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**Social behaviour of the red swallowtail perch *Anthias anthias*
(Linnaeus, 1758) (Perciformes: Anthiadidae)**

Candidata:
Valentina Grippo

Relatore:
Dott. Federico Betti

Correlatore:
Dott.ssa Sara Ferrando

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Abstract

Anthias anthias (Linnaeus, 1758), commonly known as the swallowtail perch, is a widely distributed species in the Mediterranean Sea, typically associated with coralligenous formations. Despite its abundance, scientific studies on this species remain limited. *A. anthias* is known to be a protogynous hermaphrodite, and it has long been presumed to exhibit a harem social structure. This assumption likely arises from its classification within the family Anthiadidae, whose members share key characteristics, including protogyny, small body size, gregarious behaviour, and, in some cases, harem formation. However, empirical evidence directly supporting these aspects of *A. anthias*' reproductive and social behaviour remains scarce, underscoring the need for further research to clarify its social organisation. This study aimed to investigate the social behaviour of *A. anthias* by analysing the sex ratio within groups and conducting morphometric assessments to identify potential sexual dimorphism. These analyses provide essential insights into the species' social structure and contribute to a better understanding of its reproductive strategies.

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1. Introduction

1.1 —*Hermaphroditism in fishes*

In the animal kingdom, no group of vertebrates matches the extraordinary diversity of fishes, which, with over 36,000 valid species described to date, dominate the aquatic ecosystems across the globe (Nelson et al. 2016). Approximately 99% of all vertebrates are gonochoristic, meaning they consist of separate sexes, i.e., distinct males and females. The remaining 1% of vertebrate species are hermaphroditic, almost all being fish. (Awise 2011; Ashman et al. 2014).

While it is true that members of most sexually reproducing species can be defined as either male or female, the biological world presents a more complex picture for other species. Hermaphroditic organisms provide a fascinating glimpse into alternative reproductive strategies in nature; their ecological and evolutionary successes and failures show how adaptable they can be, and this adaptability is often advantageous in certain environments or ecological contexts.

The ancient Greeks were fascinated by the concept of hermaphroditism in their mythologies. The term “hermaphroditism” indeed derives from the mythological fusion of Hermaphroditus, the child of Hermes and Aphrodite; according to the myth, he was fused with the nymph Salmacis after she prayed to the gods for eternal union. As a result, the two became one being, possessing both male and female physical traits, thus giving rise to the concept of hermaphroditism — a phenomenon in which an organism develops both male and female reproductive organs.

Since the 18th century, in botanical and zoological context, the term has referred to species in which the presence of both male and female organs in the same individual is not an anomaly but a natural condition, as seen in flowering plants and certain species of invertebrates, where hermaphroditism represent a well-established and advantageous reproductive strategy (Ghiselin, 1969; Leonard, 2006). However, for a long time, the presence of both sexes within the same organism was interpreted through an evolutionary framework that regarded hermaphroditic as a primitive trait, characteristic of “lower” organisms. According to a now outdated perspective, ancestral metazoans were assumed to be hermaphroditic, with the separation of sexes seen as a progressive evolutionary step toward greater complexity (Haeckel, 1874). This view, supported by the theories of Haeckel (1874), reflected a hierarchical view of evolution, suggesting that the development of distinct sexes was a necessary advancement in biological organization. Modern scientific evidence has refuted this notion,

demonstrating that the evolution of sexual reproduction does not follow a single, predetermined direction Altenberg (1934).

Transitions between hermaphroditism and separate sexes have occurred multiple times throughout evolutionary history, driven by specific ecological and selective pressures (Jarne & Auld, 2006; Schärer, 2009). While there may be a general tendency toward the division of reproductive roles, as suggested by early theorists (Haeckel, 1874; Müller, 1932), no universal rule explains all instances of shifts between these reproductive modes.

Thus, hermaphroditism is no longer regarded as an inherently "primitive" trait but rather as an adaptive strategy that can evolve or be lost depending on environmental and selective conditions. This shift in perspective has led to a reevaluation of hermaphroditism's role in biological diversity, acknowledging its complexity and evolutionary significance (Leonard, 2018).

In fish, hermaphroditism has been observed in around 2% of extant species (Pauly, 2004), spanning more than 40 taxonomic families across 17 orders (Breder and Rosen, 1966; Smith, 1975; Mank et al., 2006). This reproductive strategy is particularly advantageous in environments with low population densities or where encounters with mates are infrequent, allowing individuals to increase their reproductive success by performing both male and female roles. Furthermore, hermaphroditism can enhance genetic diversity and adaptability, offering evolutionary benefits in certain ecological contexts (Jarne & Auld, 2006).

Hermaphroditism can be divided into two primary types: *simultaneous hermaphroditism*, in which individuals possess both male and female organs at the same time, and *sequential hermaphroditism*.

1.1.1 — *Simultaneous hermaphroditism*

Only around 0,2% of fish species are simultaneous hermaphrodites (SH), suggesting that nearly 1 in 500 species show simultaneous hermaphroditism. Among fish, we have, e.g. the painted comber *Serranus scriba* (Linnaeus, 1758). This fish (Fig. 1a), which belongs to the family Serranidae, is found in the Mediterranean and Black Sea, along with the eastern Atlantic areas. It is commonly found on rocky bottoms and *Posidonia oceanica* meadows up to depths of 150 m, though it typically resides around 30 m depth (Lokovšek, 2022). It is easy recognizable by its vibrant coloration; on the upper part of the head and throat, there are thin blue and orange lines that can resemble writing, from which the specific name "scriba" is derived. While its appearance is quite distinctive, what is even more fascinating to us is the fact that this species exhibits simultaneous hermaphroditism. This characteristic is also observed in many other species: e.g., the order Aulopiformes, consisting of 13 families, many of which are deep-sea species exhibiting SH as a reproductive system, as well as many species from the Anguilliformes order (Kuwamura et al, 2020). It is often stated that simultaneous hermaphroditism is common among deep-sea fishes (Warner, 1984; Herring, 2001). More than one-third of fish species confirmed to be simultaneous hermaphrodites are deep-sea dwellers, and this proportion is remarkably high, considering the challenges and scarcity of studies on deep-sea species. In contrast, only six out of approximately 400 (1.5%) species confirmed to exhibit sequential hermaphroditism are found in deep-sea environments (Kuwamura et al., 2020).

However, this does not necessarily indicate that simultaneous hermaphroditism has evolved frequently in the deep sea, as most of these species belong to a single evolutionary origin within the order Aulopiformes. Among the three extant suborders of Aulopiformes (Nelson et al., 2016), only Alepisauroidei (Fig.1b) exhibit hermaphroditism, whereas the other two (Aulopoidei and Paraulopoidei) are gonochoristic (Smith, 1975; Davis & Fielitz, 2010). Within the Aulopiformes order, e.g., *Chlorophthalmus borealis*, a demersal fish that is expected to be a simultaneous hermaphrodite. This species is commonly known as “green-eyes” due to their large and iridescent eyes.

Within Anguilliformes, particularly in the family Muraenidae, hermaphroditism has been confirmed in several species of moray eels from the genus *Gymnothorax*. In the case of *Gymnothorax griseus* (Fig. 1c) simultaneous hermaphroditism has been confirmed through diagnostic criteria such as histological analysis of the gonads (De Micheson & Liu, 2008). However, in other species of the genus *Gymnothorax*, simultaneous hermaphroditism does not seem to occur; in particular, some

species, such as *G. fimbriatus*, are suspected to be protogynous, but this has yet to be confirmed by scientific studies.

Simultaneous hermaphroditism is also found in some freshwater fish species. One important family to mention is Cichlidae, within which a notable example is *Satanoperca jurupari* (Fig.1d). Commonly known as the "demon eartheater," this species is native to the Amazon River basin in South America and typically inhabits calm waters, including freshwater estuaries. It is known for digging into the muddy substrate in search of food, a behavior that gives rise to its name. Histological analyses have confirmed the presence of ovotestes containing developing oocytes alongside spermatogenic cysts with maturing spermatozoa (Matos et al., 2002). In addition to its hermaphroditic nature, *S. jurupari* engages in *mouthbrooding*, a form of parental care common among cichlids. After external fertilisation, one or both parents protect the fertilised eggs in their mouths for extended periods of time, protecting them from predators until the larvae are capable of independent swimming. This strategy enhances offspring survival, compensating for the high predation pressure typical of these early stages of life.

Another remarkable example in the freshwater environments is the genus *Kryptolebias*, which includes the well-known mangrove killifish. This genus is closely associated with mangrove forests, environments that are particularly challenging to colonise due to their stagnant, warm, and ipoxic waters. These fish are highly resilient, capable of surviving the dry season by seeking refuge in moist microhabitats such as crab burrows or decaying branches, where they can also rely on cutaneous respiration. One of the most intriguing aspects of this genus is found in *Kryptolebias marmoratus* (Fig.1e). This species is unique among vertebrates as it exhibits **sufficient hermaphroditism**, meaning it can reproduce without the need for a partner. Individuals of *K. marmoratus* can be either male or hermaphroditic, with no naturally occurring females known. Males are rare, comprising only about 5% of the population at birth. However, after 3–4 years, approximately 60% of self-fertilizing hermaphrodites transition into secondary males, losing their female reproductive structures and functions (Harrington, 1961; Avise & Tatarenkov, 2015). The proportion of males in a population is strongly influenced by environmental temperature: at temperatures above 25°C, the population consists entirely of hermaphrodites (Turner et al., 2006). ***K. marmoratus* remains the only known vertebrate capable of regular self-fertilisation** (Avise & Tatarenkov, 2015; Earley et al., 2012).

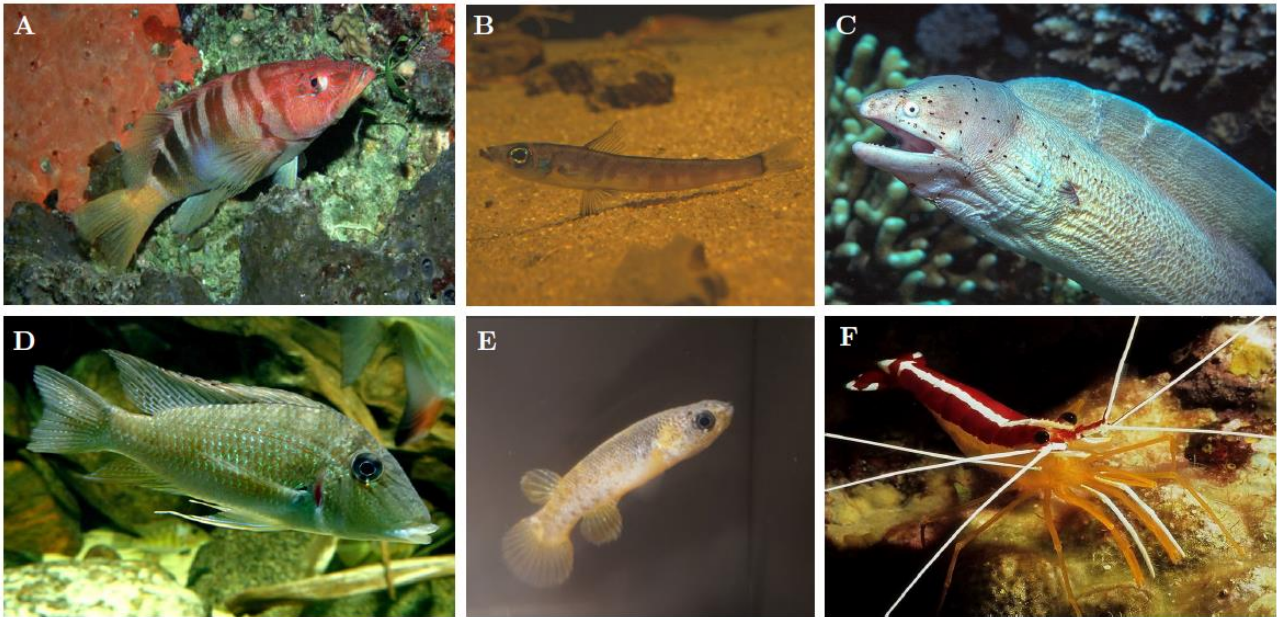


Figure 1: Some of the species in which simultaneous hermaphroditism has been observed: *Serranus scriba* (a); *Chlorophthalmus borealis* (b); *Gymnothorax griseus* (c); *Satanoperca jurupari* (d); the mangrove killifish *Kryptolebias marmoratus* (e) and *Lysmata amboinensis* (f).

In addition to fishes, simultaneous hermaphroditism is found in few other animal groups, and this reproductive system offers both genetic and ecological advantages, particularly in species where mates may not be readily available. For instance, barnacles (Crustacea: Thoracica) are sessile crustaceans that adhere to hard surfaces (like rocks, whale skin or ship hulls). To cope with the challenges of their environment and depth distribution, this group shows diverse sexual systems, including simultaneous hermaphroditism, androdioecy (hermaphrodites-males), and dioecy (females-males) (Yusa et al., 2013). Additionally, they possess exceptionally long penises, allowing them to mate with any nearby barnacle and engage in internal fertilisation, enhancing their reproductive success in stationary conditions (Yusa et al., 2013). Another marine example is the cleaner shrimp *Lysmata amboinensis* (Fig. 1f). These shrimps exhibit a unique form of hermaphroditism known as **protandric simultaneous hermaphroditism**. Individuals first mature as males and, with an ovo-testis that produces only sperm. They later become females with a functional ovo-testis, producing both eggs and sperm but not self-fertilising or storing sperm. The female phase involves one male contributing sperm and the other acting as a female as they grow, and develop female reproductive capabilities while retaining their male functions. This adaptation enables them to perform both roles during mating, increasing flexibility in reproductive partnerships. (Costa et al., 2023).

However, simultaneous hermaphroditism is not exclusive to aquatic environments. Terrestrial organisms also exhibit this reproductive strategy, e.g., many land snails and slugs are simultaneous hermaphrodites, allowing for reciprocal mating where both partners exchange sperm, leading to fertilization in both individuals; terrestrial oligochaete *Lumbricus terrestris*, a member of the Lumbricidae family is also known for simultaneous hermaphroditism and engage in reciprocal sperm exchange during mating.

1.1.2 — Sequential Hermaphroditism

Most non-gonochoristic fishes species are *sequential as opposed to simultaneous*, meaning they can change sex during their lifetime (Awise, 2011; Leonard, 2019). Sequential hermaphroditism is a widely documented phenomenon in fishes, including at least 41 families of teleost orders (Kuwamura et al. 2020). Sequential hermaphroditism is further classified into protandry — male to female sex change (*from Greek "protos" = first, "andr-" = male*), protogyny — female to male sex change (*from Greek "protos" = first, "gyn-" = female*), and bi-directional sex change, in which an individual may switch back and forth between functional male and female. The latter form of sex change, though fascinating, is rarer in nature compared to the unidirectional change, e.g., in certain coral reef species, a dominant male may transition to a female when a more dominant competitor appears in the social hierarchy.

