females in the group (Hattori 2005). In contrast, the expected reproductive success via female function will increase with increasing body size (Hattori 2012). If anemonefish lived in haremic groups with dominant males, the growth of males would retard the growth of females. Thus, the total fecundity of all the small females within a group may be lower than the potential female fecundity of the dominant fish. Moreover, the fecundity of females may increase exponentially with increase in body size. The relationship between female fecundity and body size directly influences the fecundity of the dominant fish in haremic groups (Muñoz and Warner 2004). Although no study has yet assessed the relationship between female fecundity and gonad weight in anemonefishes, gonad weight is linearly associated with female fecundity in some fishes (Rhodes and Sadovy 2002, Sivakumaran et al. 2003). If the fecundity of an anemonefish female is linearly proportional to its gonad weight, then female fecundity will increase exponentially with body size (Fig. 4; Hattori 2012). When the dominant fish is female, accordingly, this large female can

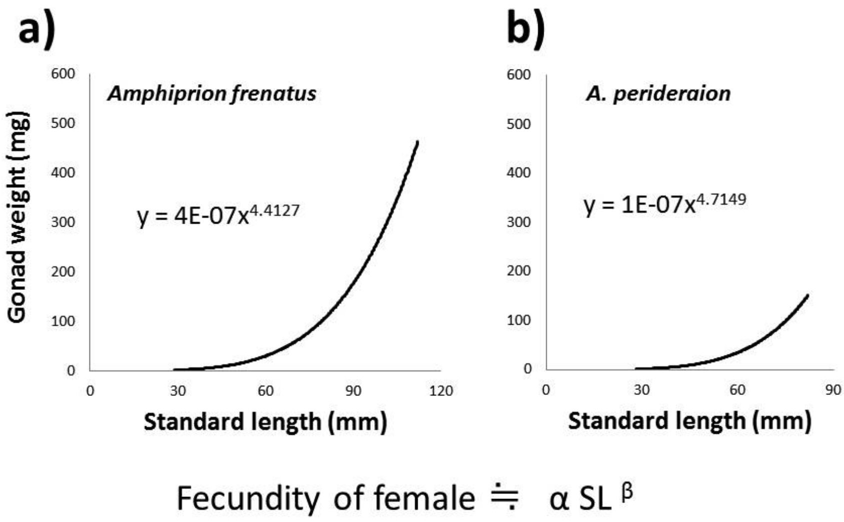

Fig. 4. Gonad weight increases exponentially with body size in *Amphiprion*: (a) *A. frenatus*, (b) *A. perideraion*. Fecundity of female, accordingly, can be assumed to increase exponentially with body size.

enjoy high fecundity. If the dominant fish was male, however, the total fecundity of small females might not be high because his growth retards the growth of females in a limited shelter space. Thus, the combination of exponential female fecundity functions and large body size differences between group members adjacent in rank can explain the function of protandry and monogamy under limited shelter space (Hattori 2012). Moreover, larger and older females, in addition to being more fecund, often produce high-quality offspring that grow faster. For example, the larger size of *Amphiprion chrysopterus* females was associated with a disproportionate increase in population replenishment (Beldade et al. 2012). Thus, when space is limited and the resulting group size is small, one large female would have the highest reproductive value in this small group meaning that a protandrous strategy with a monogamous mating system should be expected. The body size composition model with non linear female fecundity does not contradict the SAH and explains the function of monogamy and protandry when anemonefishes form small groups within small refuges.

On aggregational hosts, where shelter space is *not* limited, adult pairs hold territories (including several hosts) that are almost contiguous with each other, with subadults and juveniles usually having home ranges on the outskirts of or in the interstices between the pairs' territories in order to avoid aggressive behavior from adult pairs (Fricke 1979, Hattori and Yanagisawa 1991, Kobayashi and Hattori 2006). *Amphiprion akallopisos* living within the aggregational form of *H. magnifica* (Fricke 1979), *A. frenatus* living within the aggregational form of *E. quadricolor* (Kobayashi and Hattori 2006) and *A. clarkii* living within the densely distributed single form of *E. quadricolor* (Moyer 1980, Ochi 1989a,b, Hattori and Yanagisawa 1991) are typical examples. After the disappearance of an adult female from a breeding pair, the remaining mate rarely changes sex because a neighboring adult female usually immigrates to pair with the male in the habitats of high host density. Similar phenomenon is observed in *A. melanopus*, although no information on host anemone density is available (Godwin 1994a). In *A. clarkii*, the subsequently vacant female breeding post is frequently occupied by one of the subadults, which lives solitarily preparing to be a female. This type of subadult, which are named as subadult females, can be distinguished from other subadults based on the caudal fin coloration and behavior (Hattori and Yanagisawa 1991, Hattori and Yamamura 1995). When an adult male disappears, one of the subadults (except the subadult females) becomes the male breeder. This type of subadults, which are named as subadult males, may pair with each other and, when their host grows sufficiently large, the larger member becomes an adult female through the subadult female phase. Because the individuals, except for the small juveniles, are *not* confined to single hosts, these individuals can select mates with pair formation occurring in a size-assortative manner (Ochi 1989a,b, Hattori and Yanagisawa 1991, Hattori and Yamamura 1995). Similar phenomenon is described for *A. frenatus* in a habitat of high host density (Kobayashi and Hattori 2006). If they were polygynous, the fecundity of the male may not be high due to the high cost of territorial defense. One example is the monogamous longnose filefish *Oxymonacanthus longirostris*, in which the males defend territories so as not to decrease the females' feeding rates and fecundity (Kokita and Nakazono 1999). Polygynous territories are too large to be defended by males and consequently the cost of defense does not pay for the polygynous males. This explanation is applicable to anemonefishes in habitats of high host density.

