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**Reproductive and Parental Behaviour in the
Chameleon Cichlid *Australoheros facetus***



UNIVERSIDADE DO ALGARVE

Faculdade de Ciências e Tecnologia

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Chameleon Cichlid *Australoheros facetus***

Mestrado em Biologia Marinha

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Título do trabalho: Reproductive and parental behaviour in the chameleon cichlid *Australoheros facetus*.

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Resumo

Cuidado parental é um comportamento reprodutivo altamente espalhado entre diversas espécies animais. O ato de prover os cuidados e as condições necessárias à sobrevivência dos descendentes até que estes se desenvolvam por completo representa uma “troca” entre os progenitores e as crias. Ao mesmo tempo que as crias beneficiam da proteção e cuidados dos pais, a sua sobrevivência assegura que os genes dos pais estejam presentes em gerações futuras. Contudo, esta “troca” tem pesadas consequências na maneira como os progenitores vivem e, por isso, não é uma característica universal e uniforme entre espécies. Em peixes, as estratégias reprodutivas diferem significativamente dependendo de cada espécie e podem ser categorizadas de acordo com a localização na qual os pais cuidam da prole ou com qual o sexo é que providencia o cuidado parental. Particularmente, os peixes ciclídeos são muito bem conhecidos por apresentarem um comportamento reprodutivo e parental bastante complexo, com diferentes estratégias de acasalamento e divisão de tarefas durante a parentalidade, fazendo deste um dos melhores grupos taxonômicos para o estudo de cuidado parental. O *Australoheros facetus*, espécie focal desta tese, é um ciclídeo neotropical nativo da América do Sul que, localmente, é conhecido por “chanchito”. Este peixe está descrito como altamente invasor e, presentemente, está estabelecido em várias drenagens do tipo mediterrânico no sul de Portugal, Espanha e Chile. O *A. facetus* é caracterizado como um reprodutor de substrato com cuidados biparentais, isto é, ambos os progenitores cuidam em conjunto da sua prole num ninho localizado no substrato. Apesar desta espécie não apresentar dimorfismo sexual evidente, em ciclídeos que apresentam comportamentos de cuidado biparental da prole, o macho é geralmente maior que a fêmea. No “chanchito”, durante a execução de comportamentos reprodutivos e parentais, as fêmeas apresentam uma coloração temporária mais escura do que os machos, permitindo a distinção de sexos. Mesmo que o principal mecanismo pelo qual esta espécie cuida da sua prole já estar relativamente bem descrito, informações sobre as táticas reprodutivas e a contribuição de cada sexo durante as fases parentais estão ainda em falta. Esta tese tem como principal objetivo caracterizar o comportamento reprodutivo e parental da espécie *Australoheros facetus* nos rios e ribeiras do sul de Portugal nas quais ele é invasor, fornecendo informações importantes para o controlo de populações atuais e a sua eventual dispersão.

Todos os animais utilizados na realização do trabalho experimental foram capturados em sessões de pesca elétrica nos rios Foupana, Odelouca e Vascão e mantidos nas instalações

experimentais do Centro de Ciências do Mar (CCMAR) da Universidade do Algarve, no Campus das Gambelas. O trabalho experimental foi dividido em quatro fases (fase de formação de casais, fase de ovos, fase de larvas recentemente eclodidas e fase de larvas com natação livre) nas quais os perfis comportamentais dos pares reprodutivos foram quantificados seguindo um etograma previamente estabelecido. Todo o trabalho de avaliação direta de comportamento foi suportado por gravações de vídeo. Na fase de formação de casais, a frequência de comportamentos sociais de cada um dos membros de cada casal foi avaliada e foi criado um índice de agressividade com a finalidade de conhecer qual dos progenitores apresentava um caráter mais agressivo nesta primeira fase reprodutiva. Para as restantes fases foi feita a análise das frequências de ocorrência e tempos de execução de comportamentos reprodutivos para cada um dos sexos e foi criado um índice de “bom pai” a partir do comportamento mais relevante em cada uma das fases. Este índice permitiu uma melhor perceção da influência e investimento de cada um dos sexos em todo o período reprodutivo. Por fim, de modo a clarificar os níveis de agressividade de cada sexo ao longo de todo o período reprodutivo foi feito um teste de introdução de um coespecífico em cada uma das fases estudadas e um índice de agressividade foi de novo calculado, englobando a frequência de ameaças e ataques em todas as fases.

Os resultados obtidos com as frequências de ocorrência e tempos de execução revelaram que o comportamento reprodutivo do *A. facetus* se enquadra no comportamento parental típico de um ciclídeo neotropical com reprodução de substrato. O comportamento parental deste peixe revelou, de facto, ser biparental. Todo o cuidado parental prestado à prole durante a fase reprodutiva foi uma atividade cooperativa entre machos e fêmeas. Contudo, a execução de deveres parentais não foi igual para ambos os sexos. Machos e fêmeas apresentaram divisão de tarefas parentais ao longo das três fases reprodutivas estudadas, com as fêmeas mais investidas no cuidado direto dos ovos e larvas, e os machos com a proteção da prole e defesa do território. Relativamente aos índices de agressividade, os resultados demonstraram uma ligeira tendência para os machos apresentarem uma postura mais hostil enquanto defendem o seu território, porém sem diferenças significativas quando comparados com as fêmeas. Esta divisão de tarefas parentais entre os sexos já foi descrita em outros ciclídeos biparentais e pode ser explicada pela especialização na execução de certas tarefas parentais, isto é, pelas diferentes habilidades que ambos os sexos possuem para realizar algumas tarefas em particular.

Adicionalmente, em termos evolutivos as fêmeas tendem em permanecer com os descendentes até à sua completa formação para compensar a ausência do macho no caso deste desertar ou escolher começar uma nova tentativa de acasalamento com outra parceira. Consequentemente, machos tendem a valorizar mais a defesa de um bom território de maneira a atrair mais fêmeas para múltiplas tentativas de acasalamento. No final do trabalho experimental e após análise comportamental, foi possível elaborar um etograma complementar com dois novos comportamentos reprodutivos descritos para esta espécie. Estes incluem a transferência de ovos e larvas por parte dos progenitores para diferentes locais do território por eles protegido (visto nas fases de ovos e larvas recentemente eclodidas) e a alimentação das larvas com pedaços de ração partidas pelos progenitores (detetada nas fases de larvas recentemente eclodidas e larvas com natação livre).

Estudar a direção e influência do comportamento parental do “chanchito” no seu processo evolutivo como espécie invasora é um passo importante para compreender a evolução do cuidado parental desta espécie de modo a criar soluções eficazes para lidar com o seu carácter invasor que ameaça uma disseminação global.

Abstract

Parental care is a reproductive behaviour widely spread among animals. The reproductive strategy of providing better conditions for the survival of the young represents a costly trade-off between the parents and their offspring. In fish, these strategies can be significantly distinct. Cichlid fishes are well known for their complex reproductive and parental behaviour, with different mating strategies and division of tasks during parenting, making them one of the best taxonomic groups for parental care studies. The *Australoheros facetus* is a neotropical cichlid native to South America that is highly invasive in southern Portugal. As an American cichlid, it is known that *A. facetus* is a substrate spawner that displays biparental care, but there is still a lack of information about the reproductive tactics and the contribution of each sex during parental stages. This thesis aims to characterize the reproductive and parental behaviour of the species *Australoheros facetus* in southern Portuguese drainages, providing important information to control the current populations and their spreading. The experimental work was divided into four stages (pair formation, egg, newly hatched larvae, and free-swimming larvae) in which the behavioural profiles of the reproductive pairs were quantified following a previously established ethogram. The reproductive behaviour of *A. facetus* revealed to be typical of the substrate brooder neotropical cichlids. Males and females presented the expected behaviours, with females being more invested in the direct care of the eggs and larvae, while males were concerned with young protection and territory defence. This division of parental tasks between sexes has already been described in other biparental substrate-brooding cichlids and it is an important step in understanding the evolution of parental care in this species to create solutions to deal with its global spreading as an invasive species. Furthermore, two new reproductive behaviours were described for the first time for this species.

Keywords: fish aggressiveness, ethogram, invasive species, parental care, video recordings.

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1. Introduction

Caring for the own offspring is a reproductive behaviour widely spread among animals. This reproductive trait attracts a lot of scientific attention, especially in birds and mammals, in which it is exceptionally well described for various species (Klopfer, 1981). In both these groups, parental care arises as a necessity for the survival of the newborns since in the first months after birth they are dependent on the parents for basic survival actions such as feeding and protection. The most conventional reproductive strategy observed in mammals and birds consists of the parents choosing to have fewer succession to invest in providing parental care to a small number of infants and ensure their survival. In some cases, it is even common to see parental care functioning as an innate behaviour, like, for example, in “aunting”. This phenomenon, observed in primates (see McKeena, 1979), occurs when an adult individual takes care of infants of other animals until they are fully developed (Klopfer, 1981). On the other hand, in groups like reptiles, fish and insects, many species choose to invest in large spawns to compensate for the high mortality of the offspring and guarantee greater recruitment.

Parental care is defined as any form of behaviour that is likely to enhance the fitness of the offspring (Trivers, 1974), and represents a trade-off between the survival of the offspring and the parents’ future reproduction (Huxley, 1938; Birba et al., 2015), meaning that parents have less time, resources, and energy available to search for or attract reproductive mates (Royle et al., 2012a). Because of this costly trade-off, parental care is not a universal and uniform attribute among every species (Klopfer, 1981). This reproductive strategy has different benefits for different levels of parental investment, and it is not compatible with some mating strategies. The evolution of parental care represents an important step in the evolution of animal sociality and, consequently, the survival of species (Royle et al., 2012a), especially in more susceptible animal groups, like insects (see Shuker and Simons, 2014) and fish. According to Royle et al. (2012b), a set of conditions must be met for parental care to evolve from an ancestral state of “no care”. Factors like the costs and benefits of care, life-history conditions, and environmental dynamics can highly affect the origin of parental care in a certain species (**Figure 1.1**).

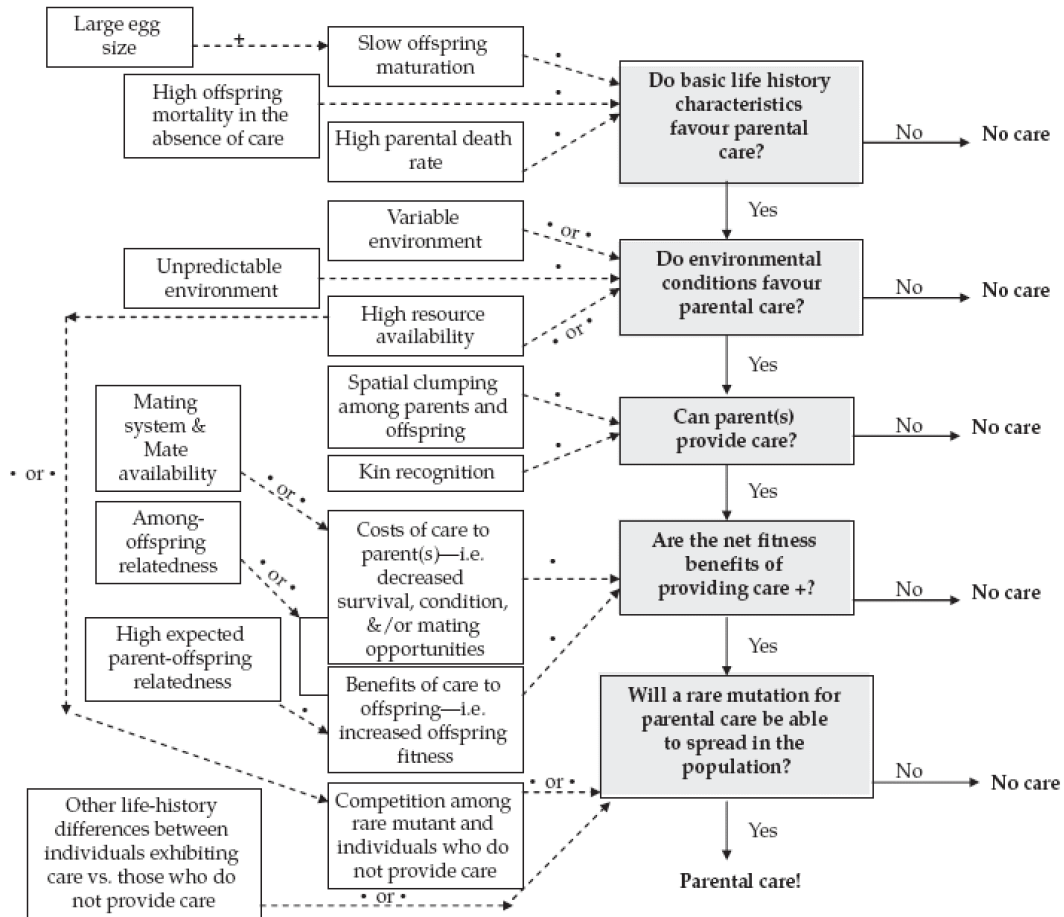


Figure 1.1. Schematic representation of the evolution of parental care (retrieved from Royle et al., 2012b).

1.1. Parental care in fish

The ways that animals care for their young vary radically across the different taxonomic groups and species. Particularly in fish, these strategies can differ immensely in the complexity of activities and amount of care provided to the offspring. Parental care can range from simple tasks to more complex behaviours. Oxygenation of the laid eggs with fan movements from the caudal fin, as occurs in several salmonid species (Sternecker et al., 2012), incubation of eggs and fry in the mouth or pouch, such as in African tilapias (Panikkar and Tampi, 1954) or seahorses (Linton and Soloff, 1964), and provision of food from their skin mucus, such as in the discus cichlids (Sylvain and Derome, 2017), are just a few examples of the variety of behaviours already documented. Therefore, such behaviours can be significantly distinct depending on the mating strategy present in each species.