▪ *Protogyny*

Protogynous sequential hermaphroditism— (PG) is common in marine fishes. Among the roughly 480 hermaphroditic fish species, at least 314 species—from 20 different teleost families—have been confirmed to undergo protogynous sex change (Kuwamura et al., 2023). The best-known examples of protogynous fishes—such as the bluestreak cleaner wrasse, *Labroides dimidiatus* (Fig.2a), from Indo-Pacific reefs, and the bluehead wrasse, *Thalassoma bifasciatum* (Fig.2b), from Caribbean reefs—serve as model organisms for various topics in behavioral ecology. In addition, a Mediterranean example is *Coris julis* (Linnaeus, 1758), a small protogynous labrid species found in both the Mediterranean Sea and Eastern Atlantic coasts. *C. julis* is known for its complex sexual dynamics, where individuals typically start life as females, transitioning to males later in life. The family Labridae includes species in which males and females display distinct differences in coloration. The coloration typically associated with females is referred to as the "primary livery," while the coloration linked to males is called the "secondary livery" (Tortonese, 1970). In *Coris julis*, the primary livery, which is typically associated with females, features more subdued colors, often with a mix of greenish and brown hues. The secondary livery, linked to males, is more vibrant and striking, with brighter colors such as blue, yellow, and orange, which are used to attract mates and assert dominance during the breeding season (Fig.2c). Interestingly, among *C. julis*, there are individuals that, despite being males, exhibit female-like coloration, the primary livery (Reinboth, 1967; Warner and Robertson, 1978). This dual sexual system enables them to adapt to different ecological and social conditions, making *C. julis* an excellent subject for studies of reproductive

behavior and sexual dimorphism in marine environments. Another example of protogyny is the well-known Mediterranean dusky grouper *Epinephelus marginatus* (Lowe, 1834) (Fig.2d), along with other members of the family Epinephelidae.

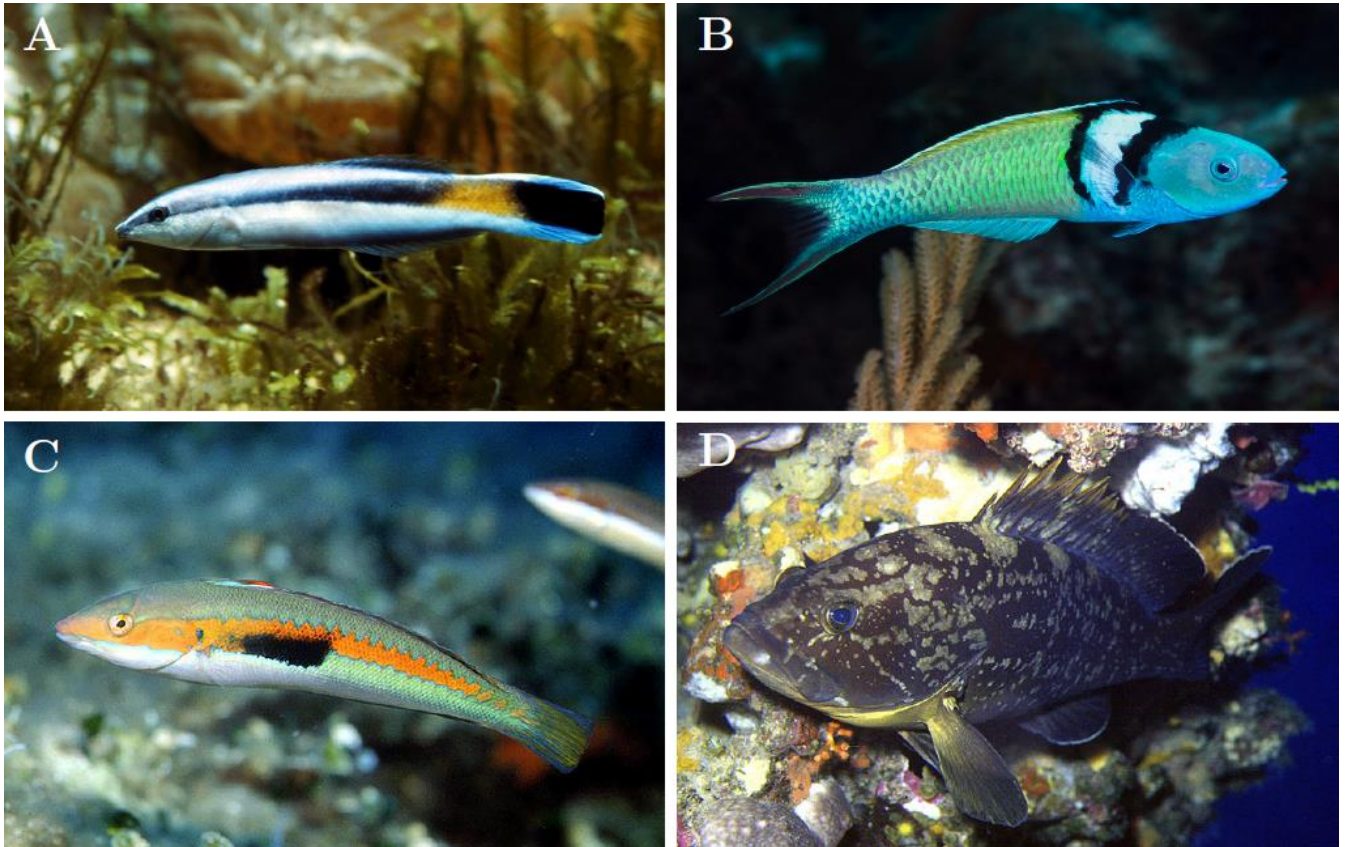


Figure 2: At the top one of the several species of cleaner wrasses, *Labroides dimidiatus* (a); *Thalassoma bifasciatum* (b); a male specimen of *Coris julis* (c); *Epinephelus marginatus* (d).

▪ **Protandry**

Protandry, male-to-female sex change, has been documented in 62 fish species across a diverse range of taxa that are not closely phylogenetically related (Gonçalves and Erzini, 2000; Mouine et al., 2007; Pajuelo et al., 2008). According to the Size-Advantage Model, this reproductive strategy is favored in random mating systems, where the reproductive success of one sex increases more rapidly with size compared to the other. Studies on the mating systems of protandrous fishes are limited to a few species. Among anemonefish (*Amphiprion spp.*, Pomacentridae), monogamy is the dominant system, with females being larger than their male partners (Fig.3a). It is suggested that, in the evolutionary past, pairing occurred randomly in their gonochoristic ancestors before protandry evolved. In flatheads (Platycephalidae) (Fig.3b), individuals change partners after each spawning event, reinforcing a random mating strategy.

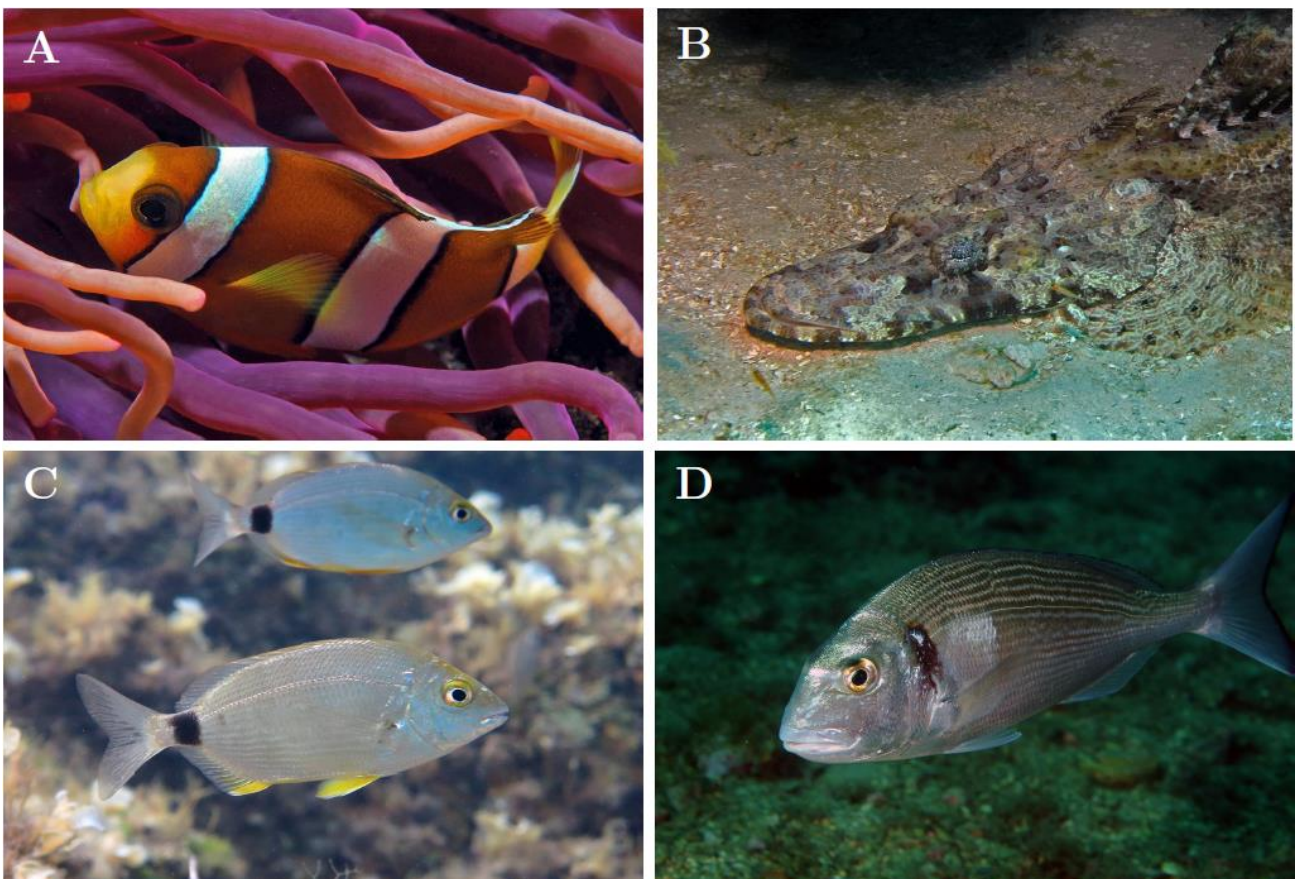


Figure 3: belonging to the Pomacentridae family, a specimen of *Amphiprion clarkii* (a); *Cymbacephalus beauforti*, also known as crocodile fish is a marine ray-finned fish belonging to the family Platycephalidae (b); the annular seabream *Diplodus annularis* (c); a specimen of *Spaurus aurata*.

A similar pattern is observed in the annular seabream, *Diplodus annularis* (Linnaeus, 1758) (Fig.3c) or the gilthead seabream *Sparus aurata*, (Sparidae) (Fig.3d). The apparent lack of male competition in these groups supports the idea that random mating plays a key role in the evolution of protandry (Gonçalves and Erzini, 2000).

▪ ***An interesting case: Bidirectional Sex Change***

Typically, as previously discussed, individuals of sex-changing species are either born as female and then switch to male, or vice versa. Bidirectional sex-change allows individuals to transition between male and female sexes multiple times throughout their lifespan, depending on social and environmental conditions (Kuwamura & Nakashima 1998; Munday et al., 2006). One of the first documented cases of bidirectional sex change in fish was observed in the protogynous grouper *Epinephelus akaara* (Fig.4a), where males appeared to undergo sex reversal when housed together in an aquarium (Tanaka et al., 1990). Later experimental studies further validate this phenomenon; for example, controlled manipulations with the hawkfish *Cirrhitichthys aureus* (Kobayashi & Suzuki, 1992) and the goby *Trimma okinawae* (Sunobe & Nakazono, 1993) (Fig.4b) demonstrated that individuals of both sexes were capable of changing sex when placed in same-sex pairs. However, evidence for this phenomenon occurring in natural populations remains limited. The controlled conditions of captivity may create social and environmental circumstances that facilitate sex reversal, which may not necessarily be replicated in the wild (Kuwamura et al., 2020). Bidirectional sex change was then confirmed in nature within a population of the coral goby *Paragobiodon echinocephalus* (Kuwamura et al., 1994) (Fig.4c). The families where bidirectional sex-change has so far been detected are commonly found on coral reefs and many species have a small body size and cryptic lifestyle (Munday et al., 2010).

While bidirectional sex change offers reproductive flexibility, its rarity suggests associated costs. The physiological and energetic demands of maintaining functional gonadal tissues for both sexes may constrain the evolution of this phenomenon to species under specific selective pressures. (Yamaguchi & Iwasa, 2017). For instance, the energetic cost of sex change has been studied in the bidirectional sex-changing fish *Gobiodon histrio* (Fig.4d), highlighting the significant energy investment required for gonadal transformation.

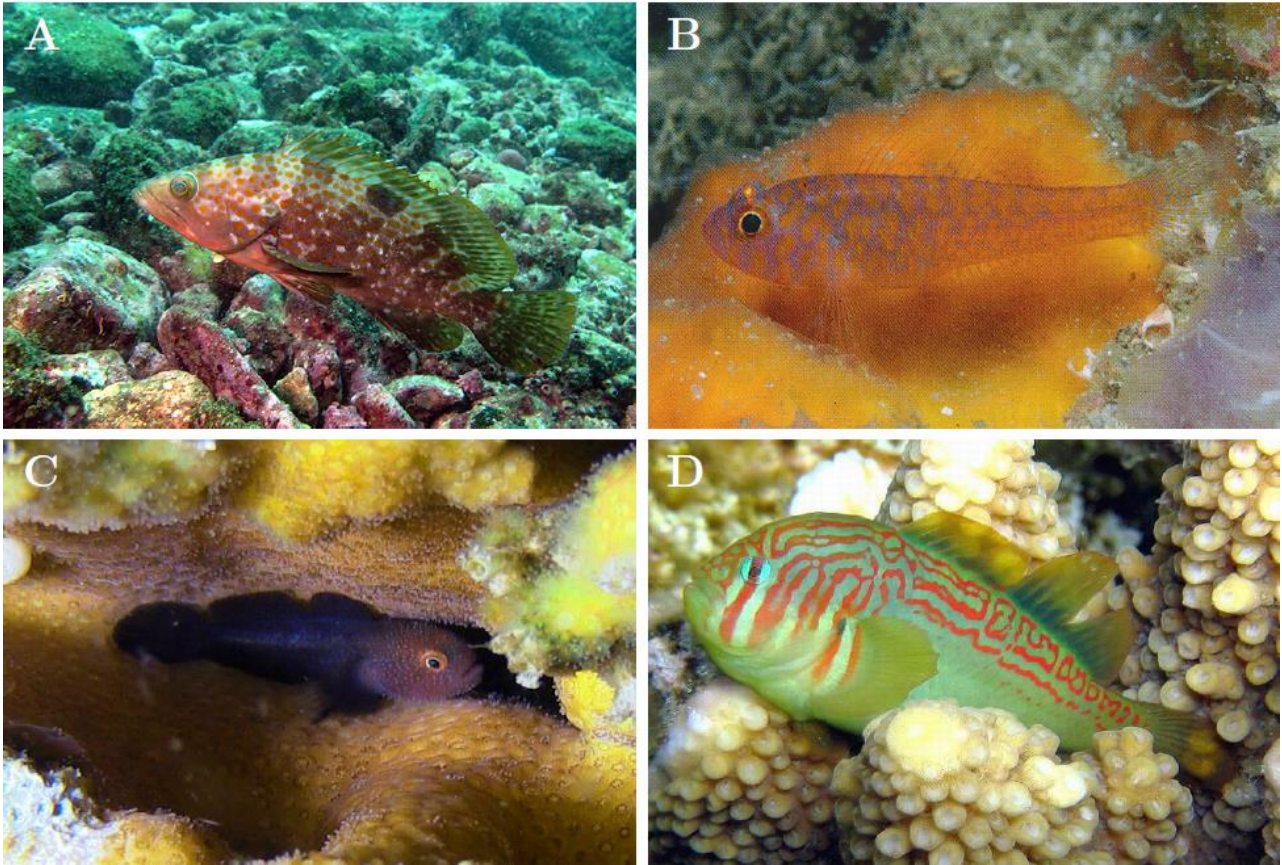


Figure 4: the Hong Kong grouper, *Epinephelus akaara* (a); the orange-red pygmygoby *Trimma okinawae* (b); the readhead goby, *Paragobiodon echinocephalus* (c); a specimen of *Gobiodon histrio* (d).