Sex Change Mechanism and Gonad Development in Different Group Structure

The process of sex inversion involves a reorganization of the reproductive system, including replacement of gonadal cell types, reorganization of duct systems and behavioral changes. Changes can begin immediately upon a shift in the social status of an individual, and, depending on conditions, can be completed quickly in a few weeks, as it has be shown in *A. melanopus* (Godwin 1994a). The following section describes the most remarkable features of these gonad changes.

Sex changers, pre-maturational sex changers and non-sex changers of protogynous Dascyllus

Some species of *Dascyllus* have been reported to be non sex-changers, since protogyny might be demonstrated only via observation of individuals undergoing sex transition. In fact, in a study of *D. flavicaudus* (Parmentier et al. 2010), which is the exceptionally large species in the reticulatus complex (Godwin 1995), we have not found any mixed stage gonad in our samples, so we would have supposed that it was a gonochoric species. Actually, *D. flavicaudus* was reported as a diandric species (i.e., males can develop directly from the juvenile phase as well as by sex change from functional females, Table 1) since secondary testes were already observed (Asoh 2004). Godwin (1995) had also observed one individual of *D. flavicaudus*, the largest of its group, with atretic oocytes together with proliferating spermatogenic tissue, a clue of a recent sex change.

The testes of primary males develop directly from the juvenile state, which had not yet developed an ovarian cavity. However, the testes of secondary males develop from gonads with an ovarian lumen (Shapiro and Rasotto 1993). Species certainly reported with this secondary structure are *D. aruanus* (Cole 2002, Asoh 2003), *D. reticulatus* (Schwarz and Smith 1990) and *D. carneus* (Asoh and Yoshikawa 2003).

Secondary testes have also been observed in *D. albisella* (Asoh et al. 2001) and *D. trimaculatus* (Asoh and Kasuya 2002), previously considered to be gonochoric species (Yogo 1987, Godwin 1995). In fact, they both show a protogynous pattern of gonadal development, although they are non-functional hermaphrodites in the current ecological context (Asoh and Kasuya 2002). In non-functional hermaphroditism, a ratio of the population may exhibit both testicular and ovarian tissues but only reproduce as male or female (Sadovy and Liu 2008). This is the case of the *trimaculatus* complex species, considered to be *functionally* gonochoristic (Godwin 1995, Bernardi and Crane 1999, McCafferty et al. 2002).

Asoh (2004) suggests that diandric protogyny may be a common trait of *Dascyllus* species, meaning that, in a given situation, some females can switch sex and become secondary males. The trigger for the change could be a variation on the number of females in the area, for example, or perhaps changes in the environmental conditions (such as the size of the available resource patch), as proposed by Loft (1991).

It is known that a single species may have different mating systems under different ecological conditions (Ross 1990). *Dascyllus marginatus*, for example, switches from multi-male polygyny, to harem polygyny to monogamy (Fricke 1980). In the same way, some flexibility in sex change would also increase the reproductive success of a mating group member. Thus, the onset of secondary males would be the simplest strategy to change from a single male harem polygyny to a multi-male polygyny.

Dascyllus *gonadal development*

According to Strüssmann and Nakamura (2002), the rudimentary gonads of teleosts undergo first a period of somatic growth with no apparent histological differentiation. During the next period, the development of cellular or histological characteristics, such as the onset of intensive germ cell proliferation, signal the beginning of sex differentiation into ovaries or testes in some species. However, for other species, it marks the beginning of an all-female or intersex phase prior to the terminal differentiation into ovaries and testes.