Fish care strategies can be categorized by both the location where the parents take care of their young, and by which sex of the reproductive pair provides the parental care (Abate et al., 2021). Regarding the location, fish species can be classified as **substrate guarders** or substrate brooders, if the fish care for their young on the ground, as with the dwarf cichlid (*Mikrogeophagus ramirezi*) and the sergeant major (*Abudefduf saxatilis*); or **mouthbrooders**, if the fish carry their young in their mouths, as occurs in the sardine cichlid (*Cyprichromis leptosoma*), and the ring-tailed cardinalfish (*Ostorhinchus aureus*; Abate et al., 2021; Goldberg et al., 2020). Then again, concerning which sex provides care to the young, care strategies can be classified as **biparental care**, if both parents commonly jointly care for the young by guarding and nursing them, like in the Galilee St. Peter's fish (*Sarotherodon galilaeus*); **male-only care**, if the parental care of the offspring is exclusive for the male, such as in the black-chinned tilapia (*Sarotherodon melanotheron*), the clown anemonefish (*Amphiprion percula*; Barbasch et al., 2020) and the very well-known pipefishes and seahorses (Vincent et al., 1992); and **female-only care**, if only the female cares for the eggs and larvae, for example, in Lake Tanganyika African cichlids (such as *Tropheus annectens*; Abate et al., 2021). Among the 422 known families of bony teleost fish, 87 show post-fertilization care of the eggs, and, of these, biparental care is found in 13%, male-only care is found in 49%, and care by females in 7% of families (Blumer, 1979, 1982; Gittleman, 1981; Gross and Sargent, 1985; Goodwin et al., 1998). However, the literature supporting this information is more than two decades old since its original publication and possible changes to these numbers may have been described in more recent studies.

1.2. Cichlid fishes

Cichlid fishes are well known for their complex reproductive and parental behaviour, with different mating strategies and division of tasks during parenting (Barlow, 1974, 2000; Ripley and Lobel, 2005; Balshine and Buston, 2008; Khoda et al., 2009; Teresa and Gonçalves-de-Freitas, 2011). With approximate 195 genera and more than 1700 species (Abate et al., 2021), cichlid fish are one of the best taxonomic groups for studying the connection between mating systems and parental care, because they show diverse forms of parental care and variation in which sex provides care (Blumer, 1982; Kuwamura, 1986; Keenleyside, 1991b; Wickler, 2010). Unlike other teleost fishes, it is known that among neotropical cichlids, monogamy and biparental care are the most common

strategies (Keenleyside, 1991a), while the biparental substrate guarding is widely considered to be the ancestral care state (Lowe-McConnell, 1959; Iles and Holden, 1969; Peters and Berns, 1982; Goodwin et al., 1998).

All cichlids whose reproductive strategies are known provide parental care for the eggs and fry (Kuwamura, 1986), but the ways that they care for their young vary a lot across all the different species (Abate et al., 2021). In literature, there are two predominant breeding strategies among cichlids: monogamic biparental substrate-brooding typical of American and Asian cichlids (82% of all cichlid genera are biparental; Abate et al., 2021), and polygamic maternal mouthbrooding common among African cichlids, although other patterns have been reported (Breder and Rosen, 1966; Fryer and Iles, 1972; Barlow, 1974; Loiselle and Barlow, 1978; Keenleyside, 1979; McKaye, 1984). The bonds established between parents and offspring during parental care can be long-lasting and often involve sophisticated communication and signalling (Breder and Rosen, 1966; Fryer and Iles, 1972; Keenleyside, 1991; Sefc, 2011). Kuwamura (1986) comparative studies described the breeding habits of 52 African cichlid species, including 22 mouthbrooders and 4 substrate-brooders genera. These studies were an important foundation for future researchers providing new information regarding African cichlid species' reproductive ecology and behaviour.

More recently, some researchers have focused their studies on neotropical cichlids' biology, social and reproductive behaviour, and parental roles. For example, Vieira et al. (2009) analysed the reproductive biology of the *Cichla piquiti* in the Itumbiara Reservoir, southeast Brazil, and demonstrated the reproductive plasticity and adaptation capacity of this species in neotropical reservoirs, providing important information on the invasive potential of cichlid fishes. Moving deeper in behaviour studies, Teresa and Gonçalves-de-Freitas (2011) worked with the *Laetacara araguaiae* in two Brazilian streams and hypothesized that this species showed reproductive cooperation and division of tasks between males and females like other substrate-spawner cichlids. The study was divided into 3 different phases and demonstrated that: in the pre-spawning stage, both sexes were directly involved in nest building and territorial defence; at the egg/wriggler stage, males and females alternate between rearing eggs and defending the nest; and finally, at fry stage, both parents jointly stay closer to the nest to take care of the fry. These results showed that the reproductive cooperation between males and females was marked by labour partition in early reproductive phases and by sharing parental tasks as the offspring

develops. These are in accordance with other studies on parental behaviour that show that this trait is a key factor for invasive success in cichlids (see Ribeiro et al., 2013).

Lavery and Reeb (1994) tested the flexibility of sex roles of the convict cichlid *Cichlasoma nigrofasciatum*. In this work, a series of removal experiments were done, and the time spent by each individual in several parental activities was assessed. They concluded that female roles are less flexible when the male is absent, while male roles adapt more easily when the female is absent. This happens because females normally are more involved in the direct care of the young and may not perform other roles related to territory protection, while males can adapt and perform both tasks. Still about the *Cichlasoma* genus, Fiszbein et al. (2010) studied the relation of photoperiod variations in the reproductive behaviour of *Cichlasoma dimerus*, commonly known as “chanchita”, and pointed out the importance of the photoperiod as a relevant environmental signal to reproductive behaviour and physiology of this cichlid. Briefly, the behavioural experiments showed that male aggression in territory selection and defence is lower when animals are exposed to short photoperiods, indicating that bigger photoperiods may influence aggressiveness in cichlid species. Ramallo et al. (2014) explored the social and reproductive behaviour of the same fish, *C. dimerus*, and gathered all the new information about the social and reproductive behaviour of this species, with a focus on the endocrine regulation of its behaviour and stress physiology. Other studies have been done regarding the reproductive behaviour and physiology in males and females of “chanchita” (see Tubert et al., 2012; Birba et al., 2015), however, studies on its parental behaviour are still very few.

1.3. Species of study, *Australoheros facetus*

The neotropical chameleon cichlid *Australoheros facetus* (Jenyns, 1842, **Figure 1.3.1**) was the used model in this work. This fish, locally known as “chanchito”, is native to the South American river drainages of southern Brazil, Uruguay, Paraguay, and northern Argentina (Rican and Kullander, 2006), and is presently established in several Mediterranean-type drainages in southern Portugal, Spain (Ribeiro et al., 2007) and Chile (Iriarte et al., 2005) as an invasive species. This species has been introduced into the southern regions of Portugal since 1940 (Ribeiro et al., 2007), and humans are most likely responsible for the spreading of this cichlid (Baduy et al., 2020). Recent studies based on

cytochrome *b* similarity show that *A. facetus* populations screened in Portugal show high genetic similarity to specimens collected in the Uruguay River (Carecho et al., 2018), meaning that the current populations established in Portugal maybe derive from Uruguay River populations. However, the real factors that promote the invasiveness of *A. facetus* in Mediterranean-type rivers remain unclear, and knowledge about its biological attributes is still limited.



Figure 1.3.1. Illustrative representation of the chameleon cichlid *Australoheros facetus* (Jenyns, 1842) in southern Portuguese river drainage.

Currently, there is a lack of updated information regarding the ecology, physiology and features that allow the species *Australoheros facetus* to establish in new habitats and compete with native fish, however, other scientific studies had been carried out, contributing to understanding this species. Ribeiro et al. (2007) showed that the generalist feeding strategy of *A. facetus* may play a significant role in the establishment of these non-native fish in Mediterranean-type rivers, where they feed predominantly on insects, small molluscs and crustaceans, and plant materials (Ribeiro et al., 2007; Ribeiro, 2008, Carecho et al., 2018). Baduy et al. (2020) detected the presence and expansion of this cichlid in Portuguese continental waters, confirming its high invasive potential, and Alzamora and Lobos (2021) registered the same tendency in the entire Chilean Mediterranean region.

As an American cichlid, *A. facetus* is a substrate spawner that shows biparental care of the eggs and fry (Baduy et al., 2020). This means that after egg fertilization both parents care for the young by guarding and nursing them (Abate et al., 2021). In most biparental substrate-brooding cichlids, either sex is capable of providing all aspects of care, however, it is known that females and males play different roles and do not share the care duties equally: males are usually more concerned with territory defence and spend more time away patrolling, while females are normally more engaged in the direct care of the offspring (Itzkowitz, 1984; Keenleyside, 1991b; Lavery and Reeb, 1994). Whether this is also true for *A. facetus* is still to be characterised. Moreover, this benthopelagic freshwater fish exhibits diurnal activity (Baduy et al., 2020), and, in Portugal, its size reaches up to 20 cm (Ribeiro et al., 2007). In cichlids with biparental care behaviour like “chanchito”, the male is usually larger than the female, but there is no evident sexual dimorphism or dichromatism (although the latter can occur temporarily during reproductive and parental behaviours, with males being paler and females darker). Such differentiation in colourations and size between sexes has also been observed in African cichlids (Kuwamura, 1986). Although there is a lack of information about the reproductive tactics of *A. facetus* in its native range, its reproduction seems to be triggered most directly by water temperature (Baduy et al., 2017) though other environmental or social cues cannot be disregarded.

With studies on chemical communication, Hubbard et al. (2017) investigated the olfactory system of “chanchito” for the first time and showed that conspecific intestinal and bile fluids are strong olfactory stimuli for this species. Similarly to many other fishes (see Hara, 1994), this fish exhibits olfactory sensitivity to amino acids and bile acids. This scientific work also showed that the olfactory potency of these fluids is greater when taken from dominant males than those taken from subordinate males, supporting the involvement of chemical signals in the social behaviour of *A. facetus*, in addition to eventual visual and acoustic signals.

Regarding the social behaviour of the chameleon cichlid, Baduy et al. (2017) defined the social organization and endocrine profiles of *A. facetus* in southern Portugal, contributing with very important and updated knowledge on this topic. In particular, the description of usual agonistic behaviour, territorial behaviour and social status, and dominance hierarchy of individuals were essential for the arrangement of this thesis. This study correlated hormonal profiles with social behaviour to identify the factors behind the

invasive success of this freshwater fish. Their results showed that this cichlid only exhibits reproductive behaviour under long photoperiods and when the water temperature is above 24°C. The reproductive pairs were stable during all the experiments, even when larvae were removed from the pair. The dominant individual in a social group was usually a male that assumed a typical reproductive colouration (dark stripes and bright background) and presented territorial strategies like guarding the territory and chasing submissive fish (Baduy et al., 2017). Many studies show that the formation of social hierarchies with different degrees of dominance in cichlid fishes can be triggered when intra-specific aggressive interactions occur (Morse, 1974; Dewsbury, 1982; Colléter and Brown, 2001; Ramallo et al., 2015), with size being the main cue for the establishment of the hierarchical position, although dynamic social interactions can also influence the rank of an individual within a hierarchy (Beacham and Newman, 1987; Chase et al., 2002). In “chanchito” hierarchy development it is normal that a female joins the dominant male and forms a reproductive pair that defend the territory and reproduce within a week of pairing (Baduy et al., 2017). However, how the two select each other and whether any specific courtship behaviour/communication exists is unknown. Thus, although this study provides substantial information about the reproductive behaviour of these species, there is still a lot to be characterized about its reproductive strategies and parental roles.

1.4. Invasive success of *Australoheros facetus*

Non-native fish have long been implicated in the decline and extinction of native freshwater biota (Kolar and Lodge, 2000). The introduction of a species into a new environment can lead to the establishment of thriving populations expanding their distribution and causing negative impacts on the environment, public health, and the economy, thus acquiring the status of invasive species (Lowe et al., 2000; Mack et al., 2002; Clavero and García-Berthou, 2005; Casal, 2006; Gallardo et al., 2015). An invasive species is defined as one that arrives in a new habitat it had not previously occupied, establishing a population and spreading autonomously (Ehrlich, Sodhi and Simberloff, 2011). This phenomenon can happen naturally when a certain species makes its way to a new habitat or with human assistance when, intentionally or accidentally, humans introduce a certain species into a new environment. Invasive species can have a bewildering range of impacts, but the greatest impacts of these species involve modifying entire ecosystems (Ehrlich, Sodhi and Simberloff, 2011).

As mentioned above, *A. facetus* is presently established as an invasive species in many Mediterranean-type drainages in southern Portugal, Spain (Ribeiro et al., 2007) and Chile (Iriarte et al., 2005). This is corroborated by the recent surveys carried out by Baduy et al. (2020) in several southern Portuguese river drainages, that confirmed the presence and spreading of *A. facetus* populations in these areas (**Figure 1.4.1**).