The evolutionary pathways leading to bidirectional sex change are complex and not yet fully understood. Phylogenetic analyses suggest that this trait has evolved multiple times independently across different lineages, indicating a significant adaptive advantage in certain ecological contexts (Sunobe et al., 2017).

The evolutionary advantages of these strategies are strongly tied to the dynamics of the species' reproductive environment. In protandrous species, males have a competitive advantage at smaller sizes, as they can begin reproducing earlier and with a lower investment of energy. As these individuals grow larger, their transition to females allows them to increase their reproductive output significantly, as larger females typically produce more eggs. Conversely, in protogynous species, where larger males have reproductive benefits, the ability to change from female to male ensures that individuals can maximize their reproductive potential when they reach a size that allows them to compete effectively with other males (Kuwamura et al., 2023).

Thus, hermaphroditism, whether simultaneous or sequential, provides a valuable mechanism for maximizing reproductive output, especially in environments where encounters with mates are not guaranteed or where the cost of reproductive effort varies with size.

1.2 — Phylogeny of Hermaphroditic Fishes and Evolution

In recent years, the phylogeny of hermaphroditism in fish has attracted considerable attention as researchers aim to understand the evolutionary pathways that have led to the emergence of this reproductive strategy. Phylogenetic studies indicate that these reproductive modes have evolved independently multiple times within teleosts, suggesting a strong adaptive response to specific ecological pressures (Ghiselin, 1969; Mank et al., 2006).

As discussed before, early theoretical work by Ghiselin (1969) proposed that hermaphroditism might evolve as an adaptation to low population densities and limited opportunities for mate encounter. Later empirical research has supported this hypothesis by demonstrating that the distribution of hermaphroditic traits is closely related to both the social and environmental conditions in which species live (Warner, 1975; Sadovy de Mitcheson & Liu, 2008). For example, sequential hermaphroditism is commonly observed among coral reef fishes, where social structures such as harems or pair-bonding systems create a favorable context for sex reversal (Warner, 1975).

Recent phylogenetic studies indicate that hermaphroditism occurs exclusively among teleost fish.

While gonadal histology in hagfishes (Myxinoidea) initially suggested the possibility of functional hermaphroditism (Gorbman, 1990), later studies have not confirmed these findings (Adolfi et al., 2019).

Among fish families including over 30 species, the percentage is relatively high in Sparidae (84%), Scaridae (35%), Ipnopidae (28%), Lethrinidae (26%), Pomacanthidae (26%), Labridae (19%), Cirrhitidae (18%), and Serranidae (17%) (Kuwamura et al., 2020).

One major barrier to the evolution of functional hermaphroditism in many vertebrate groups is the pronounced anatomical differences between the sexes. For instance, in groups such as sharks and rays (Chondrichthyes) as well as mammals and reptiles (Tetrapoda), the presence of large copulatory organs makes it energetically costly to either maintain both reproductive systems simultaneously or to undergo a complete morphological transition from one sex to the other (Warner, 1978).

1.2.1 — Phylogenetic studies

Phylogenetic studies indicate that hermaphroditism in teleost fishes has evolved independently multiple times across different lineages. A comprehensive analysis identified at least 27 independent transitions to hermaphroditism within teleosts, with 15 transitions to protogyny and five to protandry (Sunobe & Nakazono, 1993). These evolutionary shifts are often linked to specific mating systems and ecological pressures that favor reproductive plasticity.

The distribution of hermaphroditism across teleost fish families is non-uniform. For instance, simultaneous hermaphroditism has been documented in families such as Serranidae and Gobiidae, whereas sequential hermaphroditism is more prevalent among Labridae and Scaridae. This pattern suggests that hermaphroditism evolved as an adaptive response to factors like mate availability and social structure (Kuwamura et al., 2020).

Both simultaneous hermaphroditism and protandry have evolved multiple times across various, unrelated lineages within Teleostei. In contrast, protogyny has been observed solely in the Percomorphaceae family. Simultaneous hermaphroditism has been documented in four major lineages: Elopomorpha (1 family), Aulopiformes (8 families), Ovalentaria (2 families), and Eupercaria (2 families), while protandry in six major lineage: Elopomorpha (1), Clupeiformes (1), Cypriniformes (1), Stomiatiformes (1), Ovalentaria (1), and Eupercaria (9) (Kuwamura et al., 2023) (Tab.1).

Protogyny has independently evolved in four major teleost lineages: Gobiaria, Anabantaria, Ovalentaria, and Eupercaria within Percomorphaceae, with the latter group exhibiting the highest diversity of protogynous species. Additionally, bidirectional sex change has been recorded in three of these groups, highlighting its evolutionary association with protogyny in certain taxa.

Within Percomorphaceae, both protogyny and bidirectional sex change are widely distributed, indicating their recurrent evolution in multiple lineages. Specifically, **protogyny has been documented in 12 out of 46 Percomorphaceae lineages (26%),** while **bidirectional sex change occurs in six lineages (13%).** **Protandry** is comparatively less frequent, evolving in **five lineages (11%),** whereas **simultaneous hermaphroditism** is the least common, observed **in only three lineages (7%).**

The predominance of protogyny among hermaphroditic fishes, particularly in Percomorphaceae, is likely linked to their mating systems, which often involve small, structured social groups. This pattern is particularly evident in coral reef-associated species, where dominance hierarchies and social dynamics favor female-to-male sex change as a reproductive strategy. The widespread occurrence of

protogyny in hermaphroditic fishes contrasts sharply with the prevalence of simultaneous hermaphroditism found in hermaphroditic plants and invertebrates (Avisé, 2011; Leonard, 2019), suggesting that different ecological and evolutionary pressures drive the development of hermaphroditism in vertebrates compared to other taxa.

Class	Subclass / Order	Hermaphroditism Type
Sarcopterygii	Coelacanthimorpha (Coelacanths)	Absent
Myxini	Myxiniiformes (Hagfishes)	Hypothesized
Chondrichthyes	Various orders (Sharks and Rays)	Absent
Actinopterygii	Teleostei - Elopomorpha	(1 sp.) Simultaneous
	Teleostei - Aulopiformes	(8 sp.) Simultaneous
	Teleostei - Ovalentaria	(2 sp.) Simultaneous
	Teleostei - Eupercaria	(2 sp.) Simultaneous
	Teleostei - Clupeiformes	(1 sp.) Protandry
	Teleostei - Cypriniformes	(1 sp.) Protandry
	Teleostei - Stomiatiformes	(1 sp.) Protandry
	Teleostei - Gobiaria	(1 sp.) Protogyny
	Teleostei - Anabantaria	(1 sp.) Protogyny
	Teleostei - Ovalentaria	(4 sp.) Protogyny
	Teleostei - Eupercaria	(14 sp.) Protogyny
	Teleostei - Cichlidae	(1 sp.) Simultaneous
	Teleostei - Rivulidae	(3 sp.) Simultaneous
	Teleostei - Cobitidae	(1 sp.) Protandry
	Teleostei - Terapontidae	(2 sp.) Protandry

Table 1: Systematic Classification of hermaphroditic fishes, including species counts where available (Kuwamura et al., 2023).

When examining the habitats of extant hermaphroditic fishes, it is notable that **fewer than 3% (13 species) are found in freshwater environments**, despite the fact that approximately 43% of all fish species inhabit freshwater (Nelson et al. 2016). Among these freshwater species, simultaneous hermaphroditism has been documented in Cichlidae (1 species) and Rivulidae (3 species), while protandry is found in Cobitidae (1) and Terapontidae (2) (Tab.1). The lack of hermaphroditic species in freshwater has been attributed to anatomical differences in sex (Warner 1978; Sadovy de Mitcheson and Liu 2008), mating systems (Kuwamura et al. 2020), or evolutionary history (Pla et al. 2021). However, additional research is needed to fully understand this disparity.

1.3 — Theories and Models on the Evolution of Hermaphroditism

The evolution of hermaphroditism in animals has been effectively explained by considering individual fitness, leading to two primary hypotheses.

The first hypothesis is the “classical” explanation of hermaphroditism in terms of selection theory. **The low-density model** (Ghiselin, 1969) dates back to the previous century and it is one of the earliest explanations of this phenomenon. This hypothesis accounts for the benefits of simultaneous hermaphroditism in environments where encounters with conspecifics are rare: environments where individuals are sparsely distributed, limited mating group size (Charnov, 1982) or places where encounters between potential mates are infrequent, local sperm completion (Schärer, 2009), or in the case of sessile species. Under such conditions, possessing both male and female reproductive organs increases the likelihood of successful mating. This theory can also be applied to those population of fish that lack in mobility, e.g., benthic species. In these circumstances, hermaphroditism allows individuals to maximize their mating success, and this is particularly true for simultaneous hermaphrodites compared to gonochorists (Tomlinson 1966). ***This advantage persists despite the energetic costs hermaphrodites incur from maintaining dual reproductive systems*** (Heath 1977). Moreover, the potential for self-fertilization in extremely sparse populations further augments the benefits of simultaneous hermaphroditism (Tomlinson, 1966). Despite this, the low-density model does not provide a satisfactory explanation for sequential hermaphroditism.

Another prominent theory is the **size-advantage model** (Warner, 1975; Cole, 2010). This model is a theoretical framework that explains why some fish species undergo sequential hermaphroditism. This model posits that differences in the growth-related increases in reproductive success between males and females drive the evolution of sequential hermaphroditism (Ghiselin, 1969, 1974). In other words, the reproductive success of an individual is closely related to its size or age. In many species, reproductive success (or fitness) is not constant throughout life; instead, it increases as individuals grow larger. However, the extent of this increase may be different for males and females. According to this model, the direction and occurrence of sex change are determined by the species-specific mating system: in species with **random mating**—where females do not show a preference for larger males—male reproductive success is not strongly correlated with body size. Conversely, protandry may be favored because small males achieve relatively higher reproductive success than similarly sized females. In contrast, polygynous systems, where larger males monopolize access to multiple

females, reproductive success increases sharply with size, thereby favoring protogyny (Warner, 1975, 1984).

To summarize, in protogyny larger males gain a significant advantage by controlling access to multiple females, whereas in protandry, larger females produce more eggs, making female reproductive success more strongly linked to body size.

Another intriguing phenomenon is **partial protandry**, (Gonçalves & Erzini, 2000) observed in 28 of species, where **both protandrous and gonochoristic individuals coexist**. According to the Size-Advantage Model, gonochorism is expected to evolve when reproductive success remains equal for both males and females at all sizes. In species exhibiting partial protandry, individuals may have the flexibility to spawn either randomly or through alternative **mating** strategies, further highlighting the complexity of reproductive patterns in protandrous fish.

Regarding the particular case of bidirectional sex-change, this can also be seen as an extension of the size-advantage model (Kuwamura, 2010). Bidirectional sex change evolved as an adaptive response to highly variable social and environmental conditions. Specifically, this mechanism allows individuals to optimize their reproductive success in systems where mate availability fluctuates and where the costs of searching for a new partner (maybe after a loss) are high. Kuwamura and colleagues (Kuwamura, 2011; 2014) suggest that bidirectional sex change may have emerged from ancestral protogynous species—or even from gonochoristic lineages—as an adaptation driven by social cues that mitigate the fitness costs associated with static sexual roles in unpredictable environments. ***Reversed sex change has not been observed in protandrous fish species*** (Kuwamura et al., 2020). In species where the Size-Advantage (SA) model predict protandry larger females do not monopolize multiple mates, allowing smaller females the opportunity to secure mates without the need for sex change. In contrast, in species with polygynous mating systems or those exhibiting protogyny, smaller males often face limited access to females because larger, dominant males control mating opportunities. In such cases, reversed sex exchange becomes a necessary strategy for smaller males to improve their reproductive success.

1.4— Polygyny

Closely connected to the concept of protogyny is the concept of **polygyny**, which refers to the cohabitation of multiple females within a male's territory, maintaining a stable mating relationship. Polygyny is a mating system in which a single dominant male mates with multiple females during a breeding season. In polygynous systems males often compete for access to females, leading to sexual dimorphism, where males are typically larger or more diverse than females, as these traits provide an advantage in competition. For this reason, commonly, the largest individuals in each harem group function as males and the other smaller individuals are females. Harem polygyny is shown in many species within the Labridae family, more precisely 17 out of 53 species whose mating is known (Kuwamura et al., 2023), along with many members of Scaridae, Serranidae, Balistidae (at least one species), Scorpaenidae (at least one species), etc (Kuwamura et al., 2023).

Polygyny is closely linked to the formation of harems because in polygynous mating systems is the male who controls access to multiple females, forming a harem. With this system, males maximise their reproductive output by mating with multiples females; females on the other hand may benefit from protection provided by males whose often defend resources like feeding grounds or breeding sites.