Rudimentary or undifferentiated gonads of *Dascyllus* contain only primordial germ cells and somatic cells, and have no ovarian lumen, medial sperm ducts or sperm sinuses (Asoh and Yoshikawa 2003, Asoh and Kasuya 2002).

Primary testes develop from rudimentary gonads without a cavity or lumen and, in some species, it has been observed that the sperm ducts run as simple tubes in the medial hilar regions of testes throughout their length (Asoh 2003).

The first ovarian stage, which also develops from rudimentary gonads, already shows an ovarian lumen, and contains only primordial germs cells and somatic cells (Asoh and Kasuya 2002). The second stage begins with the development of at least one oocyte. Finally, the mature ovary contains oocytes from the cortical-alveolus stage to different vitellogenesis stages, and does not have testicular tissue. In this kind of asynchronous development of secondary growth follicles (Wallace and Selman 1981), oocytes at all stages of maturation are present, without a dominant population (Fig. 5a), and batches of eggs develop synchronously for each spawning event.

***Female-to-male sex change of protogynous* Dascyllus**

In some species, the transition from female to male seems to occur both before and after the individuals have developed cortical-alveolus oocyte stage in their gonads, the former being pre-maturational sex changers. Though, in *D. aruanus* (Asoh 2003), developing spermatogenic tissue occurred only in gonads having pre-cortical alveolus stage oocytes suggesting that functional female-to-male sex change is rare or absent in this population of *D. aruanus*.

According to Asoh and Kasuya (2002), during the early reversal stage of the non-functionally protogynous *D. trimaculatus* gonads, degenerating oocytes in the primary growth stage are simultaneously observed with one or more clusters of spermatocytes, spermatids or spermatozoa. Oocytes at the mid-reversal stage, both in and beyond the primary growth, degenerate. Spermatogonia proliferate and spermatogenic cysts with

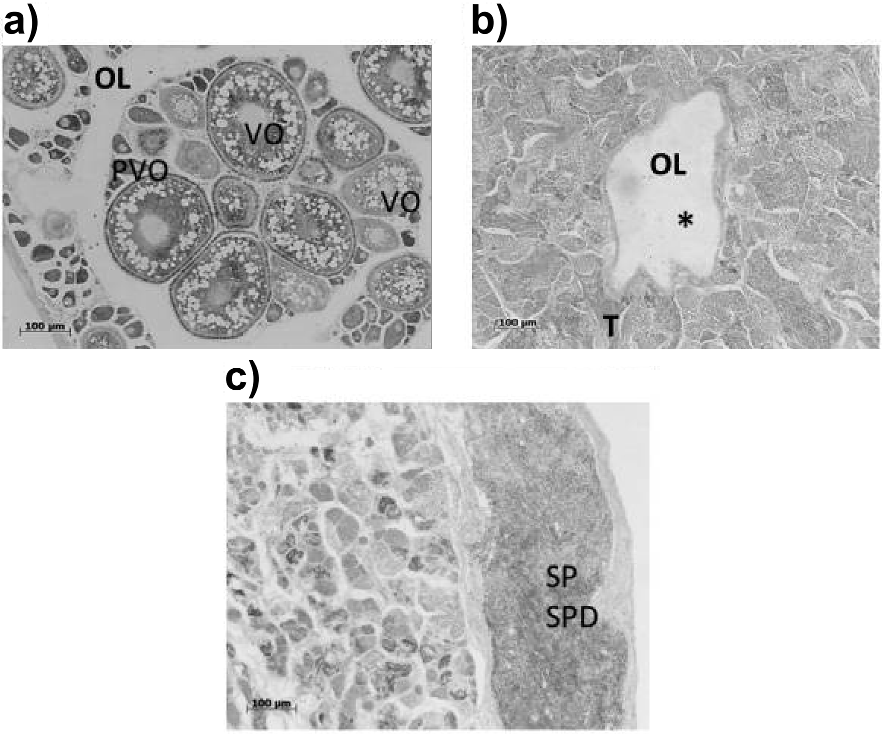

Fig. 5. *Dascyllus flavicaudus*: (a) Oocytes at different stages of development. VO: vitellogenic oocytes; PVO: previtellogenic oocytes; (b) Testicular tissue (T) showing different stages of spermatogenesis and a possible remnant of the ovarian lumen (*); (c) Male gonad with the spermduct (SPD) full of spermatozoa (SP).

cells in early stages of development predominate. They are distributed throughout the entire gonad amidst the stromal-like cells. Finally, at late reversal stage, the gonad testis of *D. trimaculatus* consists entirely of spermatogenic tissue, except for remnants of the prior ovarian phase (oocytes and the ovarian lumen) (Asoh and Kasuya 2002).