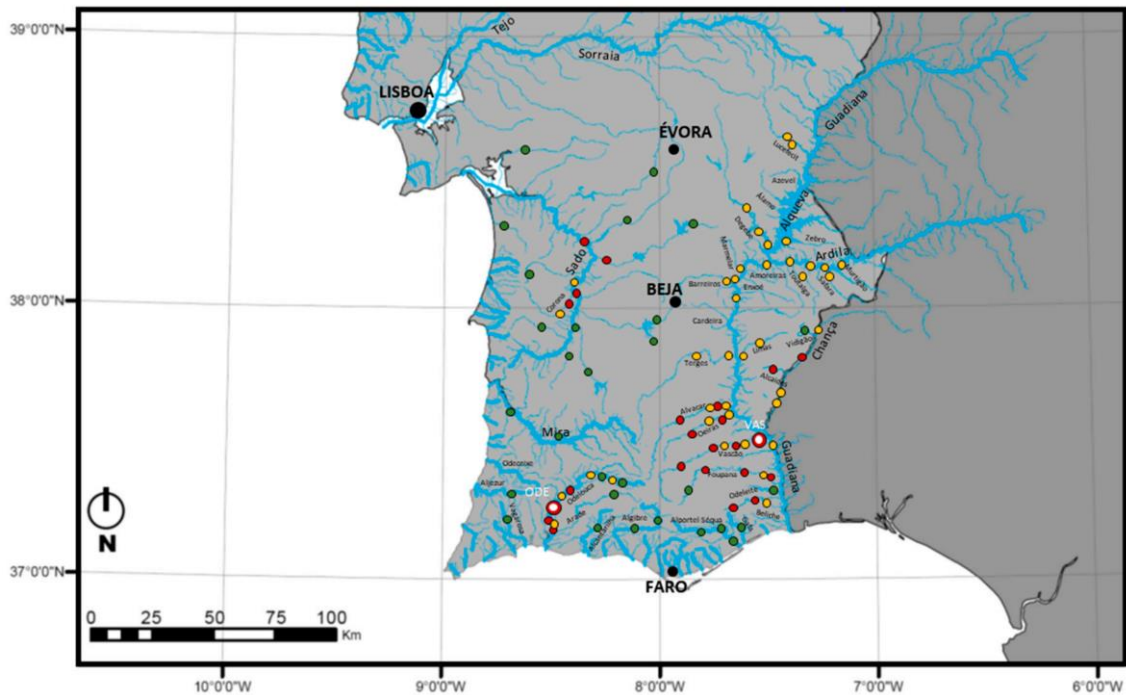


Figure 1.4.1. Distribution map of *Australoheros facetus* in southern Portugal river drainages (retrieved from Baduy et al., 2020). The map shows the locations where *A. facetus* is currently present (red), the areas where it is absent (green), and the areas where it has already been recorded in the past (yellow).

Many studies attribute the invasiveness key factor of cichlid fishes to their generalist diet (Arthington and Mitchell, 1986; Galis and Metz, 1998; Ruiz and Figueroa, 2004; Bergmann and Motta, 2005). Mediterranean-type rivers are typically shaped by strong seasonal flows, including both large winter floods and severe summer drying events (Gasith and Resh, 1999). So, this feeding attribute may be a key element in driving invasiveness in this type of biome, where food resources are strongly seasonal (Gasith and Resh, 1999) and tend to greatly limit the foraging success of native fish (Magalhães, 1993; Gomes-Ferreira et al., 2005). Summer drying events are particularly significant, as they result in major habitat contraction and loss of connectivity throughout a river network (Magalhães et al. 2002). Fishes either assemble in residual permanent waters, where abiotic conditions tend to deteriorate and become extreme with increased water

temperature and reduced dissolved oxygen (Gasith and Resh, 1999; Pires et al., 1999; Ribeiro et al., 2008) or end up dying in areas that dry up. In the absence of flow, invertebrate communities shift from high to low abundance and diversity (Pires et al., 2000), so stranded fish risk food starvation or increased competition and predation pressure for limited food resources. In these circumstances, diet generalists with opportunist feeding behaviour like *A. facetus* (Ruiz et al., 1992; Ribeiro et al., 2007) are likely to have a greater overall invasion success than diet specialists. Additionally, *A. facetus* is highly tolerant to very high and low-temperature extremes and can withstand environmental salinity at least up to 15 PSU (Practical Salinity Units), their isosmotic level (Baduy et al., 2020). This plasticity confers an important advantage to this species in adapting to the reduction of available habitats and the colonization of new ones.

On the other hand, many other studies assign the invasive success of cichlid fishes to their reproductive and parental behaviours. According to Ribeiro et al. (2013), the high level of parental care in *A. facetus* vastly contributes to high recruitment and establishment success. Additionally, this taxonomic group shows very complex social actions with different patterns of aggressiveness (Keenleyside, 1991a) which may be linked to invasive success (Baduy et al., 2017; 2020).

1.5. Objectives

The main objective of this work was to characterize the reproductive and parental behaviour of the species *Australoheros facetus* in southern Portuguese drainages, namely the roles of each parent during different stages of progeny development. To achieve this, the specific activities performed by each individual during reproductive and parental stages were observed and recorded, identifying the main tasks, and assessing the time spent on each task by each member of the reproductive pair. Ultimately, the new information gathered in this study provides important knowledge that, together with future physiological data, may be used to disrupt reproductive and parental behaviours in *A. facetus* and to control the current populations and the spreading of this invasive fish.

2. Material and Methods

2.1. Experimental animals

All animals used during the experimental work were captured by electrofishing in the Foupana, Odelouca and Vascão rivers under specific licenses provided by the Instituto da Conservação da Natureza e das Florestas (ICNF). This fishing technique is a common scientific survey method that uses direct current electricity flowing between a cathode and an anode. The direct current electricity applied in low conductive freshwater flows within a certain range until eventually encounters a fish. When in contact with high-voltage current, the fish is affected by the electricity due to the higher conductivity of its body in relation to the surrounding water and suffers galvanotaxis. Galvanotaxis (or electrotaxis) is the uncontrolled muscular convulsion caused by the electric current interruption of neurological pathways. This results in the fish swimming towards the anode pole, where a dip net is mounted. The mechanism by which this fishing technique acts relies on several technical and environmental factors, and its effectiveness is conditioned by the size and the body composition of each fish, and the conductivity of the water. In saltwater, for example, this method is ineffective, as the electricity would travel preferentially through the water rather than through the fish due to the high conductivity of saltwater.

To effectively catch the targeted fish, four people were required to execute the electrofishing sessions: an **anode operator**, that controlled the electric pulse (gas-powered) by a deadman's switch on the anode pole; an **electrofisher**, that caught the stunned fish with a dip net; and **two assistant fishers**, that received the fishes and helped the electrofisher in other matters. As a potentially dangerous technique, serious safety precautions were taken for every person involved in the activity, namely the use of rubber equipment (pants, shoes and gloves). Once captured, the animals were transported in aerated tanks to the Centre for Marine Sciences (CCMAR) experimental facilities at the University of Algarve, in Gambelas Campus. All fish were maintained in 2000L community tanks for at least 60 days before being used in the experiments, allowing them to acclimate to the holding temperature and photoperiod conditions, and were fed daily with specific cichlid feed (2% w/w, Sparos Lda.).

2.2. Animal selection

Animals were randomly selected from the community tanks (stock), and their total length and weight were measured (ranging between 11cm and 13cm), ensuring that the size of the selected fish was approximate. Since *A. facetus* does not display evident sexual dimorphism during non-reproductive stages, accurate gender identification was only possible after the formation of the reproductive pairs, when both members of the reproductive pair exhibit different colouration (during reproductive stages, females exhibit darker colouration in the chin and ventral when compared with males). Nevertheless, gender identification was attempted during this step by urogenital papillae visual inspection of all fish.

2.3. Experimental setup

As described by Baduy *et al.* (2017), one of the striking features of the behaviour of this species is the formation of territorial reproductive pairs after an intense period of aggressive displays and interactions. Knowing this, the breeding pairs were obtained as a result of the establishment of social hierarchy and territoriality. Social groups of 6 individuals of similar size and unconfirmed/suspected sex were placed in individual 250L tanks (90cm × 60cm × 50cm) fitted with a bottom biological filter for up two weeks (**Figure 2.3.1**). The water temperature was then increased to 24°C (a specific known temperature to trigger social aggression and hierarchy formation in *A. facetus*; Baduy *et al.*, 2017) at increased photoperiod (12D:12L), and a dominant territorial pair eventually emerged.



Figure 2.3.1. Illustrative representation of the experimental setup comprised of six 250L tanks fitted with bottom filters. The tanks were placed in a man-shift dark room and lights were installed directly above each tank to simulate summer light conditions in shallow waters and facilitate video recordings from underwater and external cameras.

To ensure the viability of the experiment and the well-being of all animals, all tanks were cleaned daily. A series of abiotic parameters were measured to assess the state of the water of each tank: temperature ($^{\circ}\text{C}$), dissolved oxygen (mg/L) and oxygen saturation (%), and concentrations of ammonia (NH_3) and ammonium (NH_4 ; mg/L). These were assessed daily during the first two months of experiments and weekly for the remaining three months. NH_3 and NH_4 concentrations were maintained between 0 and 0,6 mg/L in all tanks throughout the experimental activities.

At this point, social behaviours were recorded and characterized according to the ethogram established by Baduy *et al.* (2017) (**Table 2.3.1**). Once the reproductive pair's social behaviour was well characterized and the animals started to present courtship behaviour, the four neighbours were removed from the tank, allowing the experiment to proceed to the reproductive phases. Then, reproductive behaviours were recorded and characterized according to the same ethogram from the pre-spawning phase until the post-spawning phases (**Figure 2.3.2**).

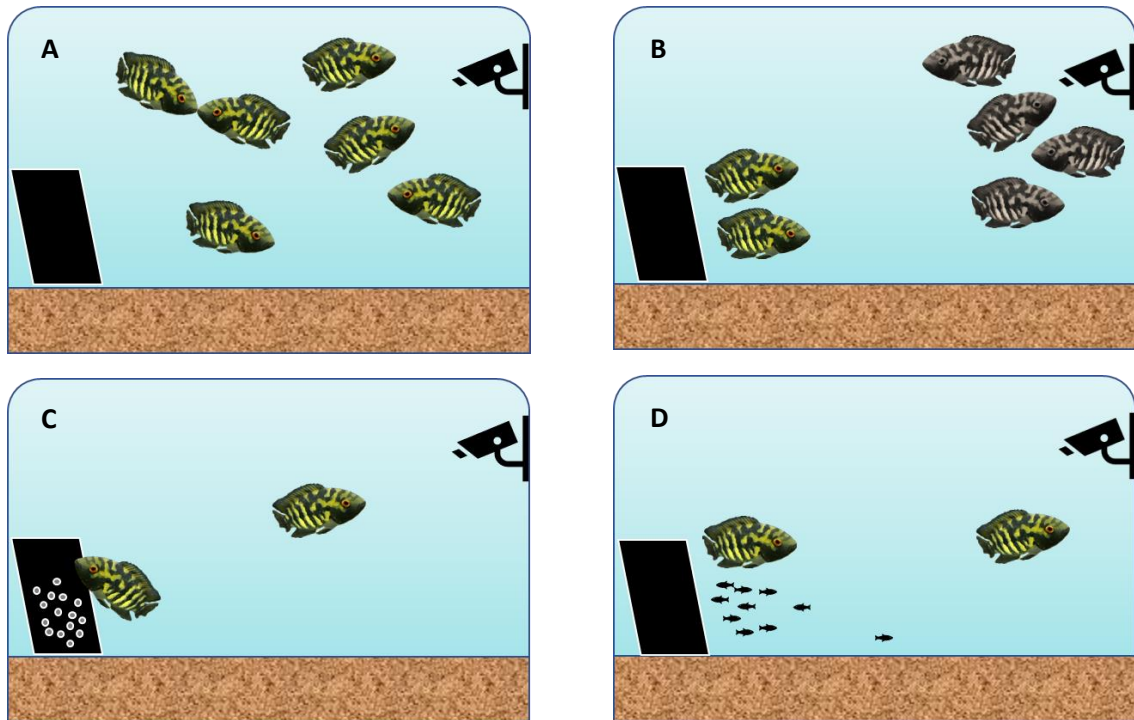


Figure 2.3.2. Schematic representation of the experimental stages. A) hierarchy establishment, which lasted between 3 and 14 days; B) pair formation, which lasted between 1 and 5 days C) spawning and egg development, which lasted between 2 and 7 days; and D) larvae development, which lasted up to 1 month.

During direct behaviour assessment work, cameras (GoPro Hero 7) were placed in and out of the tanks to support the behavioural analysis, described below. To ensure that behaviours were appropriately recorded, a system with two cameras per tank was devised. An underwater camera was placed on the tank side opposite to a black tile slab that was provided as a “nest” to the reproductive pairs. This underwater camera was mounted on a platform that could move freely in the vertical direction to obtain the best angle and cover the largest area possible. At the same time, another camera was placed outside the tank facing the glass window. This outside camera was mounted on a cus-made hanging pole in a certain position that granted the record of the whole length of the tank, allowing a better estimate of distances as fish moved away from the nest. Together, both cameras covered over 90% of the tank area/volume.

Table 2.3.1. Ethogram of *A. facetus* (adapted from Baduy *et al.*, 2017).