1.4.1 Harem formation

Another important aspect that has not been fully addressed yet is the formation of **harems**. This is often associated with hermaphroditic species, particularly protogynous ones. In fish, a harem is characterized by the presence of multiple females within a dominant male's territory, who maintains exclusive mating rights over that group of females. Typically, the largest individuals within each harem function as males, while smaller individuals serve as females. This arrangement not only influences individual reproductive success but also drives sexual selection and the evolution of sex change, as the competitive dynamics of harems create strong selective pressures on both males and females (Charnov, 1982). Studies of protogynous species—including those among *Parapercis* and perches, pomacanthid angelfishes, and cirrhitid hawkfishes—have consistently demonstrated harem polygyny in their mating systems. Additionally, harem formation has been documented in other families, with 17 out of 53 protogynous species in the Labridae, 7 out of 22 in the Scaridae, and 6 out of 17 in the Serranidae exhibiting this system. Harem polygyny is also confirmed in the Gobiidae,

Malacanthidae, Scorpaenidae, and Balistidae, with at least one protogynous species recorded in each of these families (Kuwamura, et al., 2023).

Regarding the spatial relationships among females, three distinct types of group structures have been identified in harem systems (Kuwamura, 1984; Sakai & Kohda, 1997):

- **Cohabiting Female-Type Harem:**

In the cohabiting female-type harem, the home ranges of females largely overlap (Fig.5a-b). This configuration has been documented in species such as *Bodianus rufus*, *Labroides dimidiatus*, various *Centropyge* angelfishes, and *Holacanthus tricolor*—accounting for a total of nine species (Kuwamura et al., 2023). A key characteristic of this harem type is the frequent social interaction among its members. Males actively patrol their territories and maintain regular social contact with the resident females, while the females interact not only with the male but also with one another during the daytime. Within these groups, the dominance hierarchy is typically determined by body size, with larger females usually exerting dominance over smaller ones, thus forming a linear-type harem structure (Kuwamura, 1984; Sakai & Kohda, 1997). Such social interactions are considered crucial for the regulation of sex change in these species. Moreover, even in this type of harem, females of similar body sizes tend to become territorially exclusive. While individuals of different sizes continue to share overlapping home ranges, those of similar sizes establish distinct territories. This phenomenon can sometimes result in a male's territory being subdivided into two female subgroups (Robertson, 1974; Kuwamura, 1984; Hoffman, 1985; Hourigan, 1986; Sakai & Kohda, 1997; Munday et al., 2009). This pattern of female territoriality, which is observed in species like the cleaner wrasse *L. dimidiatus* as well as in *Centropyge* and *Holacanthus* angelfishes, has been described as a branching-type harem.

- **Territorial Female-Type Harems:**

In this harem structure, females establish and defend individual territories within the overall area controlled by a dominant male (Kuwamura et al.,2023) (Fig. 5c). This arrangement has been observed in a variety of species—including parapercis and perches, *Halichoeres spp.*, *Iniistius spp.* and *Xyrichtys spp.* wrasses, the epinephelid *Cephalopholis miniata*, species of *Malacanthus* and tilefish, cirrhitid hawkfishes, and the triggerfish *Sufflamen chrysopterus*—comprising a total of 14 species (Kuwamura et al., 2023).

Exclusive territoriality among females in this system is thought to be driven by the need to secure critical resources: shelter in open habitats (Clark, 1983; Baird, 1988), mating opportunities (Ishihara & Kuwamura, 1996; Seki et al., 2009), and food resources (Shpigeland & Fishelson, 1991; Kadota et al., 2011). Unlike the cohabiting female-type harem, where social interactions among females are frequent, interactions among females in territorial female-type harems are rare. Nonetheless, dominant males frequently interact with females during territory patrols and courtship.

▪ **Aggregating Female-Type Harems:**

Fishes are often seen swimming in groups in the water column to feed on zooplankton (Shapiro, 1981; Moyer, 1984; Yogo, 1985; Sakanoue & Sakai, 2019). In these systems, females do not establish exclusive territories or exhibit a size-based spatial arrangement. This harem configuration has been documented in coral-dwelling *Dascyllus spp.* damselfishes, the anthiadidae *Pseudanthias squamipinnis*, and various *Genicanthus* angelfishes (a total of 11 species) (Kuwamura et al., 2023). Although the lack of territoriality among females in this harem resembles that seen in cohabiting female-type harems, females still maintain stable spawning interactions with territorial males (Fig.5d). Notably, all 11 protogynous species forming aggregating female-type harems display a multi-male structure, where two or more territorial males share dominance over the female group. For example, in *P. squamipinnis*, a single male harem may include up to nine females (Shapiro, 1977; Yogo, 1985), whereas larger groups can feature multiple males—as one study recorded a harem with 15 males and 72 females (Yogo, 1985). The overlapping home ranges of females with those of several males allow for opportunities to change spawning partners, suggesting a potential for promiscuous mating. The male's territory is defined by the high density of females, but individual females may occasionally move in or out of the defended area. As the harem size increases, multi-male group formations become more common. High-density multi-male groups are frequently, although not exclusively, observed in coral habitats with abundant coral cover (Fricke, 1980; Shpigeland & Fishelson, 1986).

Following Kuwamura's findings, the formation of harems is considered a key factor in polygynous mating systems among hermaphroditic fishes. This social structure intensifies male competition and creates a strong size-dependent reproductive advantage. In such systems, the dominant, larger male monopolises access to multiple females, leading to a sharp increase in reproductive success with body size. Consequently, this selective pressure favors protogyny since, when the dominant male is lost or absent, the largest female may transition to male to fill that role (Kuwamura, 2010; Warner, 1984).

Furthermore, the harem system exemplifies how social organization and mating dynamics can drive the evolution of sequential hermaphroditism. In environments where male control over a group of females is critical for reproductive success, the ability to change sex ensures that the optimal reproductive role is filled at all times, maintaining the stability of the mating system. This perspective is supported by additional studies that emphasise the adaptive significance of harem formation in promoting sexual plasticity among coral reef fishes (Munday et al., 2006).

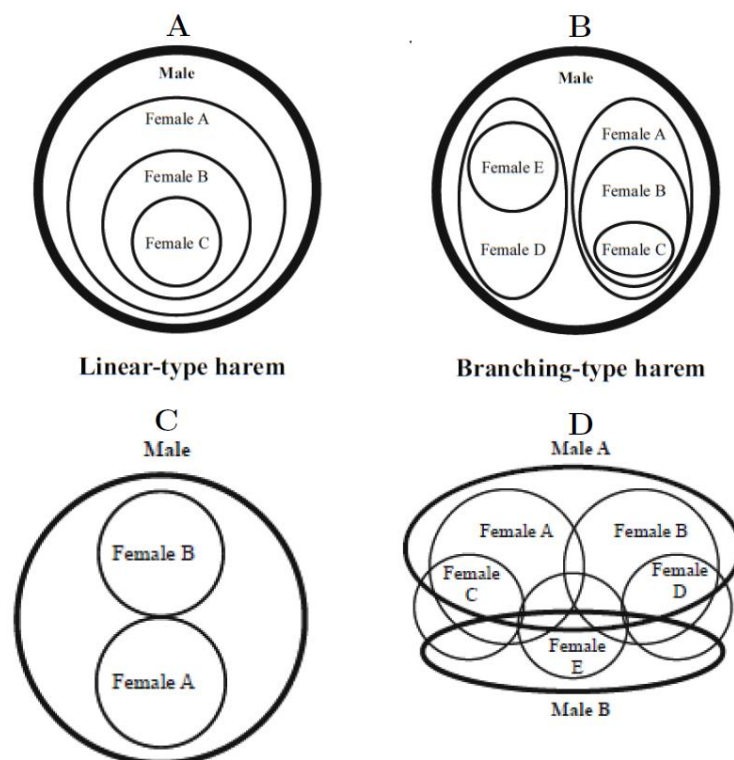


Figure 5: These diagrams illustrate the territories of males (thick lines) and the home ranges of females (thin lines). The first two (a and b) represent a cohabiting female-type harem, observed in the angelfish *Centropyge ferrugata* on the reefs of Sesoko Island, Okinawa (Kuwamura et al., 2023); on the left (a), a linear-type harem is depicted, in which female home ranges overlap within the male's territory, creating a shared space. On the right (b), a branching-type harem is shown, where two dominant females, exclusive of each other, form distinct subgroups within the male's territory. The bottom-left diagram (c) illustrates territories in two harems of the hawkfish *Cirrhitichthys falco* on the reefs of Kuchierabu-jima Island, in which each male territory encompasses two distinct female territories; the latter diagram (d) depicts the aggregating female-type harem, seen in the coral-dwelling damselfish *Dascyllus reticulatus*. In this case, females often swim together, and their overlapping home ranges (thin lines) are contained within male territories (thick lines) (Kuwamura et al., 2023).

1.5 — Case study: *Anthias anthias* (Linnaeus, 1758)

Formally described by Linnaeus in 1758 as *Labrus anthias*, *Anthias anthias* (Linnaeus, 1758), commonly known as the red swallowtail perch, belongs to the class Actinopterygii (ray-finned fishes, from the Greek aktinos = "ray" and pteryx = "fin") and the order Perciformes. This species is part of the genus *Anthias*, a name derived from the Greek and likely referring to *Sparus aurata*. Previously classified within the Serranidae family, *A. anthias* has since been reassigned to the family Anthiadidae following a taxonomic revision.

This family includes many species exhibiting gregarious behaviour, with *A. anthias* commonly found inhabiting rocky substrates and submarine caves at depths between 30 and 200 m (Paglialonga et al., 2001; Espino et al., 2007). This species is widely distributed in the Mediterranean Sea, eastern Atlantic Ocean, including several archipelagos such as the Azores, Madeira, Canary Islands, Cape Verde, and the Gulf of Guinea islands. In the Mediterranean Sea, *A. anthias* is commonly associated with the circalittoral zone, a marine region extending from the lower limit of the infralittoral zone ~40-50 m deep to approximately 200 m. Due to reduced light penetration, this habitat is dominated primarily by animal communities, with limited photosynthetic organisms. Within this environment, *A. anthias* is one of the most abundant species, yet it remains largely understudied. It is frequently observed forming large schools, particularly around coralligenous formations—complex habitats composed of encrusting red calcareous algae that create a highly three-dimensional environment with numerous crevices offering shelter and niches for marine life. Species inhabiting these areas often exhibit elongated body shapes to adapt to the intricate reef structure.

Anthias anthias is a protogynous species, meaning that all individuals first develop as female. The species exhibits pronounced sexual dimorphism: males are larger and darker than females and can be easily identified by the presence of an extended dorsal fin ray. Another distinguishing feature of males is the pelvic fin, which is relatively long, rounded at the tip, and characterised by a yellow patch near its end. In contrast, females are slightly smaller and have sharper, more triangular pelvic fins. Morphologically, *A. anthias* is a small to medium-sized fish, typically ranging between 12 and 18 cm in length, with males generally being larger than females.

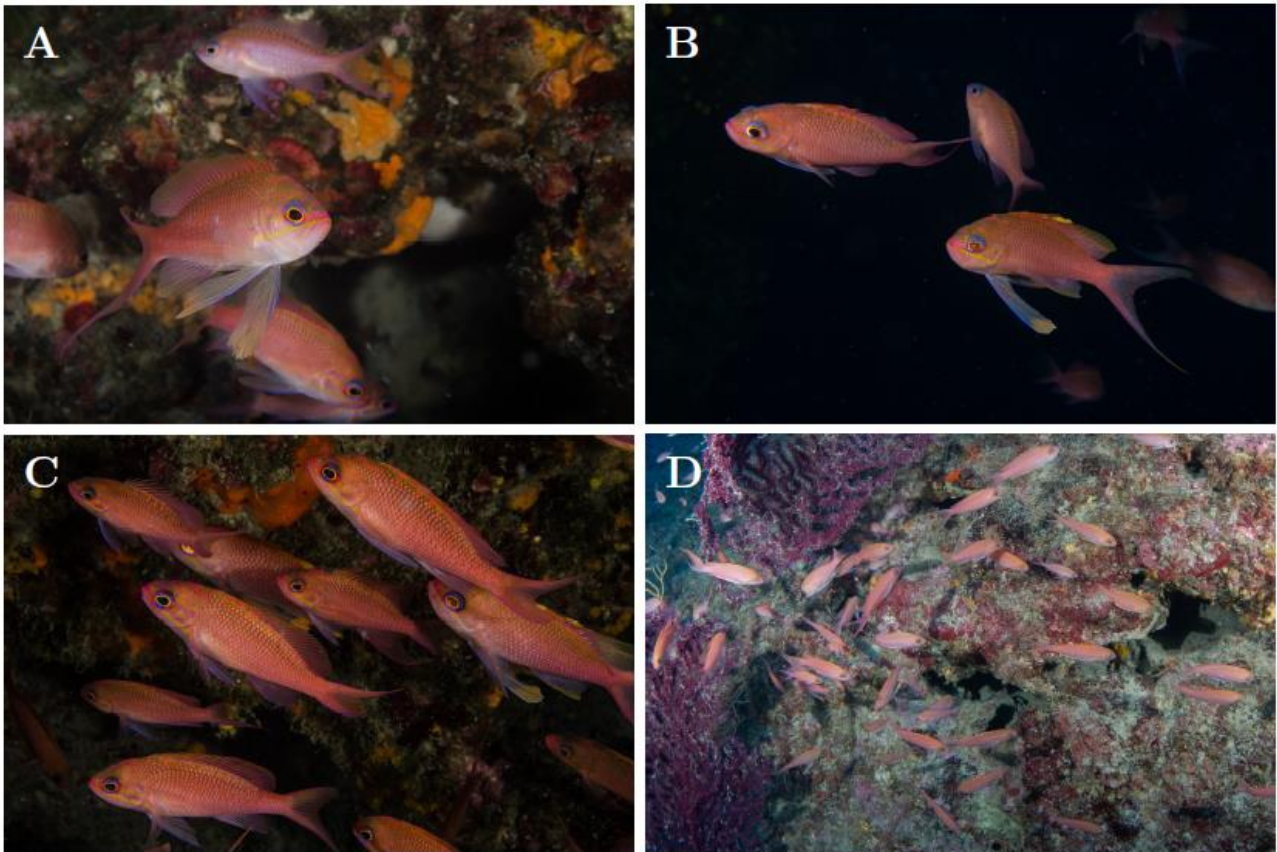


Figure 6: Panels (a) and (b) show male specimens *Anthias anthias* with distinctive characteristics, including elongated pelvic fins with a yellow marking at the tip and the presence of an extended third dorsal fin ray; panels (c) and (d) depict a group of individuals showing the typical aggregation behaviour of the species.

The caudal fin of *A. anthias* is deeply forked, with the lower lobe being longer and more pointed than the upper one, particularly in males (Fig.6 a-b). In females, however, the caudal fin appears more symmetrical. The species exhibits a striking coloration, ranging from reddish-purple to bright orange, with three yellow stripes on the sides of the head and a yellow and blue ring surrounding the eye. Large eyes, larger than the pre-orbital length.