Dimorphism in sperm duct configuration, as a criterion to distinguish between primary and secondary males (Reinboth 1962, 1970), is not clear in the genera *Dascyllus*. According to Shapiro and Rasotto (1993), in the testes of the secondary males of *Thalassoma bifasciatum* (with a lumen), the sperm duct runs within the testis wall in the form of sperm sinuses. On the other hand, the sperm ducts of primary males seem to run as simple tubes in the medial region of the gonads (Asoh 2003). The testes of the diandric protogynous species *D. flavicaudus* develop from the ovaries and exhibit an ovarian lumen (Asoh 2004). We have also seen a probable remnant of the ovarian lumen in some *D. flavicaudus* testes (Fig. 5b).

Sex changers, pre-maturational sex changers and non-sex changers of protandrous Amphiprion

In protandric species, the development of male cells is both preceded and followed by ovarian development (Shapiro 1992). It would mean that female development is primary and that male development is a temporary phase initiated by a masculinizing mechanism and terminated by its cessation. This primary ovarian differentiation has been observed at least in *A. clarkii*, *A. frenatus*, *A. perideraion, A. melanopus* and *A. akallopisos* (Miura et al. 2003, Hattori and Yanagisawa 1991, Hattori 1991, 1994, 2000, Godwin 1994b, Casadevall et al. 2010). In these species, immature gonads show predominant ovarian tissue with primary (early and perinucleolus) oocytes, while spermatogenic germ cells are not distinguishable. After the immature stage, ambisexual gonads appeared in juveniles: oocytes appear early and are a prominent part of the gonad in both juveniles and functional males (Elofsson et al. 1997). Francis (1992) suggests that female development is the "default" pattern for teleosts, with gonads developing along a female pathway unless male development is somehow induced.

In *A. polymnus*, the first sexually differentiated sex cells were hermaphrodite, having clones both of oogonia and spermatogonia (Rattanayuvakorn et al. 2006). Nonetheless, in the very young gonads of *A. ocellaris*, we observed a bigger proportion of ovarian tissue (M.C. unpublished data; Fig. 6a).

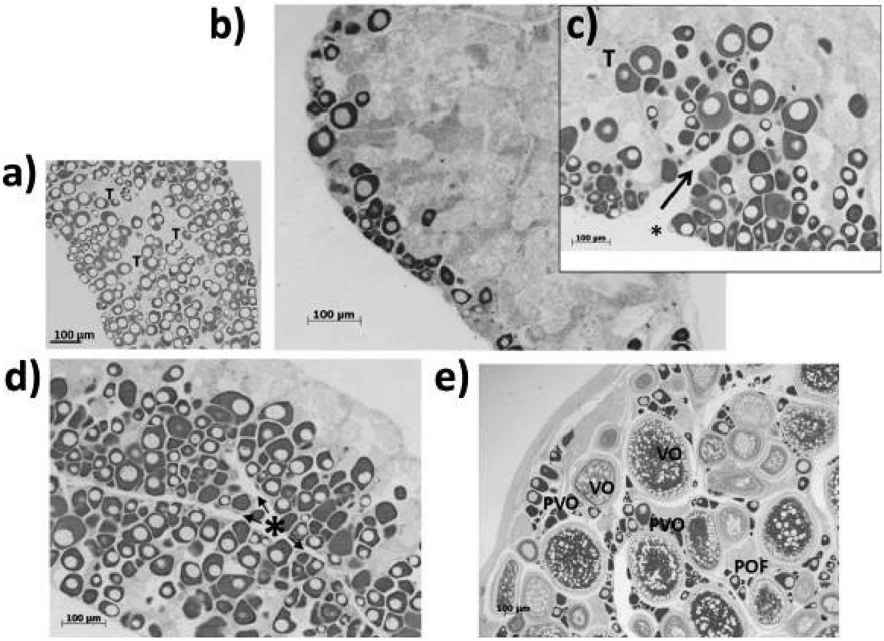

Fig. 6. (a) *Amphiprion ocellaris*. Early gonad, with not many clones of spermatogonia (T) between oocytes; (b) *A. akallopisos* male gonad. Gonad consisting mainly of testicular tissue, with some primary growth phase oocytes at the periphery; (c) *A. akallopisos*. Early reversal stage. Invagination of the ovarian tissue (*) in lateral view; (d) *A. akallopisos*. Early reversal stage. Invagination of the ovarian tissue (*) in frontal view; (e) *A. akallopisos* female gonad. Oocytes in different stages of development, previtellogenic (PVO) and vitellogenic as well (VO). Post ovulatory follicles are also observed (POF).