Class	Behaviour		Description
Social	Agonistic	Threats/low aggression	Frontal display Lateral display
		Attacks/high aggression	Strike
	Chase		Swimming at high speed after another fish. This can occur briefly or last longer, usually culminating in physical contact.
	Bite		Bites usually occur following a chase or a strike. The most affected area is the head region but can be anywhere in the body of the opponent, and the grip can last for several seconds.
	Tail beating		A fish performs rapid anteroposterior waving of the body at the side of the opponent, touching the opponent with its tail.
	Mouth fighting		This is a symmetric agonistic interaction, usually following a symmetric frontal display. Both opponents rapidly extend their jaws and bite each other simultaneously on the mouth and frontal region. Once they engage in a bite, the grip can last up to 1 min.
	Submissive display		Flee
		Freeze	The fish remains stationary during an attack, with no reaction.
		Courtship	The larger fish of the pair approaches the smaller and they touch their heads. Both fish exhibit darkened vertical bars. The smallest fish exhibits a darkened ventral anterior region. They swim close to each other slowly for about 1 min and repeat this behaviour several times at intervals of about 5 min. Both fish shake their bodies, the smallest doing so more often. Usually, the largest fish is the male, and the smallest is the female.
	Reproductive	Prespawning	The female prepares the place chosen for laying the eggs (nest), nibbling it, and curving its body into BS^ across the surface. This body movement is short and quick, lasts about 6 s and is repeated several times. The vertical bars in the body as well as the ventral region of the head are dark and further darken when the female approaches the nest. The female moves away, swims around the entire aquarium and then returns to the nest.

Class	Behaviour	Description
	Spawning	Female swims on the nest, slowly making a BS [^] movement with the body. The pectoral fin moves short and fast. The colour is like pre-spawning. Oviposition occurs slowly. After up to 90 min, the female moves and the oviposition ceases. Seconds later, the female returns, and maternal care begins.
	Dig	Digging a hole or a pit in the substrate with the mouth, pectoral, and caudal fins. This will be used as a refuge after hatching.
	Parental hover	The animal hovers directly above the eggs or recently hatched larvae. Can be performed by both members of the pair, but most commonly by the smallest.
	Care	The animal makes an 'S' movement repeatedly passing the entire body slowly over the entire surface covered by the eggs, moving their pectoral fin in long movements, removing dead eggs with the mouth, and cleaning and oxygenating the batch. Can be performed by both members of the pair, but most commonly by the smallest
	Patrol	The fish swims or stands at a distance greater than their standard length of eggs or larvae. Can be performed by both members of the pair, but most commonly by the largest.
	Fetch	Occurs when a larva moves away from the larvae group and one of the adults catches it with its mouth and spits it back into the group. This behaviour can be observed by both members of the pair.

2.4. Behavioural analysis

The description and quantification of individual behaviour during pre- and post-spawning phases were done by focal animal sampling (Altmann, 1974). The behavioural analysis was done in repeated sessions of 5 minutes of observations with each fish as a focal individual. The first 5 minutes of each observation session were considered a habituation period and all events observed during this time were disregarded from the analysis. Two approaches were used:

- 1) **Direct behavioural observation**, in which the observer followed the focal fish for 5 minutes, registering all events in which the fish was involved, either as an emitter or as a receptor – these were the behaviours analysed as the frequency of occurrence per time.

- 2) **Observation of video recordings**, in which the duration of behaviours was recorded and compared between fish, sex or development stage for the same time-interval as before – these were the behaviours considered as status that were analysed as the amount of time spent, as a percentage of total observation time.

The behavioural analysis was divided into four stages reflecting different phases of the reproductive process and offspring development (pair formation, egg stage, newly hatched larvae stage, and free-swimming larvae stage) with different existent behaviours described (Baduy *et al.*, 2017). During the pair formation stage (pre-spawning), social behaviours of male and female individuals were evaluated considering the main activities observed within social groups, during territory establishment and when the breeding pairs were isolated during courtship. The social behaviours registered during this stage included threats (frontal and lateral display), attacks (strike, chase, bite, tail beating, and mouth fighting), and submissive display (flee and freeze). At this stage, the frequency of occurrence of each behaviour was assessed.

As reproductive activities started and the pairs started to spawn, reproductive behaviours of male and female individuals were evaluated considering the main actions observed during the three different stages of offspring development (post-spawning): egg stage, newly hatched larvae stage, and free-swimming larvae stage. The reproductive behaviours registered during this stage included dig, parental hover, care, patrol, and fetch. As before, the frequency of occurrence of each activity was assessed. Additionally, throughout these phases, the time spent by each member of the couple in each of the identified reproductive behaviours was assessed using the recorded videos. For this, only the behaviours classified as “status” were analysed since “events” behaviours do not have a duration. As previously mentioned, males and females usually acquire strong colourations upon reproductive activities that are slightly different between sexes, allowing gender distinction during these stages.

Furthermore, the data regarding behaviour frequency were organized to evaluate the evolution of each of the reproductive behaviours in both sexes throughout the three analysed post-spawning phases. Additionally, for the pre-spawning phase, an aggressiveness index was created to check the aggressive levels of each sex during pair formation and territory defence. In the same way, a “good parent” index was created to see the influence and investment of each sex in each of the three reproductive stages explored in this work.

For the pair formation stage, the aggressiveness index was assessed according to the following formula:

$$\textit{Aggressiveness Index} = \frac{\textit{Sum of aggressive behaviours frequencies}}{\textit{Sum of all social behaviours frequencies}}$$

For the remaining three phases, the “good parent” index was created using the most relevant behaviour in each stage following the formula:

$$\textit{"Good parent" Index} = \frac{\textit{Most relevant behaviour frequency}}{\textit{Sum of all reproductive behaviours frequencies}}$$

2.5. Challenges to parental care

In addition to the general characterization of the animals' natural behaviours during pre- and post-spawning stages, reproductive pairs integrated a test where their tasks and roles as parents were challenged. This test consisted of a simulation of a natural challenge faced by *A. facetus* individuals in the wild and aimed to evaluate the commitment of each sex to parental tasks when exposed to intruders in their established territory, i.e., if when exposed to a threat the parents continued to present parental care of the young invested only in territory defence and nest protection or would aggressively attack the intruder. Due to time and animal limitations tests with different species/predators were not carried out. Reproductive pairs previously established in different reproductive stages (courtship stage, egg stage, or larvae stage) were selected and submitted to the introduction of a conspecific fish with different sizes in the tank. These conspecifics were placed in the tanks inside a transparent cubic box (20cm × 20cm × 20cm) with small holes (**Figure 2.5.1**) that allowed the passage of water and any type of chemical signal that these fishes could release to alert the reproductive couple to their presence. The individuals' behavioural response was evaluated using the same behavioural analysis methods as before, and the frequency of occurrence of threats and attacks in 5 minutes was calculated covering all stages.



Figure 2.5.1. Illustrative representation of the experimental setup with the transparent cubic box. In the image, both parents attack the intruder inside the box while there are eggs (white spots) laid on the stone slab, to the left.

2.6. Data analysis

Once the data was collected and organized, a statistical analysis was executed to validate the differences in the behaviours observed between the sexes. All the statistical analysis work was performed using IBM SPSS Statistics software (IBM Corp, 2021). Firstly, the normality of all the collected data was assessed using the Shapiro-Wilk normality test (confidence interval percentage of 95%). According to the results, a comparative statistical test was performed (independent Student's t-test if the data was normal or Kruskal-Wallis H test if the data deviates from a normal distribution) to assess whether there were differences in the frequency and time of occurrence of social and reproductive behaviours between males and females in all stages of the experiment. The same was done for obtained values in the created indexes (in these, a dependent paired-samples Student's t-test was performed). The listed behaviours were used as dependent variables, while the sex of the individuals was treated as the independent variable. In both tests, the confidence interval percentage was set to 95%.

3. Results

In total, 32 fish were directly observed, including 12 reproductive pairs, corresponding to 1840 minutes of direct behaviour observations throughout the pre- and post-spawning stages. For the evaluation of the time spent by each member of the couple in each of the identified activities during the post-spawning stages, 32 videos were analysed, including 7 reproductive pairs, corresponding to 320 minutes of video recordings. With the retrieved data the frequency and time of occurrence in 5 minutes of social and reproductive behaviours were obtained and are presented below at the corresponding stage. The evolution of each of the reproductive behaviours throughout the egg, newly hatched larvae, and free-swimming larvae stages, and the aggressiveness and “good parent” indexes are shown at the end. Finally, the registers of abiotic parameters are also included in this section.

3.1. Pair formation stage

As previously described, at this stage only the frequency in 5 minutes of social behaviours obtained via direct behaviour observations was evaluated. Here only the interaction received/caused by the emerging dominant male and female were analysed. Overall, 8 fishes (4 females and 4 males) were observed during the pair formation stage, corresponding to 4 reproductive couples. These were the focal individuals to which the attention was directed. Results show the sum and nature of their interaction with all other fish in the tank before they emerge as a reproductive male and female through the hierarchy establishment. This process took approximately 3 to 10 days.

This stage was marked by the establishment of social hierarchies within each of the experimental groups. In most of the groups, bigger males established a single territory and defended it with threats and attacks against other fish (**Figure 3.1.1**). It was noted that this behaviour was the main mechanism to attract females. When a female engaged with the male and helped in nest digging and territorial defence the reproductive pair was formed, and courtship behaviours started to happen. The couple then assumed a territory that would usually account for half or more of the tank volume adjacent to their “nest”, relegating all other fish to the remaining volume, and attacking those that would intrude in their territory.

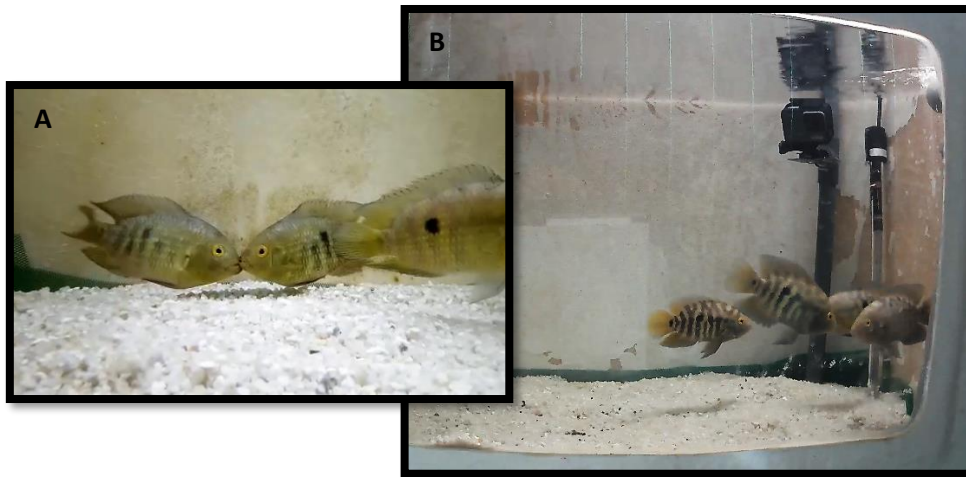


Figure 3.1.1. Examples of social behaviours observed during the pair formation stage. A) mouth fighting, and B) frontal display.

The frequency of social behaviours showed no evident differences between males and females ($n = 4$; **Figure 3.1.2**). Even though there was a higher tendency for males to perform “high aggression”/attacking behaviours, like striking ($f_{\text{male/strike}} = 1$) and biting ($f_{\text{male/bite}} = 0,3$) the statistical analysis results indicated that there was no significant difference between sexes in the frequency of all social behaviours. The comparative statistical test significance values are presented hereafter (**Table 3.1.1**). Since all significance values were greater than 0,05 ($p\text{-value} > 0,05$), the null hypothesis was accepted, meaning the data was not significantly different.

Table 3.1.1. Significance values of social behaviours during the pair formation stage.

Behaviour	p-value
Frontal display	0,508
Lateral display	0,850
Strike	0,166
Chase	1
Bite	0,405
Tail beating	0,317
Mouth fighting	1
Flee	0,850
Freeze	0,131
Courtship	0,850

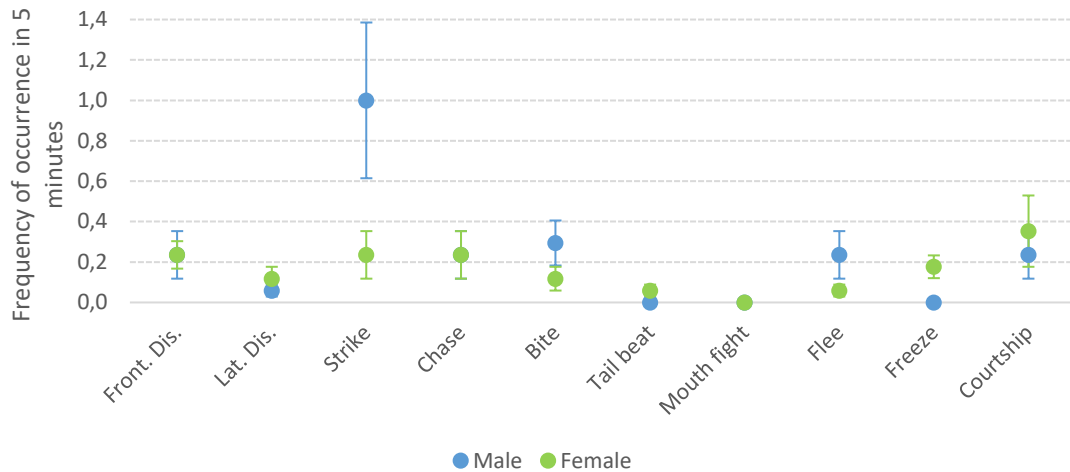


Figure 3.1.2. Average frequency (and respective standard deviation) of occurrence of social behaviours of *A. facetus* males and females in 5 minutes of observations during the pair formation stage (n = 4).