Small caniniform teeth are present, two above and four below. *Anthias anthias* is a planktivorous species, feeding primarily on zooplankton. As a **pelagic spawner**, it releases its eggs and sperm simultaneously into the water column, allowing them to disperse with ocean currents. This reproductive strategy enhances the distribution and survival chances of the offspring, and it is highly common among marine fishes (Duarte & Alcaraz, 1989). Spawning typically occurs from June to September. The fertilised eggs remain planktonic, developing while suspended in the water, before hatching into free-floating larvae that eventually settle in suitable habitats.

This species is commonly observed during scuba dives at various depths, where its gregarious behavior and vivid coloration make it an easily recognizable species. Despite its fascinating nature, it has not been extensively studied, particularly in terms of its social behavior and whether it truly forms harems, as commonly assumed. Many other Anthiadidae species are known to form harems, but they have received greater scientific attention due to their higher commercial value or greater relevance to human interests, while *A. anthias* remains relatively understudied. The family Anthiadidae includes numerous species of marine fishes, many of which are small, brightly colored, and gregarious. Among them, tropical species are particularly well known for their striking appearance and complex social behavior. Many members of this family are known for forming harems, one example is *Pseudanthias squamipinnis* (Peters, 1855), and many other species from the genus *Pseudanthias*.

The genus *Pseudanthias* was originally described by Bleeker (1871, 1873); however, Boulenger (1895) later considered *Pseudanthias* a synonym of *Anthias*, a classification that remained widely accepted until Katayama & Amaoka (1986) revalidated *Pseudanthias* as a distinct genus. *P. squamipinnis* is widely distributed across the western Indo-Pacific, ranging from the Red Sea to South Africa, extending north to Japan and south to Australia. Adults of *P. squamipinnis* are typically found hovering above coral outcrops and outer reef slopes, often forming large aggregations. They are zooplanktivores, primarily feeding on planktonic organisms. This species is a protogynous hermaphrodite, with males being territorial and haremistic, typically remaining within 20 meters of their "home" rock or coral outcrop. (Popper & Fishelson, 1973). The transition from female to male in *Pseudanthias squamipinnis* occurs through a series of gradual behavioural changes that mirror those typically exhibited by mature males. As observed by Shapiro (1981), this transformation does not happen immediately but follows a progressive sequence. Just two days after the removal of the dominant male, the largest females in the group begin to display an increase in aggressive interactions, particularly *nose rushes*—swift movements directed at other fish, often females or potential rivals. By days 4-5, these transitioning individuals start performing *U-swims*—characteristic courtship display in which the fish moves in a looping trajectory. Subsequent stages include the erection of the third dorsal spine (Fig.7b) and, ultimately, gonadal transformation. Notably, females that do not undergo sex change do not exhibit these behaviours.

Another study from Shapiro and Boulon (1987) suggests that these fish live in well-defined groups that are evenly dispersed across the coral reef, with significant spaces separating neighboring groups; the placement of these groups is not dictated by resource availability, but rather by the reproductive and social dynamics of the species. The further a group is from others, the more it can monopolise juvenile settlers, allowing for a steady influx of new members that eventually mature and influence the group's social balance. This balance is particularly important because *P. squamipinnis* is a protogynous hermaphrodite, and the *trigger for sex change is related to the female-to-male ratio within a group*. When this ratio surpasses a critical threshold, one of the dominant females transitions into a male. The frequency of this transition is directly tied to the number of new juveniles joining a group since their maturation into females affects the overall sex ratio. Groups that attract more juveniles experience more frequent sex changes, allowing dominant females to secure reproductive advantages by becoming males sooner. According to Shapiro (1977), on average, *P. squamipinnis* groups contain eight adult females per male.

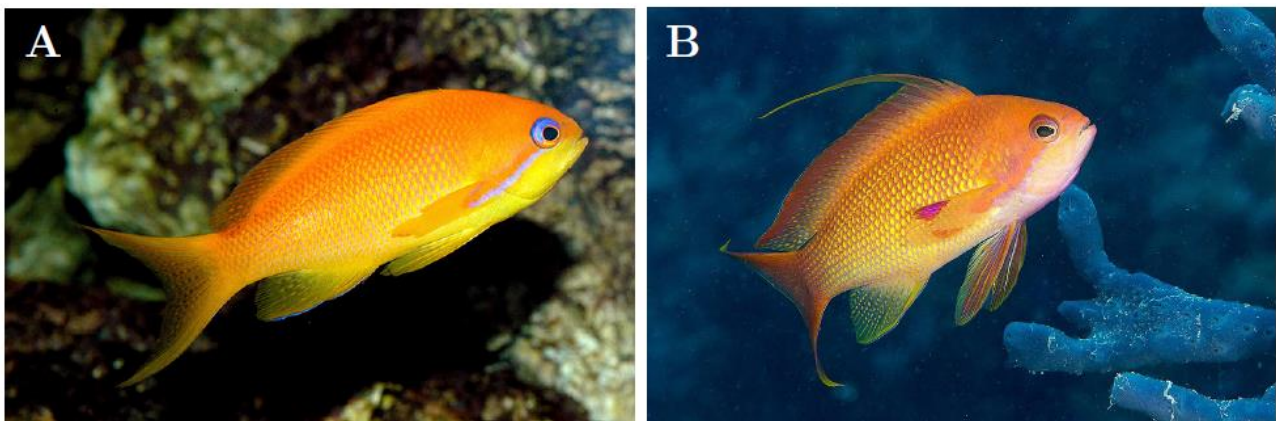


Figure 7: comparison between a female specimen (a) and a male specimen (b) of *Pseudanthias squamipinnis*.

Numerous other scientific studies confirm the formation of harems in other species belonging to the family Anthiidae, e.g., *Pseudanthias huchtii*, the red-cheeked fairy basslet, reported to be territorial and harem (Lieske & Myers, 1994), *Pseudanthias heemstrai*, the orange-head anthias (Baensch & Debelius, 1997), *Pseudanthias ignitus* (Allen, 2004) and *Pseudanthias calloura*, in which each harem is reported to occupy a vast area of 20-25 square meters, and no fighting between males on the boundary of the harem occurs (Ida & Sakawe, 2001).

2. Aim of the Study

The aim of this study is to determine whether *Anthias anthias* exhibits a harem social structure, a behavioral pattern commonly observed in other members of the Athiadidae family. While *A. anthias* has long been hypothesised to form harems, no scientific confirmation of this reproductive strategy currently exists. Several related species of this family, particularly those inhabiting tropical environments, have been documented to establish harems, supporting the plausibility of this social organization in *A. anthias*. Given its protogynous hermaphroditism, understanding whether this species follows a harem-based structure is essential to understanding its social dynamics, reproductive strategies, and population structure. This study aims to fill this knowledge gap by systematically analysing sex distribution patterns in both natural and controlled environments, examining differences in body length and coloration to confirm the presence of strong sexual dimorphism, and assessing potential changes in sex ratio in relation to environmental factors such as water temperature, depth and latitude. If *A. anthias* does not form harems, its protogynous nature would still be confirmed, further supporting its classification as a sequential hermaphrodite. By providing key data on its social behaviour, this study contributes to a better understanding of *A. anthias*, a widely distributed yet poorly studied species.

In detail, the present study aimed to investigate:

- i) Temporal variations in social structure
- ii) Latitudinal and bathymetric variations in social structure
- iii) Social behaviour and sex change in controlled conditions

3. Materials and Methods

3.1 Data collection

3.1.1 Temporal variations in social structure

Temporal changes in *A. anthias* social structure were investigated employing monthly scuba surveys carried out between January 2024 and December 2024 inside the borders of Portofino MPA, located in the Metropolitan City of Genova, between Camogli, Santa Margherita Ligure, and Portofino (Ligurian Sea). During each survey, 12 photographs of *A. anthias* aggregations were taken using a Nikon D780 camera in Isotta housing, equipped with a Tokina 10-17 fisheye lens and two Inon z330 strobes. Pictures were taken between 20 and 30 m depth. The minimum depth reached by the fish was noted.

Additionally, data on water temperatures were collected to assess potential correlations between water temperature during the sampling months and the male-to-female ratio. The data were obtained from both a dive computer, providing temperature readings from below the thermocline, and a mareographic station, which recorded surface water temperatures. A total of 144 pictures were analysed.

3.1.2 Latitudinal and bathymetric variations in social structure

To better understand *A. anthias* social behaviour, aggregations were studied along a wide latitudinal and bathymetric gradient. Information was obtained by analysing an ample dataset of pictures collected by ROV campaigns conducted along the Italian coasts between 2009 and 2022. While this method presents some limitations, including reduced image clarity and specimens not always being assessable as these ROV surveys were originally conducted for different research purposes, it provides valuable insights into deeper populations. Only surveys with a consistent presence of *A. anthias* were selected. A total of 24 sites distributed along the Italian coasts from the Ligurian Sea to the Sicilian Channel were analysed (Tab.2). Pictures were divided into two bathymetric ranges, namely 40-80 m and 80-164 m, both deeper than Portofino MPA observations. Between 1 and 12 pictures were analysed for each site. Some sites, however, do not fall within the two selected bathymetric ranges due to their broad depth distribution, making it impossible to classify them into either category. Nevertheless, the data from these locations were not entirely excluded; they were left

out from the bathymetric analysis but included in the latitudinal assessment due to the notably high abundances of the species recorded. These sites are summarised in Table 2.

Area	Latitude	Bathymetric Range	Sex Ratio M/F
Portofino	44.3037	1;2	0.82
Imperia	43.8863	1	0.22
Sanremo	43.8198	1;2	0.25
Tuscan Archipelago	42.7902	1;2	0.47
Pontine Archipelago	40.9692	1;2	0.56
Ischia	40.7269	-	1.24
Gulf of Salerno	40.4662	-	0.64
Sardinia South Boi	39.1276	1;2	0.76
Amendolara	39.9524	1	0.35
Sant'Eufemia	38.7165	1;2	0.67
Capo Vaticano	38.6197	2	1.18
Gulf of Patti	38.1913	2	0.47
San Vito	36.8026	2	1.01
Pelagie Archipelago	35.5111	1	0.43

Table 2: Summary table of the sites analysed. In the "Range" column, the values 1, 2, and "-" refer to the bathymetric ranges considered. Bathymetric range 1: 40–80 m; Bathymetric range 2: 80–164 m; "-": indicates a bathymetric range too broad to fall within either 1 or 2.

During the study of *Anthias anthias* distribution at depth, *Callanthias ruber* (Rafinesque, 1810) (Callanthiidae) was also observed (Fig. 8a). While absent at the surface, it seems to integrate with *A. anthias* groups in deeper waters. Compared to *A. anthias*, *C. ruber* has a more symmetrical, arched, and yellow tail, along with distinctively different fins, making it easily recognisable.

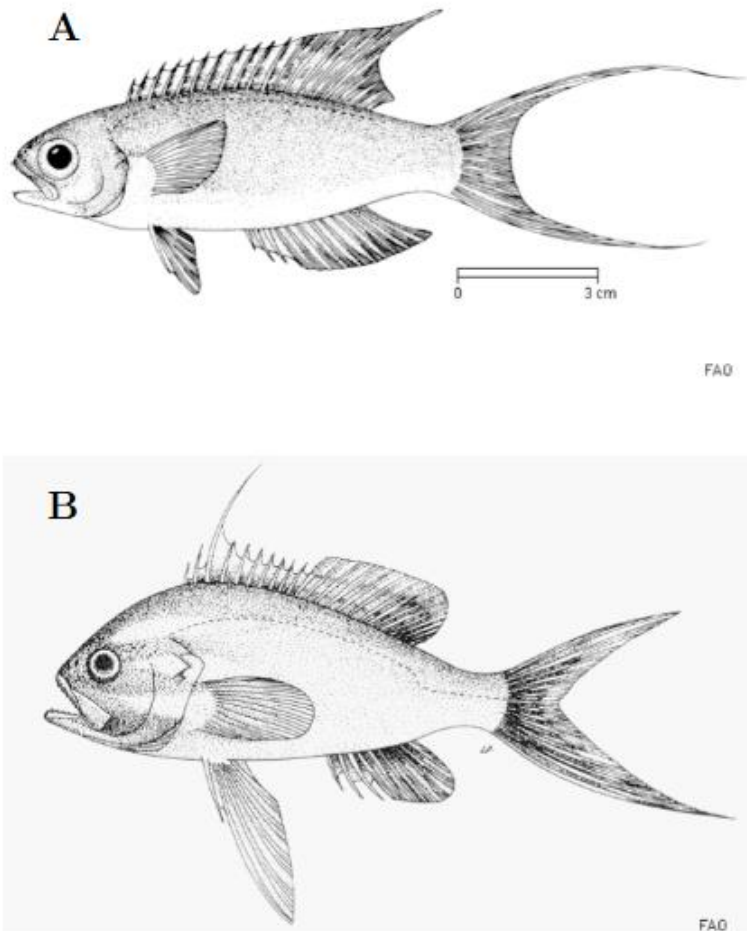


Figure 8: *Callanthias ruber* (a) and *Anthias anthias* (b) compared; the general morphology of the two species differs significantly.

3.1.3 Social behaviour and sex change in controlled conditions

The final dataset was obtained from specimens studied in a controlled aquarium environment. This dataset was obtained by visual counts recorded between December 2024 and March 2025 with observations conducted approximately every two weeks. The study took place at the Acquario di Genova aquarium, focusing on a tank where various organisms were present, the “V Terre” tank. This type of observation proved particularly useful as one of the main challenges of field research is the inability to track and monitor a specific group of individuals over time. This limitation makes it difficult to study their behavior and determine whether harem formation occurs. In this regard, the controlled environment of an aquarium allows for direct and precise observation of interactions between individuals, providing valuable insights into the social dynamics of this species. It is indeed easier to access for repeated observation; photos and videos can be conducted with higher accuracy; variables such as temperature, lighting, and water quality are controlled; it is easier to recognise and track specific individuals. However, on the other hand, a controlled environment also presents certain limitations, which must be taken into consideration and may lead to bias: the main one regards the alteration of natural behavior, stress-related changes. Despite this, the aquarium dataset provided a complementary perspective, as a possible increase in the number of males would confirm the absence of harem formation in the species, while supporting the presence of protogyny alone.

3.2 Photo analysis

Each picture was analysed using ImageJ software following the same criteria:

- **Counting individuals and determination of Sex Ratio:** the total number of individuals was recorded for each photography. Specimens were identified as either male or female to obtain an initial estimate of the sex ratio. Males were recognised based on their distinguishing characteristics, specifically the presence of an elongated filament on the third dorsal fin spine, and highly elongated pelvic fins with a distinctive yellow tint at the tip. This filament was often difficult to discern, whereas the pelvic fin was the primary discriminant used throughout the analysis. When compared to the pelvic fin of a female, the male's fin appears more rounded and exhibits a yellow coloration at the tip, whereas the female's pelvic fin is sharper and more triangular in shape.
- **Morphometric Measurements:** for each measurable specimen, total length, body depth, and eye diameter were recorded. The first two measurements were taken to assess potential differences in body size between males and females and to examine how these differences varied over time. **Total length** was measured in a straight line from the tip of the snout to the posterior edge of the caudal fin; **body depth** was measured from the dorsal fin downwards. The **eye diameter** can provide valuable information regarding juvenile specimens, as younger individuals typically have proportionally larger eyes relative to their body size. Since *Anthias anthias* is a protogynous species, juveniles were counted among the females.
- **Color Analysis (RGB):** for the previously measured specimens, an RGB analysis was conducted to evaluate potential color differences between males and females. Males are generally hypothesised to be darker than females, and this analysis aimed to highlight variations in color patterns associated with sexual dimorphism. The obtained values provided relevant data on the three color channels—**Red (R), Green (G), and Blue (B)**—allowing an assessment of which channel was more or less pronounced in males and females. Using ImageJ, body contours were selected while excluding areas that could distort the data, such as the fins and head, where a strong yellow coloration is present.