Amphiprion *gonadal development*

Juveniles are non-breeders with ambisexual gonads. Abol-Munafi et al. (2011) observed that non-breeders of *A. ocellaris* possess an intermixed ovotestis without boundaries between the testicular and ovarian regions. According to Miura et al. (2003, 2008), the ovarian development of *A. clarkii* begins two months after hatching, but testicular tissue does not begin its differentiation until the fifth month.

In *A. akallopisos*, the subadult male gonads show different stages of development of male germinal cells, including some spermatozoa (but not in the seminiferous tubules or in the sperm duct) and primary growth phase oocytes (Casadevall et al. 2010). In those males, testicular tissue gradually dominates the gonad and there is no ovarian cavity (Fig. 6b). The same has been observed in subadult males of *A. ocellaris* (M.C. unpublished data). In both species, the female region is peripheral whereas there is a larger proportion of mature testicular tissue with seminiferous tubules. In these tubules, cells in all the different stages of development are organized in cysts and show synchronous development. Upon completion of spermiogenesis, cysts open up and the spermatozoa are discharged into the lobular lumen, from where they head to the sperm duct. Only oocytes in the primary growth phase are observed in the ovarian tissue, peripherally located: oogonia, chromatin-nucleolar and perinucleolar stages. Oogenic activity of the ovotestis does not proceed beyond the perinucleolus stage either in *A. polymnus* (Rattanayuvakorn et al. 2006), *A. frenatus* (Bruslé-Sicard and Reinboth 1990, Hattori 1991), *A. clarkii* (Hattori and Yanagisawa 1991, Hattori 1994) and *A. perideraion* (Hattori 2000).

The transition to a functional male involves proliferation of spermatogenic tissue. The male tissue is composed of sexual cells in all the different stages and includes large amounts of spermatozoa. *Amphiprion akallopisos* testicular tissue has a remarkable increase in size and the spermiduct is full of ripe sperm, with clearly visible blood vessels close to this area (Casadevall et al. 2010).

The central gonadal lumen or ovarian cavity is not observed in ambisexual or male gonads, neither in *A. akallopisos* nor in *A. ocellaris*.

Male-to-female sex change of protandrous Amphiprion

In some species, sex differentiation follows a gradient from the anterior to the posterior areas of the gonads regardless of sex (Strüssmann and Ito 2005). Sex reversal in *A. akallopisos* and *A. ocellaris* also occurs in a cephalocaudal gradient (Casadevall et al. 2010, Abol-Munafi et al. 2011), with the formation of an ovarian cavity or lumen. The cavity formation has also been related to the sex reversal process in *A. frenatus* (Hattori 1991), *A. perideraion* (Hattori 2000), and *A. clarkii* (Hattori and Yanagisawa 1991).

Miura et al. (2003, 2008) observed that the initial ovarian cavity formation was indicated by the presence of two elongated aggregations of somatic cells in the basal and distal portions of the gonads. However, in *A. akallopisos*, the gonad lumen formation (Fig. 6c,d) appears to be due to an invagination of the ovarian tissue (Casadevall et al. 2010), in a very similar manner to the process described by Godwin (1994b) for *A. melanopus*; the spermatogenic tissue being rejected to the periphery of the gonad. The whole process of sex reversal in *A. akallopisos* (transverse section) is illustrated in Fig. 7.

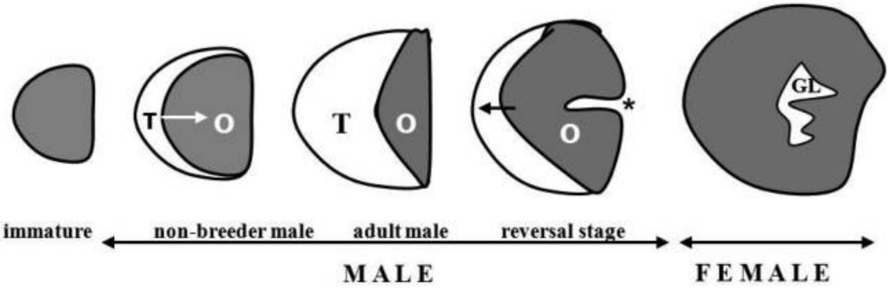

Fig. 7. *Amphiprion akallopisos*. Process of sex reversal from immature stage to female stage (in a transverse section). GL indicates the gonadal lumen formation from the ovarian tissue invagination.

During the lumen development of the latter, the ovarian tissue is mainly composed of oocytes in the primary growth phase (oogonia, chromatin-nucleolar and perinucleolar stages) but an additional stage of cortical alveoli of some oocytes, with lipid drops in their cytoplasm, is observed, indicating that vitellogenesis process is ready to start. It is possible to observe all the spermatogenic stages (from spermatogonia to spermatozoa) in the testicular tissue.