3.2. Egg stage

At the egg stage, during the direct behaviour assessment work, 20 fishes were analysed, corresponding to 10 reproductive pairs. Throughout this stage, it was observed that, when spawning, the female deposited a small number of adhesive eggs in the nest (previously prepared by both parents), that were fertilized by the male, a few at a time. This procedure was repeated several times until the entire brood was deposited and fertilized (egg-laying averaged between 100 and 300 eggs between tanks), and the eggs developed for several days before hatching (from 2 up to 7 days), while were cared for by the parents (**Figure 3.2.1**). The duration of full egg development was similar in all tanks. Sometimes the parents were seen transferring the eggs to different sites within their territory. Such transfers were done very gently using their mouths to pick up a few eggs at a time.



Figure 3.2.1. Example of reproductive behaviour observed during the egg stage. In the image, both parents care for the eggs (white spots) laid in the stone.

The results regarding the frequency of parental behaviours during this phase ($n = 10$) revealed a similar distribution of behaviours between males and females, although there was a higher rate of egg care by female individuals ($f_{\text{female/care}} = 4,3$; **Figure 3.2.2**), and a higher rate of males performing the parental hover activity ($f_{\text{male/parental hover}} = 0,6$). After the statistical analysis, the frequency of dig, care and patrol behaviours was not significantly different between sexes ($p_{\text{dig}} = 0,643$; $p_{\text{care}} = 0,059$; and $p_{\text{patrol}} = 0,848$), while there was a significant difference in the parental hover behaviour ($p_{\text{parental hover}} = 0,044$).

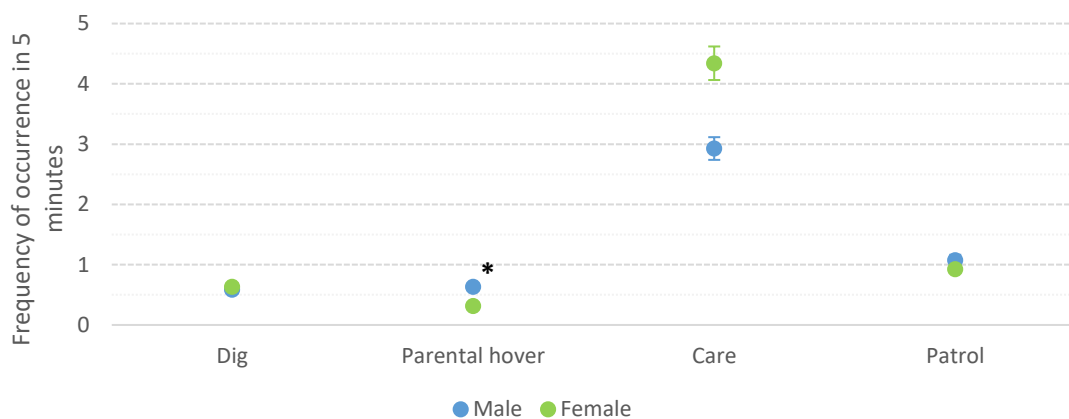


Figure 3.2.2. Average frequency (and respective standard deviation) of occurrence of reproductive behaviours of *A. facetus* males and females in 5 minutes of observations during the egg stage ($n = 10$).

Concerning the time allocation, a total of 12 videos were analysed for the egg stage, including 7 reproductive pairs, corresponding to 120 minutes of video recordings. The percentage results indicated that females spend slightly more time in parental activity than males ($\%_{\text{female/parental}} = 40,3$; and $\%_{\text{male/parental}} = 33,0$; **Figure 3.2.3**). The statistical analysis revealed yet that there were no significant differences between sexes in the time spent in parental or non-parental activity ($p_{\text{parental}} = 0,162$; and $p_{\text{non-parental}} = 0,162$; non-parental activity comprehends locomotor patterns like swimming and hovering behaviour, yawning, and quivering).

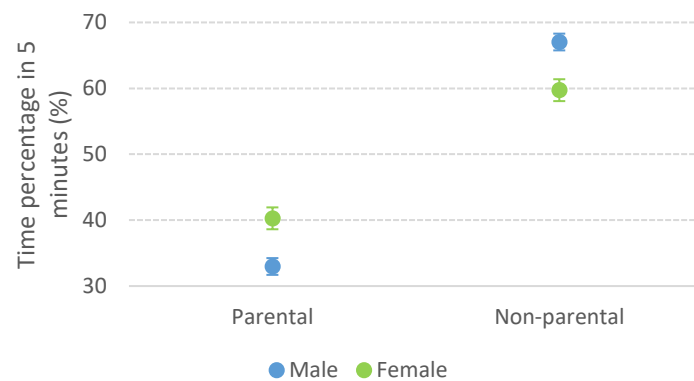


Figure 3.2.3. Time percentage (and respective standard deviation) of parental and non-parental activity of *A. facetus* males and females during egg stage (n = 7).

When the total of parental behaviours are dissected into specific actions, it becomes evident that the percentage of time spent by each sex in each of the listed reproductive behaviours presents variations. Both sexes were equally involved in digging, but there was a tendency for females to concentrate on caring for the eggs ($\%_{\text{female/care}} = 26,8$), while males were more focused on patrolling ($\%_{\text{male/patrol}} = 13,3$) and hovering over the eggs ($\%_{\text{male/parental hover}} = 6,4$; **Figure 3.2.4**). These results were in concordance with the ones obtained in the frequency in 5 minutes, sharing the same relations between sexes. The comparative statistical test disclosed that there were no significant differences between sexes in all studied behaviours ($p_{\text{dig}} = 0,819$; $p_{\text{parental hover}} = 0,835$; $p_{\text{care}} = 0,148$; and $p_{\text{patrol}} = 0,632$).

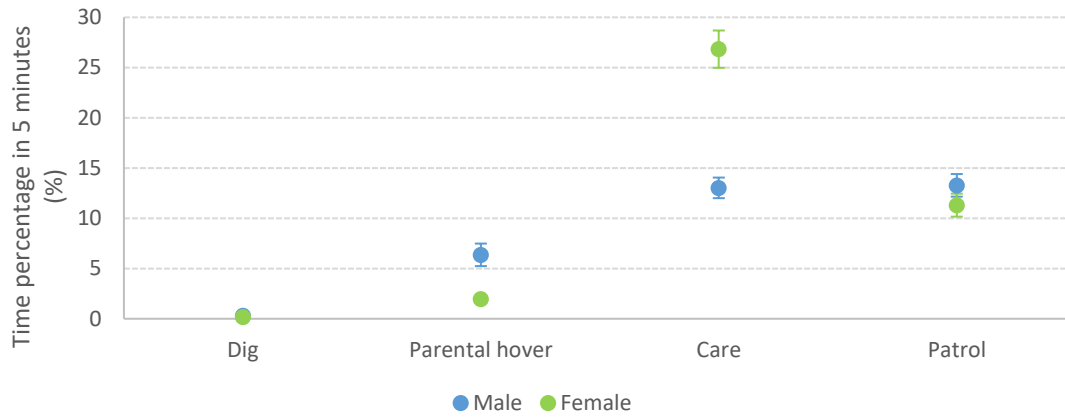


Figure 3.2.4. Average time percentage (and respective standard deviation) of reproductive behaviours of *A. facetus* males and females in 5 minutes of video analysis during the egg stage ($n = 7$).

3.3. Newly hatched larvae stage

At the newly hatched larvae stage 8 fishes, corresponding to 4 reproductive pairs, were observed during the direct behaviour assessment work. It was noted that, following hatching, the recently hatched small larvae remained attached to the nest for several days until they were able to move and swim freely. As before, the parents were occasionally seen transferring their young to different sites within their territory while performing parental hovering and patrolling tasks (**Figure 3.3.1**).



Figure 3.3.1. Example of reproductive behaviour observed during the newly hatched larvae stage. In the image, the female (on the left) patrols the area while the male (on the right) stays near the nest hovering over the recently hatched larvae (seen here as black dots enclosed in the red circle).

The results concerning the frequency of parental behaviours during this phase ($n = 4$) showed a tendency of caring and fetching behaviours being more performed by females ($f_{\text{female}/\text{care}} = 0,6$; and $f_{\text{female}/\text{fetch}} = 4,0$), while protection behaviours were a concern of male individuals ($f_{\text{male}/\text{dig}} = 0,6$; $f_{\text{male}/\text{parental hover}} = 1,6$; and $f_{\text{male}/\text{patrol}} = 2,6$; **Figure 3.3.2**). However, the comparative statistical test demonstrated that the frequency of all studied behaviours was not significantly different between sexes during this phase ($p_{\text{dig}} = 0,643$; $p_{\text{parental hover}} = 0,225$; $p_{\text{care}} = 0,089$; $p_{\text{patrol}} = 0,071$; and $p_{\text{fetch}} = 0,118$).

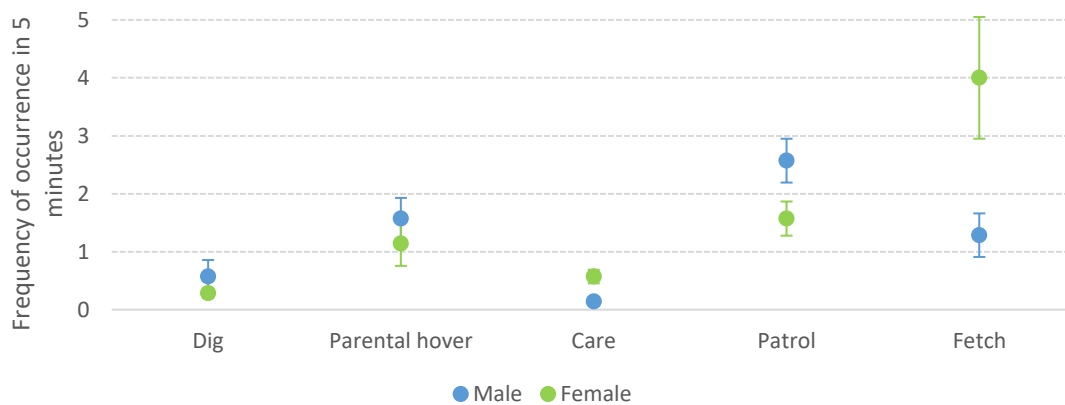


Figure 3.3.2. Average frequency (and respective standard deviation) of the occurrence of reproductive behaviours of *A. facetus* males and females in 5 minutes of observations during the newly hatched larvae stage ($n = 4$).

Regarding the time allocation, a total of 4 videos were analysed for the newly hatched larvae stage, corresponding to 4 reproductive pairs, in a total of 40 minutes of video recordings. The results suggested that, in this stage, fish spent almost twice the time in non-parental behaviours than they did in parental activities. Surprisingly, when the analysis was made for each sex, males appear to spend more time in parental activity during this stage than females ($\%_{\text{male}/\text{parental}} = 32,4$; and $\%_{\text{female}/\text{parental}} = 17,9$; **Figure 3.3.3**). The statistical analysis revealed however that there were no significant differences between sexes in the time spent in parental or non-parental activity ($p_{\text{parental}} = 0,107$; $p_{\text{non-parental}} = 0,128$).

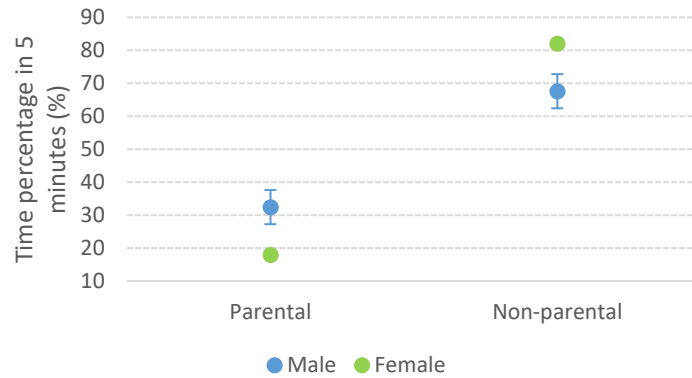


Figure 3.3.3. Time percentage (and respective standard deviation) of parental and non-parental activity of *A. facetus* males and females during the newly hatched larvae stage (n = 4).

The percentage of time spent by each sex in each of the recorded reproductive behaviours presented a similar predisposition as the frequency in 5 minutes, with females caring more for the offspring ($\%_{\text{female/care}} = 5,3$) and regrouping the larvae ($\%_{\text{female/fetch}} = 6,1$), and males patrolling a lot more ($\%_{\text{male/patrol}} = 24,1$; **Figure 3.3.4**). The statistical analysis showed that the percentage of time of the dig, parental hover, patrol, and fetch behaviours was not significantly different between males and females ($p_{\text{dig}} = 0,850$; $p_{\text{parental hover}} = 0,295$; $p_{\text{patrol}} = 0,086$; and $p_{\text{fetch}} = 0,374$), while there was a significant difference in the care behaviour ($p_{\text{care}} = 0,044$).