All three measurements were performed using ImageJ, with specific tools that enabled precise analyses. ImageJ is an open-source image processing software widely used in scientific research, particularly in fields such as biology, medical imaging, and material sciences. Since the obtained values for total length, body depth, and eye diameter were recorded in pixels, a conversion from pixels to real-world measurements was necessary for data analysis.

3.3 Laser

On three occasions, an underwater green laser was used to assess the reaction of fish. Laser chasing behaviour is characteristic of territorial species and can be attributed to their natural instinct to defend the harem from a potential threat. A particularly interesting study by Davies and Sheehan (2019) highlighted the laser-chasing behavior observed in certain fish species. Although the exact reason why some fish pursue the laser remains unclear, this phenomenon has proven to be a useful, indirect method for estimating their territoriality. Determining the territory size of a species is challenging, but this approach provides a potential estimate, as many fish appear to chase the laser only until it moves beyond their territory. Another study (Cohn et al., 2020) found that in fish already known for their strong territoriality and aggressive nature, e.g., anemonefish, typical defensive behaviors—such as biting, chasing, and lunging—significantly increased when exposed to laser light. This suggests that these species may perceive the laser as a potential territorial threat, further reinforcing the idea that laser stimulation can be a useful tool for studying space use and aggression in wild fish.



Figure 9: Laser chasing behaviour showing how fish respond to a laser stimulation following it. In the picture two specimens of *Ctenolabrus rupestris* interacting with the benthic laser scale of a towed video camera (Davies & Sheenan, 2019).

4. Results

4.1 Temporal variation

The collected data represent a monthly trend of male to female occurrence ratio in *Anthias anthias*' groups. The results indicate a distinct seasonal trend, with lower values of sex ratio recorded between January and April, (ranging from 0,03 to 0,17), a gradual increase observed from May onwards reaching approximately 0,28 in June, and during the summer months, from July to August, the ratio appears to slightly decline (0,10), and a marked increase from September to December, with the highest values recorded (0,36) indicating a shift in sex ratio composition over time (Fig.10).

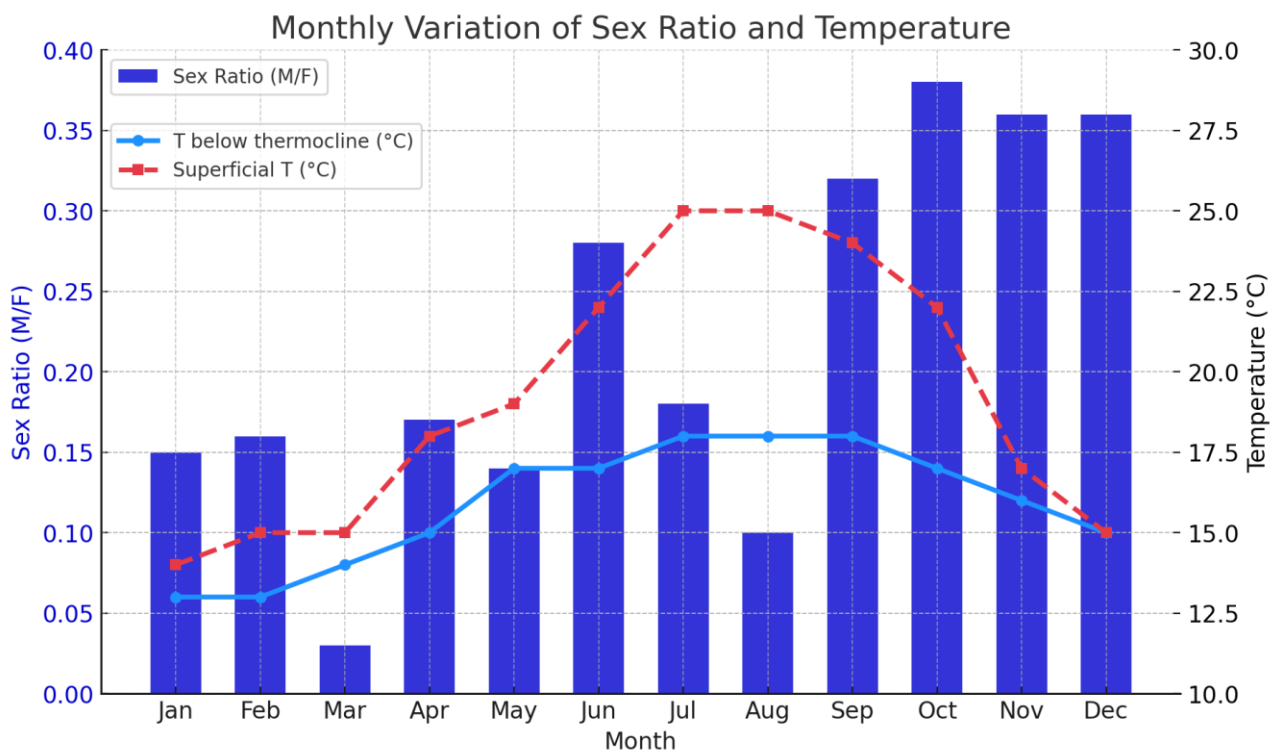


Figure 10: Average trend of sex ratio over the twelve months of sampling.

Overall, the sex ratio exhibited fluctuations, with the lowest values occurring in late winter and early spring, and the highest values recorded during autumn.

In addition to analysing the monthly variation in the male to female sex-ratio (M/F), the relationship between temperature and sex ratio was also considered (Tab.3). Temperature data were recorded for both surface water and below the thermocline to assess potential correlation with sex ratio fluctuations over time.

Month	T below thermocline (°C)	Superficial T (°C)	Sex Ratio (M/F)
Jan	13	14	0.15
Feb	13	15	0.16
Mar	14	15	0.03
Apr	15	18	0.17
May	17	19	0.14
Jun	17	22	0.28
Jul	18	25	0.18
Aug	18	25	0.10
Sep	18	24	0.32
Oct	17	22	0.38
Nov	16	17	0.36
Dec	15	15	0.36

Table 3: Monthly variation of sex ratio in relation to temperature.

The relationship between temperature and sex ratio (M/F) was assessed using the Pearson correlation coefficient. The analysis revealed a weak correlation between these two variables:

- Temperature below thermocline: $r=0,29$, weak positive correlation;
- Surface temperature: $r=0,15$, even weaker correlation.

When r is between 0 and 0.3, the correlation is considered weak, meaning that while there may be a relationship, it is not strong or reliable.

4.2 Latitudinal and bathymetric variations in social structure

To assess whether the male-to-female abundance ratio of *A. anthias* varies with latitude and bathymetry, a dataset was created containing data from the analysis of 24 sites, grouped into 12 different areas. These areas were then arranged in latitudinal order and divided into two depth ranges for further analysis.

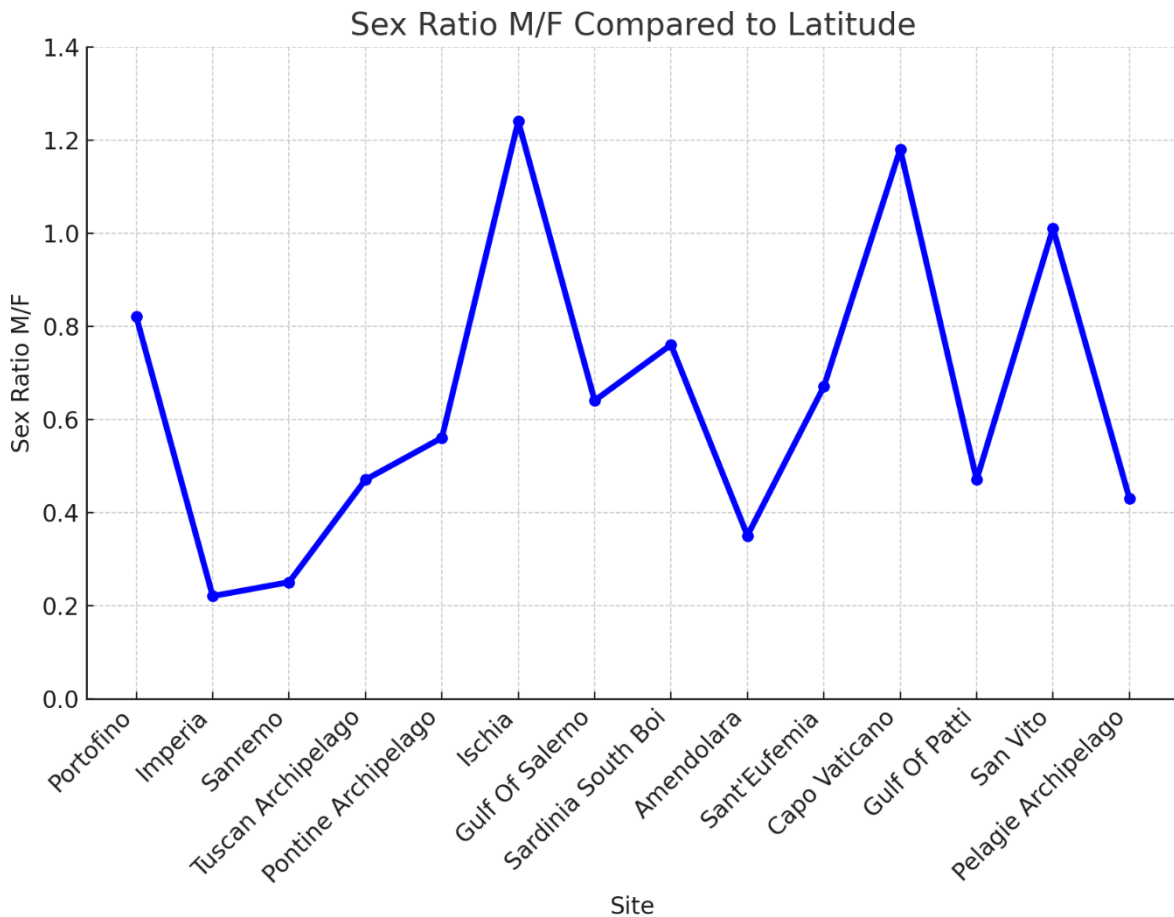


Figure 11: Sex ratio values compared to latitude.

The analysis of sex ratio (M/F) across different sites along a latitudinal gradient did not reveal a clear correlation between latitude and sex ratio distribution. The observed values fluctuated across locations, with no evident increasing or decreasing trend from northern to southern sites.

The highest sex ratios were recorded at Ischia and Capo Vaticano, while lower values were found at Imperia and Sanremo, yet these differences did not follow a predictable latitudinal pattern.

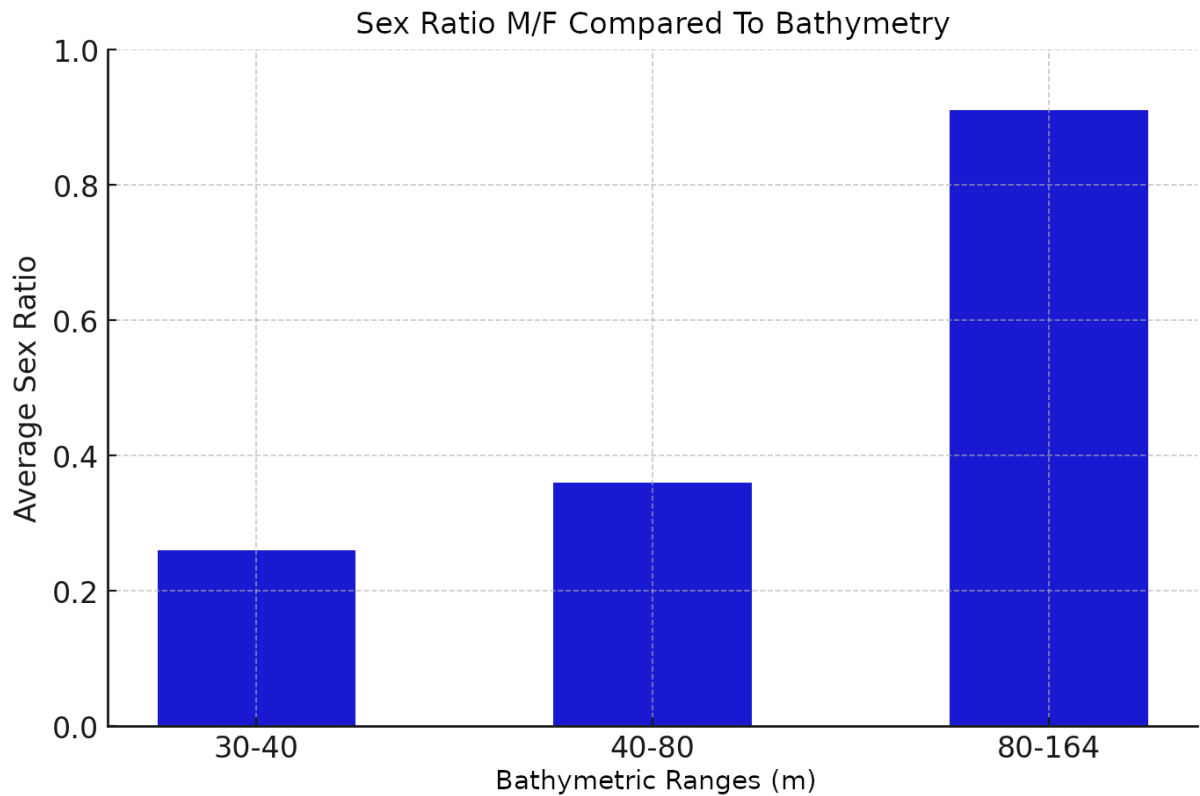


Figure 12: Sex ratio values compared to bathymetry.

The bar chart (Fig.12) illustrates the relationship between average sex ratio and bathymetric ranges, based on monthly sampling in Portofino for the shallowest range, while data for the two deeper bathymetric ranges were obtained through ROV surveys. *The trend in the data indicates that sex ratio increases with depth.* At the shallowest range (30-40 m), the sex ratio is relatively low (around 0.25). In the intermediate range (40-90 m), it increases slightly (~0.35), but the most significant change occurs in the deepest range (70-164 m), where the sex ratio approaches 0.9.