The mid-reversal stage ends with the complete delimitation of the gonad lumen. At the late reversal stage of *A. akallopisos*, the degeneration of testicular tissue is observed. Simultaneously, the ovarian tissue develops numerous oocytes in the first cortical alveoli development stage.

When oocytes begin to mature, the testicular parts of the intersexual gonads disappear in *A. akallopisos*. This was also observed in *A. clarkii*, *A. polymnus*, *A. perideraion*, *A. sandaracinos* and *A. ocellaris* by Moyer and Nakazono (1978).

In the mature female gonad (Fig. 6e), ovarian tissue is well developed around the straight luminal spaces. All the oocyte stages are found: chromatin-nucleolar, perinucleolar, cortical alveoli, vitellogenic stages, mature and atretic oocytes. The ovarian development is clearly asynchronous, with oocytes of all stages, without dominant populations (Wallace and Selman 1981).

Postovulatory follicles (POF) were also observed in active females, indicating a recent spawning event (Casadevall et al. 2010). The spawning periodicity is variable in clownfishes, but it has been related to lunar cycles in some tropical species. In *A. melanopus*, for instance, spawning peaks coincide approximately with the first and third quarters of the moon (Ross 1978a). Although the relationship between spawning and lunar cycle is not clear for all the species (Gordon and Bok 2001), it means that a period of *ca*. 15 days minimum is required between the development of the clusters of cortical alveoli oocytes to the next spawning event.

Neuroendocrine Aspects and Social Control of Sex Change

Signals used by anemonefish for social control of sex change are diverse. Such signals could be attributed to behavioral interaction between sexes, relative size, sexual proportion, and possible stimuli such as pheromones, visual cues or sound communication (Loft 1991, Guerrero-Estévez and Moreno-Mendoza 2010, Fricke 1980, Fricke and Fricke 1977, Colleye et al. 2009, Colleye and Parmentier 2012).

In *Amphiprion* species, behavioral interaction between sexes seems to be stronger and more hierarchical. According to Ross (1990), protandrous suppression refers to suppression of sex change in protandrous species, and occurs when one or more members of a social group prevent sex change in a candidate fish, normally through aggressive dominance. Sex change is initiated or completed only when the suppression condition is removed (disinhibition), normally when the single dominant terminal-sex individual dies or vacates the social group. This is the case of *Amphiprion* species, and induction by other specific stimuli does not seem necessary.

In *A. ocellaris*, sex differentiation of the upper-ranked individuals is gradually determined by long term social interactions (Iwata et al. 2008). However, Fricke and Fricke (1977) had observed that sex reversal in *A. akallopisos* also occurred in the absence of intragroup social pressure. In all those cases, females were not present, so that they concluded that females control the emergence of other females. It is also possible that some non-breeders become females without passing through a functional male state when the opportunity arises, as in *A. clarkii* (Ochi and Yanagisawa 1987, Ochi 1989b, Hattori and Yanagisawa 1991) and *A. frenatus* (Hattori 1991).

Actually, behavioral sex change can be faster than gonadal changes, and sometimes occurs between minutes and hours (Robertson 1972, Warner and Swearer 1991). According to Francis (1992), for species with environmentally labile sex determination that respond to social cues, the brain is the likely site of recognition of social events, and hypothalamic GnRH (gonadotropin releasing hormone) is the key regulator. As stated by Godwin (2009), social control of sex change in fishes must be initiated through changes in neural signalling, but the understanding of the mechanisms is still very basic.

In a recent study of *A. melanopus*, findings by Kim et al. (2012) support the hypothesis that GnRH plays an important role in the regulation of gonadal development and its sex change. It is suggested that GnRH peptides are probably involved in the regulation of gonadal function as autocrine or paracrine regulators.

Hypothalamic GnRH neurons are the control centre of the hypothalamo- pituitary-gonadal axis and reproductive function. In fact, studies of hermaphroditic fish suggest that brain GnRH and arginine vasotocin (AVT) are involved in the sex change process (Foran and Bass 1999, Godwin 2009, 2010). More specifically, AVT has been closely linked with the display of aggressive social dominance, either increasing or decreasing this behavior (Godwin 2010).

Munakata and Kobayashi (2010) propose a sexually bipotential brain for some teleost fishes: when a protogynous hermaphroditic fish is in a female phase, the female portion of the brain is active and the male portion is quiescent; at the time the individual starts to behave as a male, the male portion of the brain is activated and the female portion becomes quiescent.

According to Elofsson et al. (1997), the male sex is associated with greater numbers of preoptic GnRH hypothalamic cells; increase in GnRH cell number correlates with protogynous sex change and teleost sexual maturation, and the decrease of the GnRH cells with protandrous sex change.