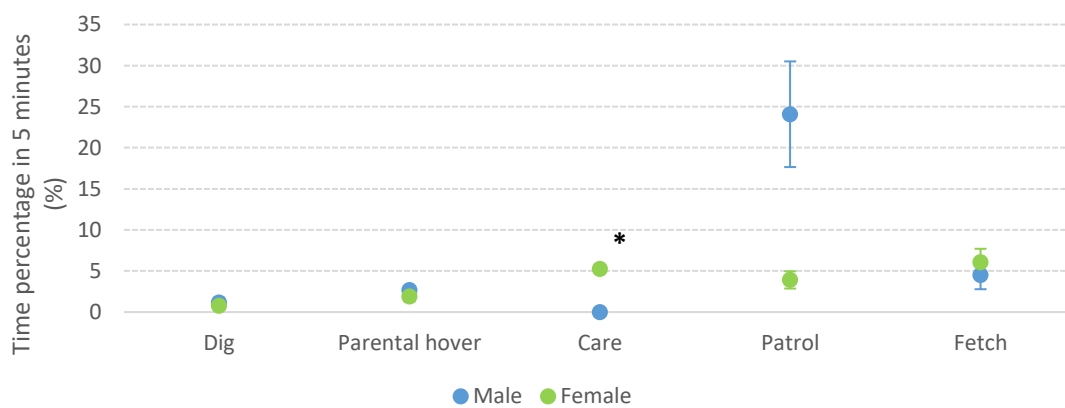


Figure 3.3.4. Average time percentage (and respective standard deviation) of reproductive behaviours of *A. facetus* males and females in 5 minutes of video analysis during the newly hatched larvae stage (n = 4).

3.4. Free-swimming larvae stage

Finally, at the free-swimming larvae stage 10 fishes, corresponding to 5 reproductive pairs, were observed during the direct behaviour assessment work. In this stage, it was observed that, initially, the unattached larvae remained together in a group very close to the ground while being protected by both parents. However, as the larvae develop, the group started to disperse within the range limited by the experimental tanks and fetching behaviour started to occur (**Figure 3.4.1**). Eventually, both parents started losing their focus on caring for them. Additionally, it was noted that, when the larvae were grouped near the ground, some parents tried to feed their young by breaking the feed into smaller particles and purposely dropping them on the tank floor so the larvae could eat them. Unfortunately, this behaviour was not initially classified in the ethogram and was not recorded systematically nor analysed.



Figure 3.4.1. Example of reproductive behaviour observed during the free-swimming larvae stage. In the image, the female (on the left) fetches a small larva (in red) while the male (on the right) stays near the nest hovering over the larvae group.

The results regarding the frequency of parental behaviours in 5 minutes during this phase ($n=5$) presented a change in the patrolling behaviour frequency, with females performing this activity more times during this phase than males ($f_{\text{female/patrol}} = 0,8$). Additionally, females performed fetching a lot more, while males continued to be in charge of nest and offspring protection ($f_{\text{dig}} = 1,2$; and $f_{\text{male/parental hover}} = 1,3$; **Figure 3.4.2**). Nevertheless, the statistical analysis revealed that the frequencies of digging and patrolling behaviour were significantly different between males and females ($p_{\text{dig}} = 0,025$; $p_{\text{patrol}} = 0,021$), though

the rest of the behaviours were not statistically different between sexes ($p_{\text{parental hover}} = 0,417$; and $p_{\text{fetch}} = 0,094$).

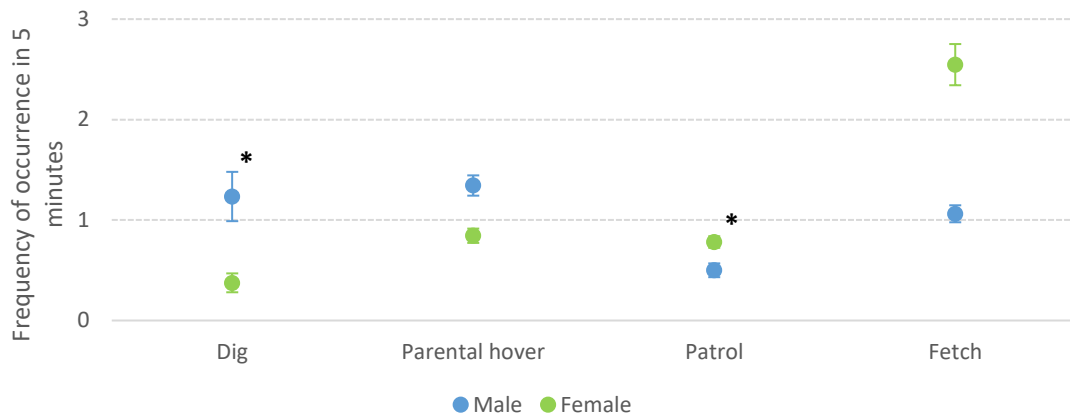


Figure 3.4.2. Average frequency (and respective standard deviation) of the occurrence of reproductive behaviours of *A. facetus* males and females in 5 minutes of observations during the free-swimming larvae stage (n = 5).

A total of 16 videos were examined for the free-swimming larvae stage in the time assessment work, including 5 reproductive pairs, corresponding to 160 minutes of video recordings. The percentage of time spent in parental activity showed that females spend slightly more time in parental activity than males ($\%_{\text{female/parental}} = 24,2$; and $\%_{\text{male/parental}} = 17,8$; **Figure 3.4.3**). The statistical analysis revealed that at this phase there were no significant differences between the two ($p_{\text{parental}} = 0,107$; $p_{\text{non-parental}} = 0,108$).

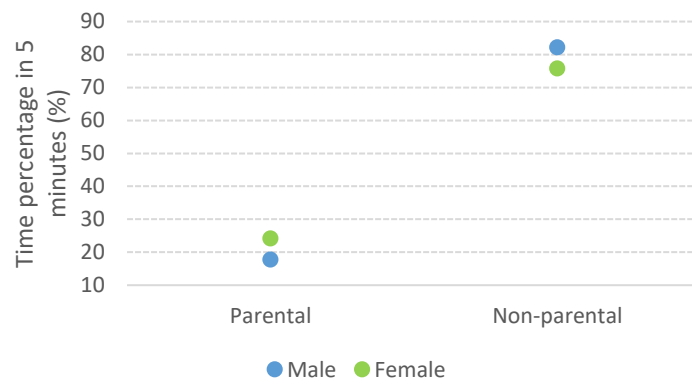


Figure 3.4.3. Time percentage (and respective standard deviation) of parental and non-parental activity of *A. facetus* males and females during free-swimming larvae stage (n = 5).

Lastly, the percentage of time spent by each sex in each of the listed reproductive behaviours displayed a similar pattern to that of the frequency in 5 minutes. Females spend more time patrolling ($\%_{\text{female/patrol}} = 8,9$) and fetching larvae ($\%_{\text{female/fetch}} = 7,9$) a lot

more than males, while males were more focused on parental hovering the larvae near the nest ($\%_{\text{male/parental hover}} = 6,8$) and digging ($\%_{\text{male/dig}} = 2,9$; **Figure 3.4.4**). Yet, the comparative statistical test disclosed that there were no significant differences between sexes in all studied behaviours ($p_{\text{dig}} = 0,054$; $p_{\text{parental hover}} = 0,594$; $p_{\text{patrol}} = 0,783$; and $p_{\text{fetch}} = 0,190$).

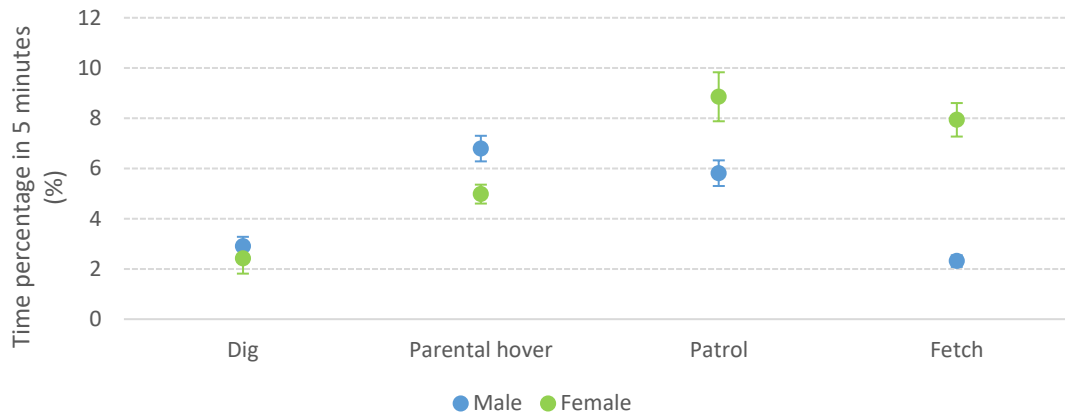


Figure 3.4.4. Average time percentage (and respective standard deviation) of reproductive behaviours of *A. facetus* males and females in 5 minutes of video analysis during the free-swimming larvae stage ($n = 5$).

3.5. Evolution of reproductive behaviours throughout post-spawning stages

The evolution of the percentage of time spent in parental activity by both sexes during the three post-spawning stages analysed in this work is graphed below (**Figure 3.5.1**). The results showed that, throughout the complete reproductive period, the time percentage of parental activity in both sexes decreased to about half of the initial value (from around 30 - 40 % in the egg stage to 20% in the free-swimming larvae stage). Despite the different levels of parental involvement in each stage, both males and females had a similar time contribution tendency in parental tasks, given that both tendency lines have close slopes ($m_{\text{male}} = -7,58$; and $m_{\text{female}} = -8,05$).

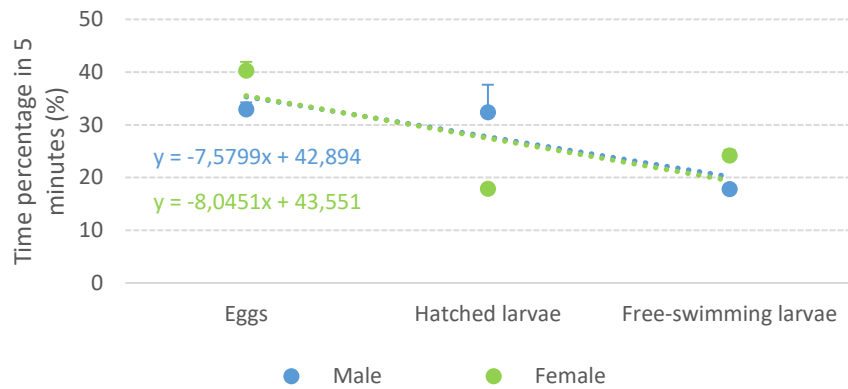


Figure 3.5.1. Time percentage of parental and non-parental activity of *A. facetus* males and females during reproductive phases.

With the focus on relating the relevance of each reproductive behaviour during egg, newly hatched larvae, and free-swimming larvae stages with the fish sex, a series of plots were created, in which the frequency of each selected behaviour was plotted against its occurrence in each development stage. The individualisation of each behaviour allowed a better understanding of the evolution of the exact actions of each sex throughout the whole reproductive period. Firstly, in the evolution of **dig** behaviour, it was noted an increase in the frequency of this behaviour when performed by males. On the other hand, females performed fewer digging tasks as the offspring developed (**Figure 3.5.2.A**). Regarding the evolution of **parental hover** of the eggs and larvae, both sexes presented a relatively similar increase in the frequency of occurrence of this task over time, even though males have performed this parental activity a lot more than females (**Figure 3.5.2.B**). As the offspring developed, the **care** of eggs and larvae decreased considerably in both sexes. This reproductive behaviour presented high frequencies of occurrence in the initial stage in males and females (almost once a minute) but was absent in the last stage of the reproductive period. This decline is particularly evident in females since they were the main parents that performed this activity in the egg and newly hatched larvae stages (**Figure 3.5.2.C**). The progress of **patrol** behaviour throughout the reproductive period was marked by a decrease in the frequency of occurrence in males, while in females this behaviour frequency tended to remain almost constant over time (**Figure 3.5.2.D**). Lastly, concerning the evolution of larvae **fetch** behaviour, it was noted an increase in the frequency of this behaviour in both sexes since this task was not performed in the initial reproductive stage of the experiment (**Figure 3.5.2.E**).