4.3 Morphometric analysis

4.3.1 Body and eye measurements

The bar chart (Fig. 13) illustrates the male-to-female (M/F) ratio for length and depth across different geographic sites, allowing for a comparison of male and female morphological differences in relation to latitude. The general trend observed is that the M/F ratio in length is consistently higher than the M/F ratio in depth, indicating that males tend to be *longer rather than deeper* relative to females.

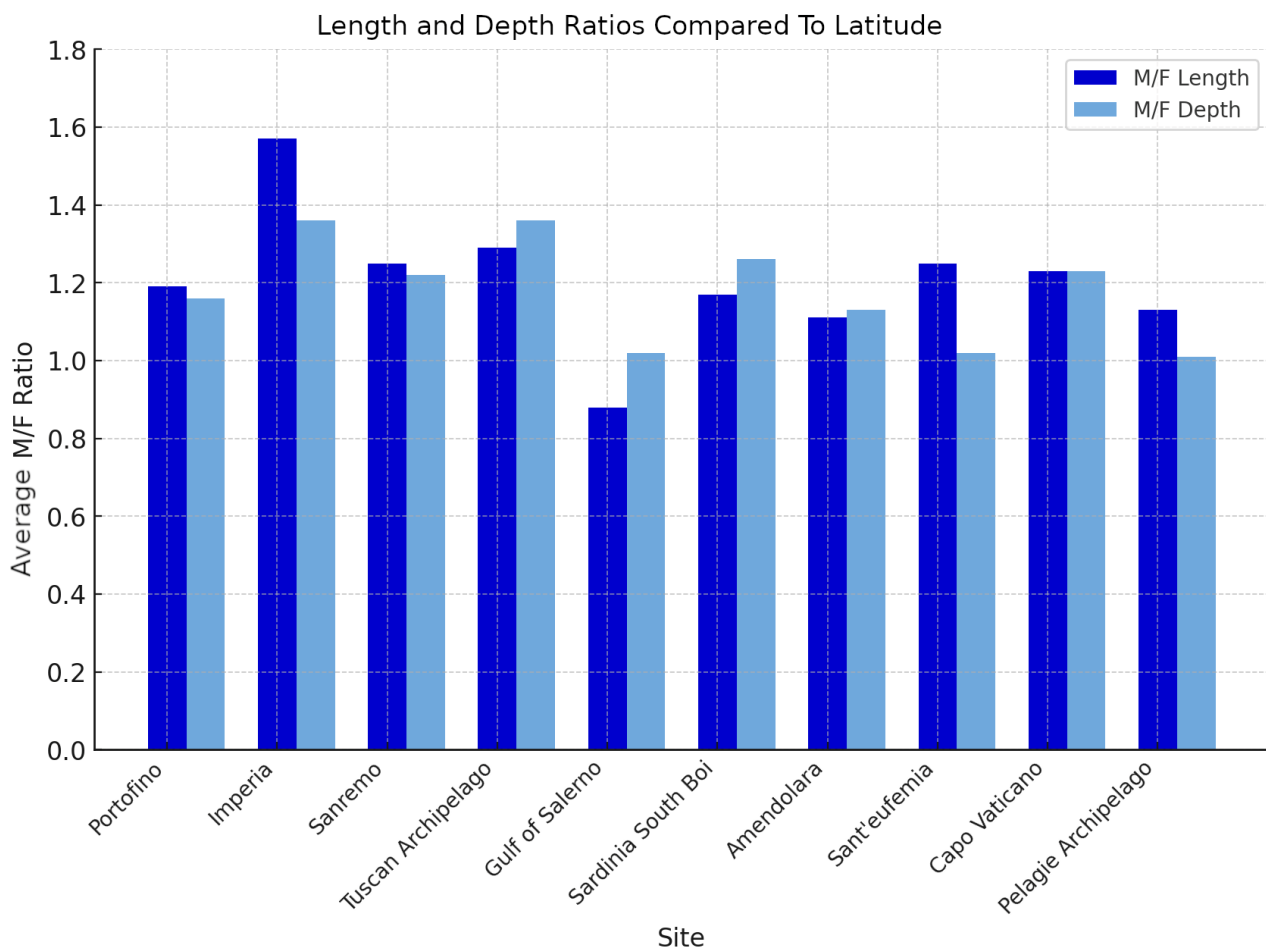


Figure 13: male-female ratio for length and depth at different latitude.

Across the various sites, no clear pattern emerges in relation to latitude. While some locations, e.g., Imperia, exhibit a slightly higher M/F length ratio, others, like the Gulf of Salerno, show lower values.

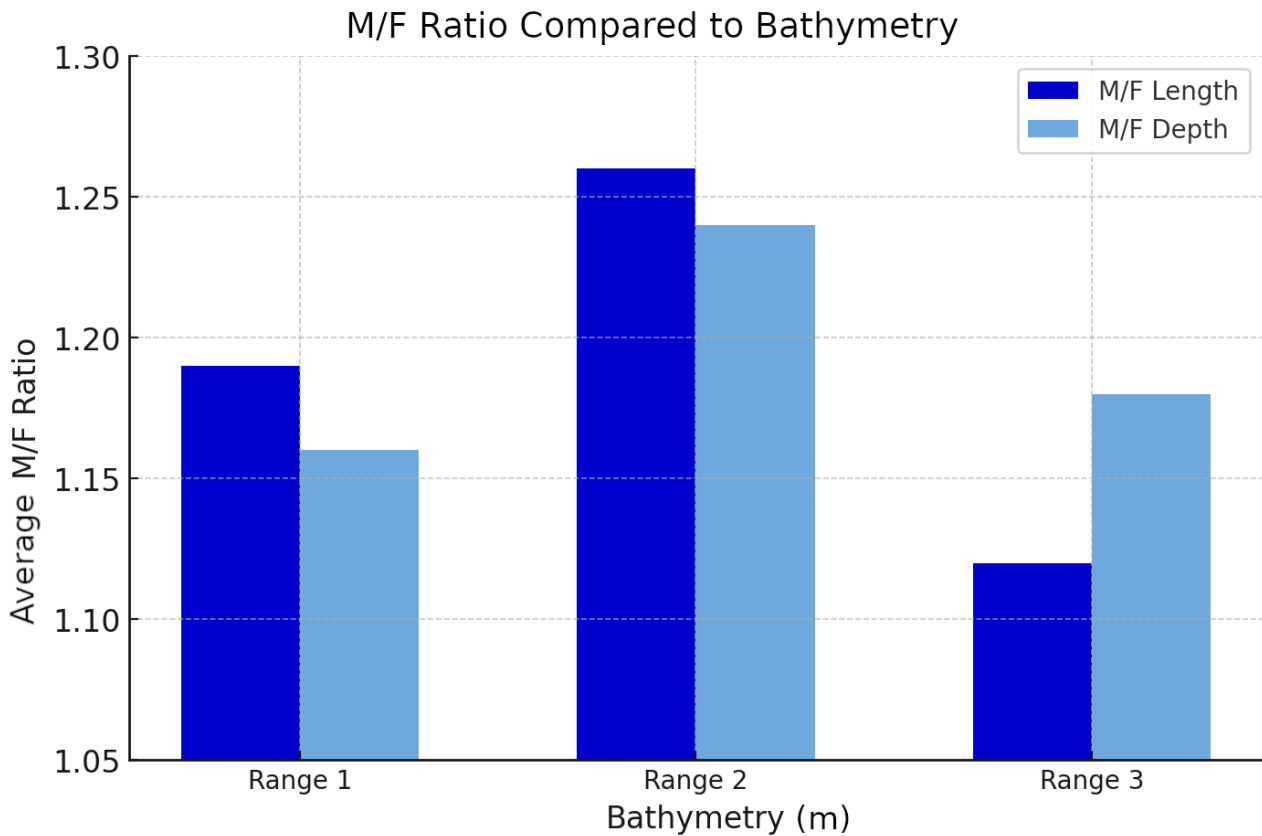


Figure 14: M/f length and depth ratios across three bathymetric ranges.

Focusing on bathymetric variations, this bar chart (Fig. 14) represents the male-to-female (M/F) ratio for length and depth across three different bathymetric ranges, as represented on the x-axis. As described in the Materials and Methods section, these three depth ranges correspond to shallow, mid, and deep waters. As previously observed, no strong correlation emerges between depth and species morphology, and in both Range 1 and Range 2, length remains more pronounced than depth. However, in Range 3, the M/F length ratio is lower than the M/F depth ratio, whereas in the other two ranges, the differences remain more consistent. To further investigate whether depth or latitude play a significant role in shaping male and female morphology, statistical analyses were conducted. By defining a null hypothesis (H_0) stating that there are no significant differences between the M/F ratio in length and the M/F ratio in depth, and an alternative hypothesis (H_1) suggesting that males are significantly longer than they are deep, the results indicate the following:

- Latitude comparisons: a paired t-test comparing the M/F ratio in length and depth across multiple geographic locations returned a **p-value of 0.511**. Since the p-value is greater than 0.05 this suggests that there is not enough evidence to reject the null hypothesis. Although males consistently exhibit greater body length than depth, this trend does not significantly vary with latitude or across different sites.
- Bathymetric influence: similarly, analysis across three distinct depth ranges resulted in a paired t-test **p-value of 0.918**, confirming that depth does not significantly influence the length-to-depth relationship in males.

Regarding other measurements, the eye diameter in relation to the total length of the organism was assessed. Specifically, the analysis focused on how and whether female length, relative to eye size, varied seasonally to determine if it was possible to identify the presence of juveniles.

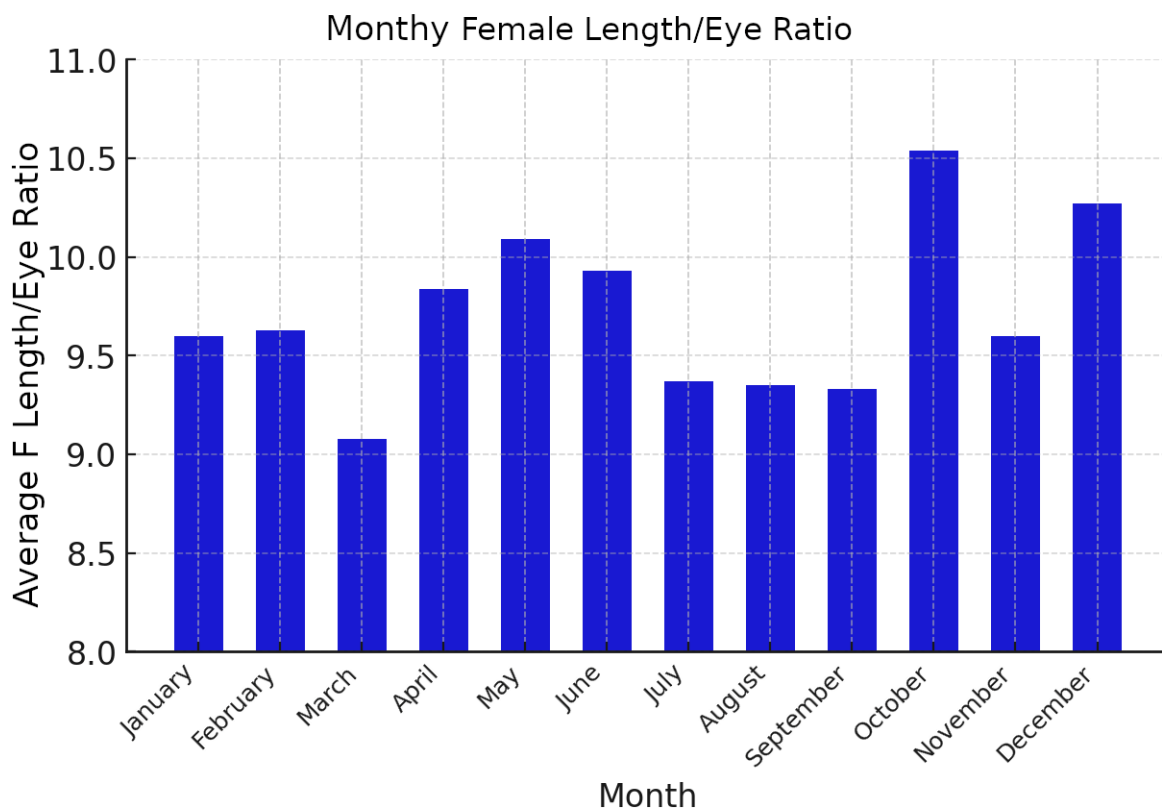


Figure 15: female length/ eye diameter ratio measured over a 12 month sampling period in Portofino.

The bar chart (Fig. 15) displays the female length-to eye ratio over a 12-month period, allowing an assessment of how this morphological trait varies throughout the year. It is known that juveniles have relatively larger eyes compared to body length, and that the species reproduces between June and September. During the reproductive period the F length/eye ratio remains relatively low, ranging between 9.93 in June, and around 9.30 between July and September.

4.3.3 Colour Analysis

The evaluation was conducted in the same way as the measures one, considering both latitudinal and bathymetric variations.