Thus, the complete gonad sex change probably involves the brain GnRH, and pituitary together with gonadotropins gonad steroid hormones (androgens and estrogens). Indeed, anemonefishes show higher levels of estradiol in females, while 11-ketotestosterone levels are higher in males (Godwin and Thomas 1993).

However, despite recent insights, the molecular mechanism underlying gonadal sex change in hermaphroditic fish is not yet well understood.

Growth, Inter-group Movement and Sex Change Strategies in Relation to Types of Refuge

We have reviewed the sexuality patterns of the genera *Dascyllus*, *Amphiprion* and *Premnas*. Sex-changing species in damselfishes are all habitat-specialists. When inhabiting small discrete refuges, sex-changing species form small groups with a size-ordered dominance hierarchy. However, the body size composition of group members largely differs between *Dascyllus* and anemonefish (*Amphiprion* and *Premnas*) species.

In anemonefishes living in isolated small groups, body size differences (or ratios) between group members adjacent in rank are large and constant, groups are rather stable, and individual inter-group movements rarely occur. When their hosts are the single form, the home ranges of subordinates are often confined to single hosts. However, aggressive interactions among group members are rarely observed (Hattori 1991, 1995, 2000, 2005, Buston and Cant 2006). When they inhabit aggregational hosts or densely distributed single hosts, where inter-host movements frequently occur, aggressive interactions between group members (or neighbors) are intense and body size differences between males and females become small (Fricke and Fricke 1977, Ross 1978b, Fricke 1979, Hattori and Yanagisawa 1991). Godwin (1994a) reported highly aggressive interactions between the members of small groups in *A. melanopus* at Madang, Papua New Guinea, but information on host anemones are unavailable: these groups may be formed at isolated small aggregations of anemones and the body size differences are small, like at Guam (Ross 1978a). Thus, the large body size differences may minimize costly conflicts among individuals that live in the isolated small groups (Buston et al. 2007, Buston and Zink 2009). In *Dascyllus*, even those inhabiting discrete branching corals, the body size differences between adjacent ranked members are small or trivial, groups are unstable, and individual inter-group movements often occur (Fricke 1977, Asoh 2003). The large body size differences must be unnecessary for *Dascyllus* group members to coexist, even those inhabiting discrete branching coral heads. We speculate that positive or negative effects of the changes in neural signalling caused by visual cues on the production of GnRH may be a crucial determinant in the social control of sex change.

The differences between *Dascyllus* and anemonefish species may be due to differences in refuge structure (Fig. 8) and distribution pattern. Giant sea anemones, in which *Amphiprion* is present, have a tentacle-grown soft basin structure, in which

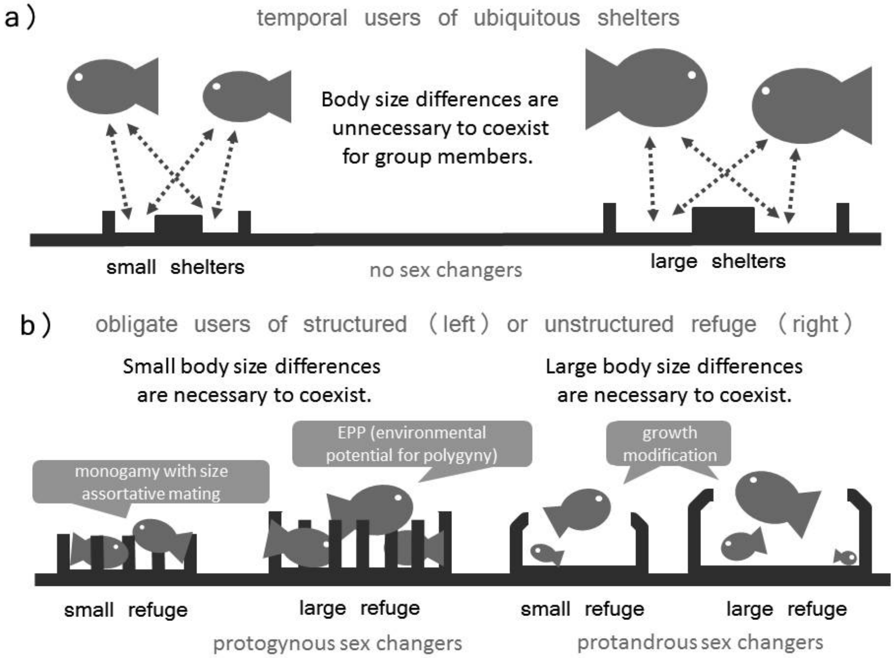

Fig. 8. Model of coexistence of group members in a damselfish species in relation to habitat use pattern. (a) Aggregational and gregarious species that temporally use ubiquitous shelters. Body size differences are unnecessary for group members to coexist in a site. (b) Social species that always use structured or unstructured refuges. Large body size differences are necessary for group members to coexist in an unstructured refuge, otherwise subordinates would receive fatal eviction by dominant fish. Small body size differences are necessary for group members to coexist in a structured refuge, although the complicated structure of refuge may provide the members with a type of screen, which may weaken the social stress by visual cues that may be able to retard the growth of subordinates.