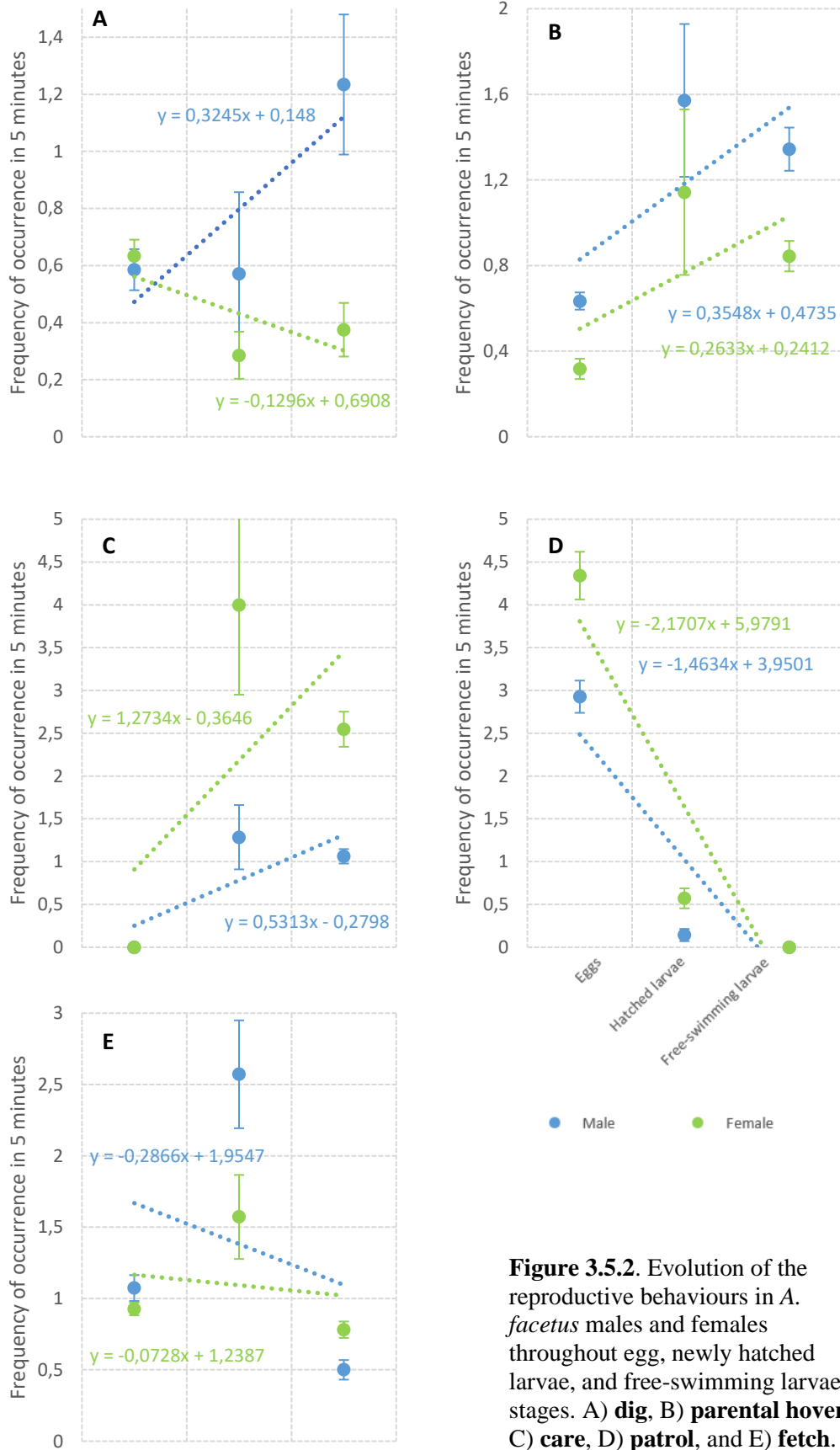


Figure 3.5.2. Evolution of the reproductive behaviours in *A. facetus* males and females throughout egg, newly hatched larvae, and free-swimming larvae stages. A) **dig**, B) **parental hover**, C) **care**, D) **patrol**, and E) **fetch**.

3.6. Aggressiveness and “good parent” indexes

Calculating the aggressiveness and “good parent” indexes allowed a better understanding of the influence and investment of each sex in each stage studied in this work. As previously mentioned, the aggressiveness index was created from the total frequency of aggressive behaviours in each individual during the pair formation stage, while the “good parent” indexes were created using the total frequency of the most relevant parental behaviour in each reproductive stage. The results are shown below for each individual according to the stage in question (**Table 3.6.1**).

Table 3.6.1. Aggressiveness and “good parent” indexes for each individual in the respective stage.

Aggressiveness Index (pair formation)		Care Index (egg)		Patrol Index (recently hatched larvae)		Fetch Index (free-swimming larvae)	
Males	Females	Males	Females	Males	Females	Males	Females
1	0,8	0,568	0,76	0,714	0,2	0,579	0,55
1	1	0,569	0,809	0,471	0,227	0,149	0,577
0,2	0,5	0,655	0,7	0,667	0,333	0,198	0,47
1	0	0	0,143	0,188	0,174	0,263	0,691
-	-	0,25	0,75	-	-	0,265	0,462
-	-	0,909	0,818	-	-	-	-
-	-	0,2	0,455	-	-	-	-
-	-	0,55	0,676	-	-	-	-

In the pair formation stage, the aggressiveness index was calculated for 4 males and 4 females that posteriorly formed reproductive couples. The results showed that both males and females presented high aggressiveness values, and even though males seemed more aggressive towards other fish in the tank there were no significant differences between the sexes ($p = 0,239$; **Figure 3.6.1**). Moreover, the most relevant behaviour selected to integrate the “good parent” index during the egg stage was “care”. In this stage, the care index was calculated for 8 reproductive pairs. The results revealed that, during this phase, females invested more in this behaviour than males. The statistical analysis confirmed that there were significant differences between the sexes ($p = 0,012$; **Figure 3.6.2**). Concerning the newly hatched larvae stage, the most relevant behaviour used was “patrol”. The patrol index was assessed by quantifying the actions of 4 reproductive pairs. It was clear that males invested a lot more in this phase with this behaviour than females, and the statistical analysis revealed that these differences were, in fact, statistically significant ($p = 0,038$; **Figure 3.6.3**). Finally, the selected behaviour used in the “good

parent” index created for the free-swimming larvae stage was “fetch”. Here, 5 reproductive pairs were analysed for the calculation of the index. The results showed a significant statistical difference between the investment level of both sexes during this last reproductive phase, with females being more invested in fetching activity than males ($p = 0,019$; **Figure 3.6.4**).

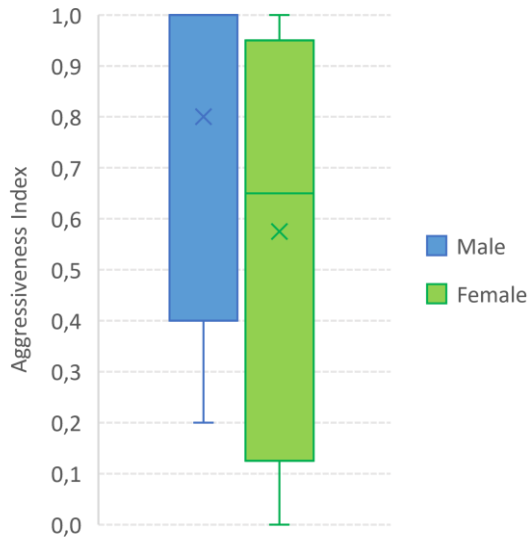


Figure 3.6.1. Aggressiveness index of *A. facetus* males and females during the pair formation stage (n = 4).

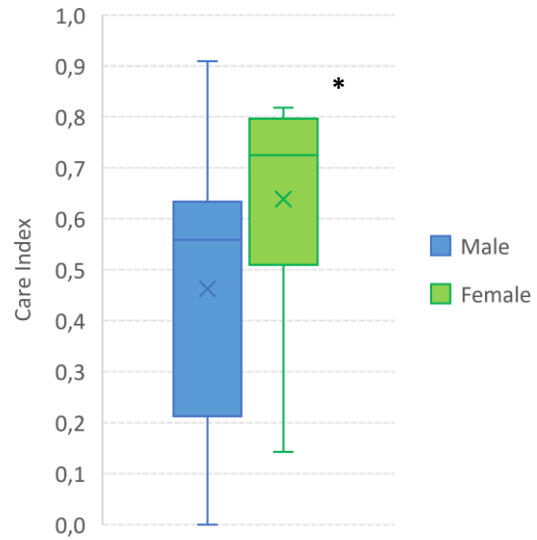


Figure 3.6.2. Care index of *A. facetus* males and females during the egg stage (n = 8).

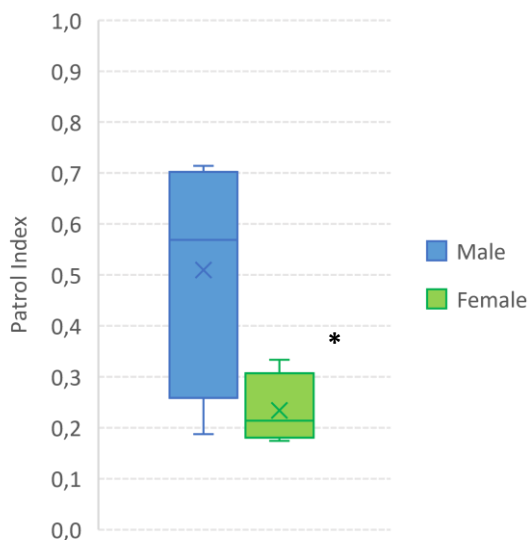


Figure 3.6.3. Patrol index of *A. facetus* males and females during the newly hatched larvae stage (n = 4).

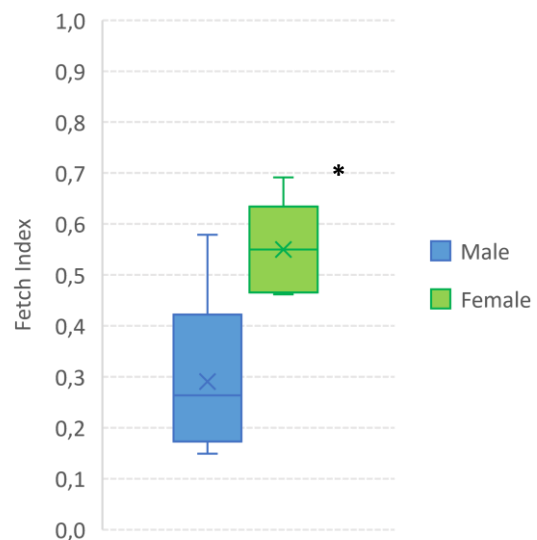


Figure 3.6.4. Fetch index of *A. facetus* males and females during the free-swimming larvae stage (n = 6).

3.7. Challenges to parental care

As previously mentioned, the response of each sex to the presence of an intruder throughout the different stages of the reproductive period was assessed to evaluate if the parents continued to care for the young ignoring the intruder or would aggressively attack the introduced individual. During the execution of these tests, it was registered a very different response of each reproductive pair to the introduction of the new individual. The two hypothesized responses occurred systematically in the same reproductive couples throughout multiple analyses. Nevertheless, the frequency of occurrence of threats and attacks in 5 minutes was calculated covering all stages and revealed no differences between sexes (**Figure 3.7.1**). The comparative statistical test significance values are presented hereafter (**Table 3.7.1**). As before, since all significance values were greater than 0,05 ($p\text{-value} > 0,05$), the null hypothesis was accepted, meaning the data was not significantly different.

Table 3.7.1. Significance values of threats and attacks.

	Behaviour	p-value
Threats	Frontal display	1
	Lateral display	0,317
Attacks	Strike	0,924
	Chase	1
	Bite	0,849
	Tail beating	0,317
	Mouth fighting	1

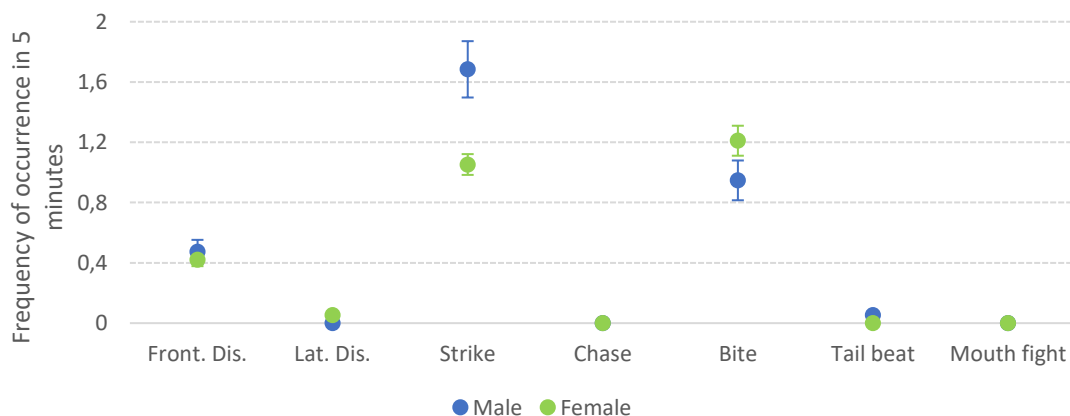


Figure 3.7.1. Average frequency (and respective standard deviation) of threats and attacks of *A. facetus* males and females in 5 minutes of observations during the whole reproductive period when exposed to an intruder ($n = 6$).

3.8. New behaviours described

At the end of the practical experiments and after behavioural analysis, it was possible to elaborate a complementary ethogram with new described behaviours for *Australoheros facetus* (Table 3.8.1). This ethogram includes the two reproductive behaviours seen during newly hatched larvae and free-swimming larvae phases and can be integrated into the used ethogram for the creation of this study (Baduy et al., 2017).

Table 3.8.1. Ethogram of *A. facetus* with the new described behaviours.

Class	Behaviour	Description
Social	Reproductive Egg/larvae transfer	Both parents gently move the eggs or the recently hatched larvae to different sites within their territory using their mouths.
	Larvae feeding	Both parents break the feed into small particles, drop them and let them sink for their young to eat.

3.9. Abiotic parameters

The registers of the measured abiotic parameters are presented below. Temperature registers (Figure 3.9.1), and dissolved oxygen and oxygen saturation (Figure 3.9.2) are represented according to their development in the course of the experience. As previously mentioned, in addition to these parameters, NH₃ and NH₄ concentrations were maintained between 0 and 0,6 mg/L in all tanks throughout the experimental activities.