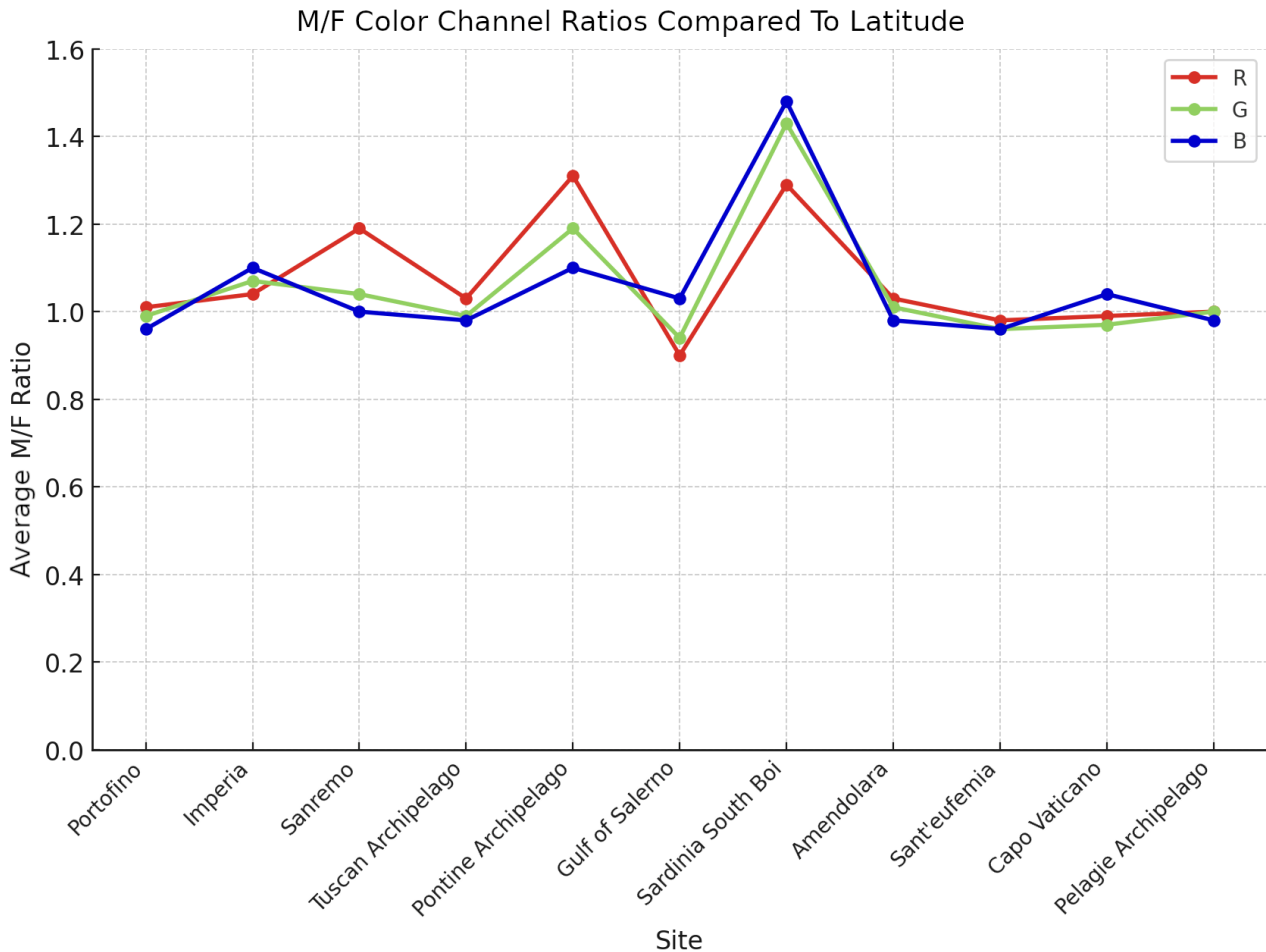


Figure 15: M/F color channel comparison across different latitudes.

The graph (Fig. 15) illustrates the male-to-female (M/F) ratio for different color channels (Red, Green, and Blue - R, G, B) across various geographic sites, allowing for an assessment of whether latitude influences color expression differences between sexes. The overall trend shows some fluctuation in M/F ratios across locations, with red (R) and blue (B) showing more variability than green (G). In certain sites, such as Pontine Archipelago and Sardinia South Boi, males appear to exhibit a stronger intensity of red and blue coloration compared to females, whereas in locations like

Gulf of Salerno and Sant'Eufemia, the M/F ratio for red is lower, indicating a potential reversal in this trend.

Regarding the color analysis in relation to bathymetry, the bar chart (Fig. 16) illustrates the male-to-female (M/F) ratio for the three color channels across the usual three distinct bathymetric ranges: this analysis aims to explore potential differences in color expression between males and females across varying depth ranges.

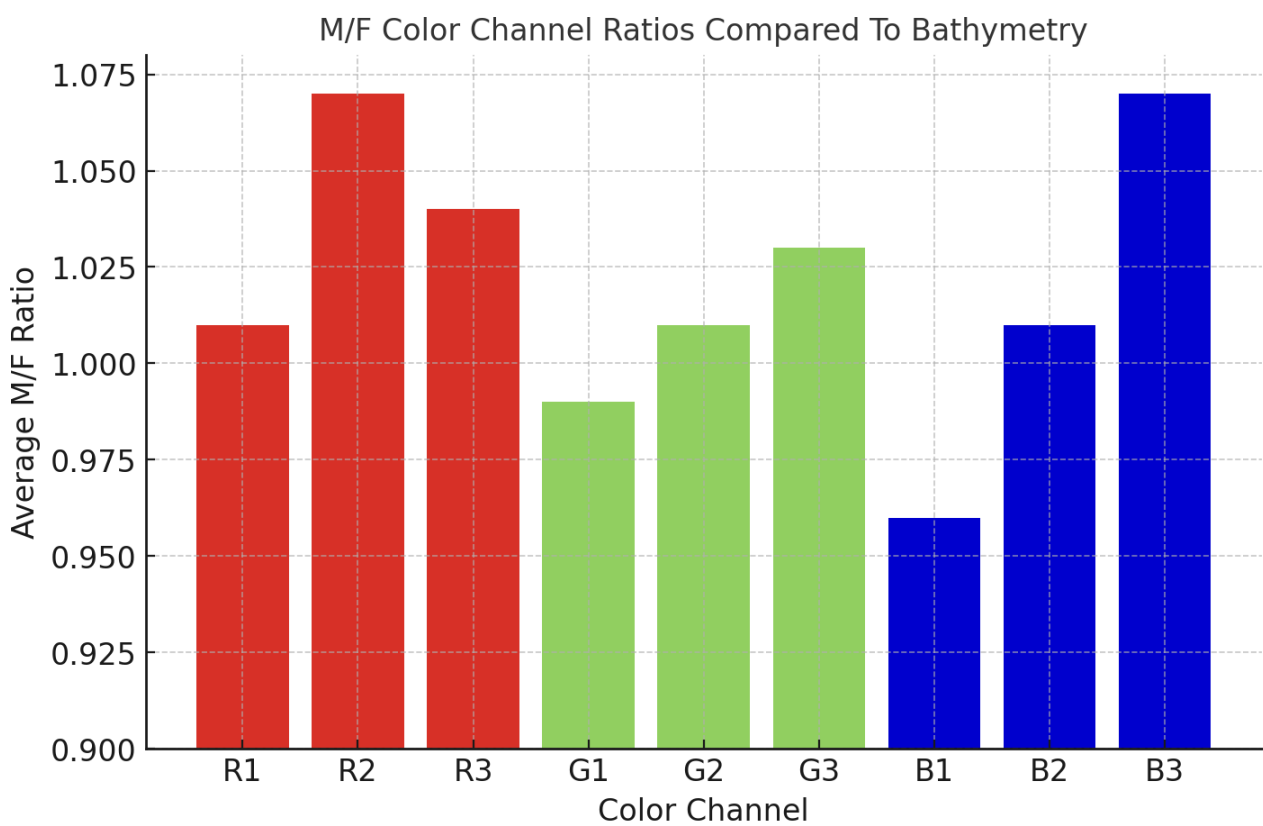


Figure 16: M/F color channel comparison across three bathymetric ranges.

The results show that, red intensity (R) appears to be higher in males than in females, with R2 (mid-depth) showing the highest M/F ratio (1.07), followed by R3 (deep, 1.04), and R1 (shallow, 1.01). This suggests that males may exhibit stronger red pigmentation than females.

On the other hand, the green channel (G) shows a more balanced distribution between sexes, with G1 slightly favoring females (0.99 M/F ratio), while G2 and G3 are close to parity (1.01 and 1.03,

respectively). This indicates that green hues are not significantly different between males and females, remaining relatively stable across depths.

The blue channel (B) presents a more variable pattern, with B1 (shallow) being lower in males (0.96), *suggesting females might have more blue hues*, while B3 (deep) reaches the highest value (1.07), indicating a stronger blue intensity in males at greater depths. The mid-depth range (B2) has a value of 1.01, suggesting that blue coloration differences between sexes may become more pronounced at extreme depths.

4.4 Social behaviour and sex change in controlled conditions

The results obtained show that the sex ratio remains variable even in a controlled environment. However, more observation are likely needed to better understand how this phenomenon works when specimens are held in captivity. On December the number of males was 8 versus 21 females. The numbers do not fluctuate that much, but maybe this is just because the data collected are poor. On January 9 males and 23 females; on February 8 males and 22 females.

4.5 Laser

The results obtained from the use of the underwater laser did not reveal any aggressive behaviour in the species. The animals appeared to be curious about the laser; a moderate level of laser-chasing behaviour was observed, though it was not associated with any form of territorial tendencies.

5. Discussion and Conclusion

The results of this study provide a comprehensive assessment of the social behaviour of *Anthias anthias*. These findings revealed extreme variability in the sex ratio (i.e., the male-to-female ratio) from a seasonal, latitudinal, and bathymetric perspective. Furthermore, the variability of the sex ratio over the 12 months of sampling at Portofino was not found to be correlated with temperature fluctuations across the different months, a conclusion further supported by Pearson's correlation analysis. If temperature were a key determinant, a stronger correlation would be expected. However, **correlation does not imply causation**; while the peak in temperature in August may explain the drop in the sex ratio, the transition across months appears irregular. Notably, between October and December, the sex ratio remains nearly constant despite decreasing temperatures. If the two factors were correlated, it would not explain why, despite the drop in temperature in January, the sex ratio remains low, whereas similar temperatures in December correspond to a higher sex ratio. Thus, it can be concluded that *temperature does not have a strong influence on the sex ratio*.

The variability observed among sites further suggests that latitude does not influence the proportion of males and females in *Anthias anthias* groups. Latitude alone does not appear to be a primary driver of sex ratio variation, and further investigations are required to assess the influence of both biotic and abiotic factors.

From a bathymetric perspective, *an increase in sex ratio was observed at greater depths* (Fig.12). Since the sex ratio is defined as the number of males relative to females (males/females), *a higher sex ratio at greater depths indicates a greater proportion of males compared to females*. This suggests that males are either more abundant or more likely to inhabit deeper waters, while females may be more concentrated in shallower depths, implying a depth-related variation in sex distribution. This pattern may be driven by ecological factors, habitat preferences, or other biological and environmental influences. For instance, the greater abundance of males at deeper depths could be associated with a reduction in natural predators or, more generally, a decrease in environmental threats. A lower predation pressure at greater depths could contribute to increased survival rates, male included, thereby leading to the observed pattern in sex ratio distribution.

Morphometric analyses also revealed considerable variability in the data, both in terms of latitude and bathymetry. Regarding body size, no significant correlation was found between latitude and differences in body dimensions between males and females (Fig.13). Consequently, the results suggest that latitude does not significantly influence the relationship between male and female length and

depth, reinforcing the idea that this morphological trait is likely an intrinsic characteristic of the species rather than a response to geographic location.

The same conclusion can be drawn concerning bathymetry and the differences in length and depth between males and females. In this case as well, the recorded values exhibited considerable fluctuations (Fig. 14). In both cases, this conclusion was supported by statistical analyses, specifically a t-test, which returned p-values too high to support the hypothesis that bathymetry and latitude influence male and female body size.

Thus, based on these findings, we can confirm a consistent pattern of sexual dimorphism in *Anthias anthias*, where males tend to be proportionally longer than they are deeper across nearly all sites and depth ranges analysed, suggesting that sexual dimorphism is a fundamental characteristic of the species rather than an environmentally driven adaptation.

Furthermore, the relationship between total female body length and eye diameter was examined to assess the potential presence of juveniles and evaluate their seasonal occurrence. The graph (Fig. 14) shows a decrease in the female length/eye diameter ratio in July, August, and September; since this ratio represents the relationship between female body length and eye diameter, a decrease in its value indicates that specimens have proportionally larger eyes relative to their body size, i.e., they are likely newly settled individuals. However, the values recorded in other months, outside of July, August, and September, do not exhibit a particularly significant difference. This may be due to the fact that, while juveniles are easily recognisable in photographs, precise measurements were not always possible, particularly when individuals were positioned too far from the camera or slightly angled, making accurate length and eye measurements challenging. Additionally, although the reproductive period occurs between June and September, juveniles from multiple spawning events may overlap, leading to individuals at different growth stages coexisting throughout the year. This could result in a more gradual shift in the female length/eye diameter ratio rather than a sharp seasonal change.

Moreover, the lack of detailed information on larval ecology further limits the understanding of this phenomenon. Factors such as settlement timing and early-stage survival rates remain largely unknown but could play a crucial role in shaping the observed patterns. Incorporating larval distribution data and early growth dynamics could provide a clearer and more comprehensive picture of how recruitment variability influences the female length/eye diameter ratio throughout the year.

The analysis of the expression of the three colour channels (R, G, B) once again revealed fluctuations in the recorded values. From a latitudinal perspective, although certain sites, such as Sardinia South Boi, exhibited peaks in R, G, and B values, in most other locations, the values remained relatively stable (Fig. 15). Despite these variations, no clear latitudinal gradient emerged, suggesting that *differences in male and female coloration are not directly linked to latitude but may instead be influenced by other factors*. Furthermore, as this analysis is based on photographic data, several aspects of image acquisition could have influenced the observed male-to-female colour ratios, potentially introducing variability that does not necessarily reflect actual biological differences.

Regarding the bathymetric analysis of colour expression, the results indicate that across all three depth ranges, males tend to exhibit a stronger red pigmentation than females. The green channel remains relatively stable, showing no significant differences between sexes at any depth range, while the blue channel appears to be more depth-dependent, becoming more pronounced at greater depths. However, this pattern may again be influenced by multiple factors, including light availability and variations in photographic methodology.

Additionally, behavioural observations conducted using an underwater laser did not reveal any signs of territoriality. The species exhibited a mild interest in the laser, displaying laser-chasing behaviour, but this was not associated with any aggressive or territorial responses.

In conclusion, the analyses of sex ratio, body size, and colour expression have provided valuable information on a species that is both well-known yet still largely unexplored. Regarding the sex ratio, given its variability across both latitudinal and bathymetric gradients, and the lack of territorial behavior, as confirmed through the use of the underwater laser, ***it can be stated with reasonable confidence that *Anthias anthias* is not a species that forms harems, contrary to previous assumptions***. These assumptions were likely based on its morphological and reproductive similarities with other members of the family Anthiidae, as discussed earlier. *While protogyny is confirmed for this species, the social behaviour associated with harem formation can be refuted.*

Morphometric analyses, along with the study of RGB channel expression, support the presence of clear sexual dimorphism. Males tend to be slightly longer and exhibit a more intense red pigmentation than females.

Despite these findings, further research is needed to explore the mechanisms driving sex change and social dynamics in the species. By expanding on these aspects, it will be possible to gain a more comprehensive perspective on the species' life and its ecological role within its habitat. Although the social behaviour of *Anthias anthias* may resemble what has been described as an aggregating-type harem, this definition appears to be slightly misleading. This is primarily due to the high variability in the male-to-female ratio, which does not follow a consistent pattern at any latitude or depth. In contrast, the classical definition of a harem implies a certain degree of stability, with dominant males maintaining control over a group of females. When a harem is defined as a multi-male structure in which females mate freely, the concept begins to diverge from its original meaning.

Furthermore, while *A. anthias* is a protogynous species, there is no clear evidence that this sex change occurs as a direct response to a rigid harem-like social structure. Instead, other factors may influence this transition.

However, further studies are needed to deepen the understanding of these aspects, particularly in relation to social behaviour in controlled environments, to capture potential behavioural variations with greater accuracy.

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