individuals of similar body size could hardly coexist while interacting aggressively, as frequently seen in a small aquarium with open space. Consequently, subordinates adopt a growth modification strategy to prevent *fatal eviction* by dominant fish outside the anemone in the field (Buston 2003a,b). In short, because of the unstructured refuge, low average density of anemones and a high risk of mobility, subordinates must adopt a mating strategy that involves waiting for vacated breeding posts (Hattori and Yamamura 1995, Hattori 2000). In contrast, live branching corals have a 3-D hard framework structure. This may look like a small aquarium in which a structured object is centered. Subordinate individuals of a group can grow gradually in one side in order to achieve dominant status in a new group because the structured refuge, the high density of the refuge and their low risk of mobility (i.e., no fatal eviction by dominant fish) allow them to escape from social stress exerted by dominants in the field. Similar reproductive tactics are described in other protogynous fishes (Munday et al. 2006). The difference in body size composition of group members between *Dascyllus* and anemonefish species is attributable to the difference in their growth and inter-group movement strategies while using different types of refuges.

Conclusion and Prospects

Comparing sex changers and non-sex changers among damselfishes, we have found that sex changers are all obligate refuge-dwelling species, while the others are basically temporal users of ubiquitous shelters. In addition, we can conclude that frequent sex changes can be observed where social species utilize isolated small patches. Relatively large and/or structurally complicated refuges may be able to weaken the social stress among subordinates because excessive space or complicated hard structure would allow them to escape from behavioral stress that influences the social control of sex change (Fig. 8). Accordingly, large body size differences between adjacently ranked group members must be necessary when they are confined to small and/or unstructured refuges. Probably, this body size difference results from their growth modification strategies to coexist with dominants and to avoid fatal eviction. In a structured refuge, as an intermediate state, only small body size differences may be necessary.

Bidirectional sex change has been recently found in some protogynous coral reef fishes (e.g., Kuwamura et al. 1994, Munday 2002, Munday et al. 2006). In fact, some species and clades have evolved the capacity to change sex in either direction because their local, social or environmental conditions can change dramatically and unpredictably in a way that favors repeated changes of sex (Erisman et al. 2013). Among demersal spawners, bidirectional sex changers are always found in small habitat specialists, such as polygynous *Trimma* and monogamous *Gobiodon* and *Paragobiodon* gobies (Kuwamura and Nakashima 1998, Wong and Buston 2013, Karplus 2014). Accordingly, one may expect that polygynous *Dascyllus* and monogamous anemonefishes also have the ability of bidirectional sex change. Cole (2002) suggests the presence of bidirectional sex changers in *Dascyllus* based on observations of the gonad structure, and Kuwamura and Nakashima (1998) conducted several experiments to find out whether *Amphiprion* have this ability. However, it has not been confirmed in the damselfishes (Wong and Buston 2013, Karplus 2014). Both-ways sex changers may need very small “structured” refuges, where EPP is unclear and pairs of similar body sizes can be formed (Fig. 8). In the bidirectional sex-changing gobies, because of the smaller body size, the burden for males in parental care must be severer than in case of the social species of damselfishes and consequently subordinate females can grow faster than males, unlike in case of the damselfishes. In addition, they mate with the first partner they encounter in a discrete refuge avoiding further risky movements (Kuwamura and Nakashima 1998, Wong and Buston 2013, Karplus 2014). Accordingly, the alternative sex-changing strategies can be observed in the gobies under various social statuses of individuals.

For anemonefishes, however, the necessity of large body size differences between pair members for coexistence may prevent bidirectional sex change. Indeed, forcible pair formation of females with a small body size difference always causes the death or emigration of one partner (Fricke and Fricke 1977, Kuwamura and Nakashima 1998). After a sex change from male to female, the rest of the previous testicular structure in the gonads disappears in *Amphiprion* (Godwin 1994b, Casadevall et al. 2010, Abol-Munafi et al. 2011). Thus, the reverse sex change does not seem to be an option. Further studies are required to confirm whether *Dascyllus* have the ability of bidirectional sex change or not.

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