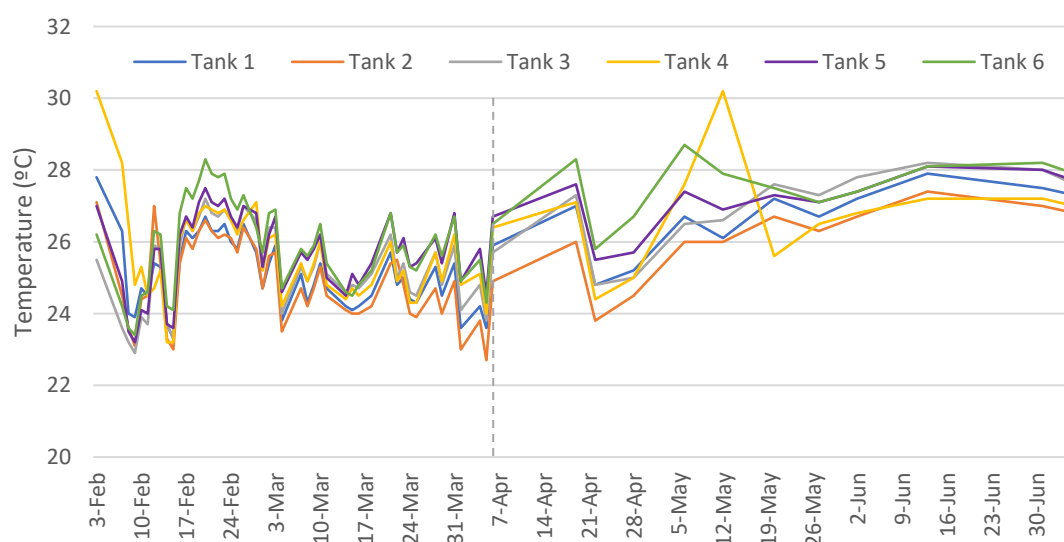


Figure 3.9.1. Temperature registers (°C) of all tanks throughout the experimental activities. The vertical line marks the transition date (6th of April) between daily and weekly measures.

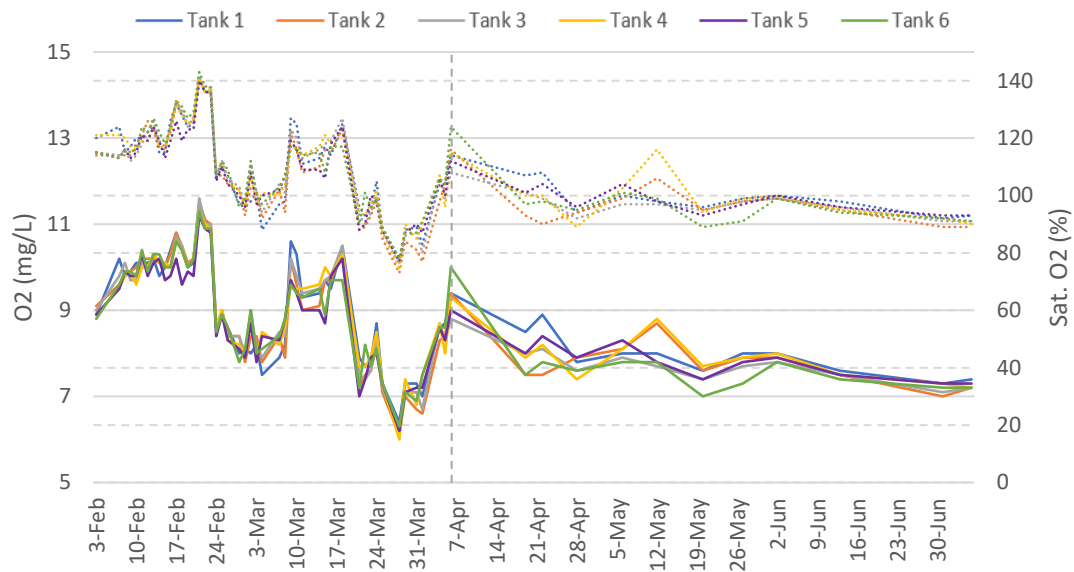


Figure 3.9.2. Dissolved O₂ (mg/L) and O₂ saturation (%) registers of all tanks throughout the experimental activities. The vertical line marks the transition date (6th of April) between daily and weekly measures.

4. Discussion

In the present work, the *A. facetus* parental behaviour was described and the possible roles of male and female parents were analysed for the first time, using frequencies of specific discrete events or time spent in activities related to the general care or defence of the offspring. The data obtained allowed us to stipulate that females are more involved in “caring” activities while males spend more time in “patrolling” activities, although both sexes share roles and engage in all the behaviours observed throughout the different stages of offspring development.

Previous studies by Baduy et al., (2017) showed that the formation of a reproductive couple in *A. facetus* results from the establishment of a social hierarchy achieved through multiple aggressions between individuals until a dominant fish emerges, to which other joins as a pair. In the same study, it was also shown that the calculated dominance index was directly related to fish size and that even minute sizes appeared to be relevant to determine the outcome of fish aggressive interactions. However, since “chanchito” does not show sexual dimorphism, it was not possible to track the specific interaction of either the dominant male and female or which would emerge as the primary dominant fish after the hierarchy formation. The actual process by which the pair is formed, and the mate chosen is still unclear, and in at least one case the dominant pair was composed of two females. Thus, size allied to other factors that relay physiological status or fitness (visual,

acoustical, or chemical communication) may determine preferences in coupling. In the present study, it was attempted the evaluation of the dominance index of each pair member by characterising all fish during hierarchy formation and after pair formation by performing a retroactive analysis of the interactions in which both the dominant male and female were involved.

Initially, both sexes showed aggressive behaviour towards other fish at the pair formation stage. Males seemed to invest more during this phase, with a higher frequency of aggressive behaviours, like striking and biting, however, these differences revealed to be not significant. Nevertheless, as previously mentioned, this higher aggression displayed by males is most likely related to territory establishment, a very typical characteristic of cichlids (Teresa and Gonçalves-de-Freitas, 2011). Curiously, the frequency of individuals that flee from attacks was higher in males too, which may indicate that, despite the lack of sexual dimorphism, there is gender recognition, and males fight more among them than with females. However, since the fish were not sacrificed and the sexes of all fish were not positively identified, it is also plausible that the number of females present in the tanks was low and consequently, so was the probability of interactions. Only the dominant male and female were conclusively identified.

Additionally, contrasting with the expected, the threatening behaviours (frontal and lateral display) were lower than aggressive behaviours. Threats are important agonistic elements that mark the territory and signal power or dominance, ensuring the defence of the area while requiring less risk of injuries (Clutton-Brock et al., 1979; Itzkowitz, 2010) and less energetic cost than aggressive behaviours (Ros et al., 2006). For these reasons, it was expected higher frequencies of threats showed by males in this early reproductive phase. Hubbard et al. (2017) demonstrated that a naive fish can discriminate between the odorants emitted by a submissive or dominant fish, which could help in the decision of whether to engage in an all-out fight with lethal consequences. However, despite this, the violence of hierarchy formation observed could be brutal and the injuries some fish suffered resulted in death. Both increased male aggressiveness and submission rates, and high occurrence of aggressive agonistic behaviours, can be explained by the fact that males may value the possession of a territory a lot more than females, mainly because they focus on attracting females to their territory for mating purposes if they fail in a first attempt to reproduce (Schwanck, 1989). This was especially noted in the present study, where the population of potential mates and territory available were limited by the

area/volume of the tanks and the fish present in each tank, at a variable female/male ratio. The high value of territoriality is particularly obvious in African cichlids, such as the Mozambique tilapia (*Oreochromis mossambicus*), in which males fight for the territory and attention of females. The winner forms a “harem” (an area of his territory where he keeps several females), chases away other males and is the one that fertilizes all the eggs (Oliveira and Almada, 1996; Oliveira and Canário, 2000). However, in *A. facetus*, territoriality is exercised by both the male and female, and contrasting with the Mozambique tilapia, both fish actively chase intruders away.

Our observations disclosed that, although dominant males display only slightly higher dominance and aggressiveness indexes than the dominant females, they appear to be more involved in the process of hierarchy formation, perhaps mainly because females may be recognised and spared in fights (Wong et al., 2004; Magurran and Ramnarine, 2005). To clearly define this, further studies should be carried out with fish undoubtedly identified in relation to their sex from time zero. So, social groups of size-matched fish with different ratios of males to females should be formed to determine if aggressive behaviours and the aim of territoriality are equally shared by both sexes.

Upon pair formation, couples initiated subtle courting behaviours and both members changed to bright yellow colouration with marked dark vertical stripes. Females and males engaged actively in digging behaviours, while the female prepared the area chosen to lay the eggs. This appeared to be a relatively short period that was not easily followed. During spawning, both fish were very close to each other, and the eggs were immediately fertilized.

At the egg stage, both sexes presented high investment in caring for the eggs. Still, females were more involved in these tasks than males, spending over twice the time on caring-related tasks than males. The higher investment of females in egg care is well correlated with evolutionary theories based on females’ low reproductive potential (Trivers, 1972). On the other hand, males performed vigilance of the territory and protection of the offspring. It is well documented that, in almost all biparental cichlids, females perform all brood care activities and remain closer to the eggs than males, which mainly defend the territory (Keenleyside, 1991b). This labour separation is very common during stationary offspring development among substrate brooding cichlids (Itzkowitz and Nyby, 1982; Townshend and Wootton, 1985; Rogers, 2010), and might be the result of the differential significance that parental roles represent to each sex (Trivers, 1972;

Teresa and Gonçalves-de-Freitas, 2011). Otherwise, the specialization in the execution of certain parental tasks may be explained by the different abilities that both sexes have to perform some particular tasks (Barlow, 1974; Schwanck, 1989; Awata and Khoda, 2004; Itzkowitz et al., 2005). For example, *A. facetus* males are usually bigger than females and, therefore, could be more effective in territorial defence since body size is an important feature to threaten and fight other fish. For this reason, males would choose to perform this activity rather than females. Although it was aimed to evaluate the relevance of “size vs gender” as proximate causes for specific behaviours, the random composition of the used reproductive couples resulted in a large bias towards pairs with larger males and smaller females, and the lack of enough independent data did not permit for this possibility.

Furthermore, the behaviour observed in the parents when transferring the eggs to different sites has already been reported in cichlids. It is known that some cichlid species use their mouths to perform various activities related to parental care. For example, some parents use their mouths to taste the laid eggs, receiving chemical signals that indicate a diseased or dead egg, thus stimulating their removal. Also, it is thought that this behaviour is used to turn the eggs as well, enhancing optimal embryo development by moving lipids around (Keenleyside, 1991a; Sopinka et al., 2009; Abate et al., 2021). Yet, the actual reasons why parents move their brood around are not well understood, but it has been hypothesized that it serves as an antipredator manoeuvre (Keenleyside, 1991a). Concerning offspring development duration, according to Abate et al. (2021), the exact duration of egg development observed in all tanks can be explained by the similar water temperature present in all tanks.

At the newly hatched larvae stage, it was noticed a decrease in care behaviour by both sexes, even though females were still involved a lot in this task. In contrast, the patrolling and fetching behaviours increased a lot in males and females, respectively. This transitional stage from eggs to larvae is exceptionally short but is when the young are more vulnerable to predators and other environmental factors, for example, strong water currents (Fitzgerald and Keenleyside, 1978; Nagoshi, 1987). This happens because recently hatched larvae are not able to swim freely yet so are unable to escape predators' attacks; additionally, it is in this phase that the larvae are more conspicuous due to the high fetching and regrouping of the young by the parents. In this sense, higher rates of close parental behaviours by both parents near the young would be expected. Nevertheless, the

adopted parental strategy of more vigilance of the territory to ensure a larger area free of predators still guarantees the safety of the young (Annett et al., 1999).

The observed phenomenon by which the larvae remain attached to the nest surface is explained by the presence of adhesive mucus threads found on the embryos' head region (Peters and Berns, 1982; Courtenay and Keenleyside, 1983). While stuck, the recently hatched larvae absorb the yolk sac (rich in nutrients) for several days before their sticky threads dissolve and allow them to swim freely (Barlow, 2002).

A drastic decrease in parental activity from around 30 - 40 % in previous stages to 20% was noticed at the free-swimming larvae stage, especially in males. Reductions in male parental care are very common in advanced parental stages among various animals and have been shown in shorebirds (*Charadriides*) (Székely and Reynolds, 1995). In this particular case, the loss of caregiving behaviours in shorebirds is attributed to possible benefits to males for additional mating opportunities (Reynolds and Székely, 1997). In *A. facetus*, the upcoming end of the parental care phase in advanced parental stages may influence the males' behaviour, causing a loss of interest in the last reproductive partner and arousing the preparation for a new courtship. For this reason, females invested a lot more during this phase, particularly in tasks previously executed by males, for example, parental hover and patrol. This shift in parental behaviour in females during advanced parental stages may predispose females to remain with the young until they are fully developed if males choose to desert (Keenleyside, 1991b; Teres and Gonçalves-de-Freitas, 2011).

Additionally, the occurrence of parents feeding their young has already been described in cichlids. Some cichlid species have been reported to feed their young by producing mucus or by exposing aquatic insects near the bottom for their young to eat (Hildenmann, 1959; Townshend and Wootton, 1985a, b; Wisenden et al., 1995; Schütz and Barlow, 1997; Balshine and Sloman, 2011). The phenomenon observed during this phase by which the parents break the feed into smaller particles and purposely drop them on the bottom so the larvae could access and eat them complements this series of reported parental/feeding behaviours in cichlids.

Regarding the response of each sex to the presence of an intruder throughout the different stages of the reproductive period, no differences between sexes were noted, although the frequency of attack behaviours like striking and biting revealed to be higher than the rest.

Additionally, the fact that the two hypothesized responses occurred systematically in the same reproductive couples throughout multiple analyses may happen due to the different natures observed in different reproductive pairs. It is known that animals have different “personalities” that influence their response to different scenarios (Briffa and Weiss, 2010). For instant, a shy couple may choose to seek refuge when faced with a threat, while a bolder couple may choose to face the opponent and attack him in order to defend their current territory. This is just one of the possible reasons to justify this pattern. Factors such as the sex and size of the intruder fish, the area available for territorial dispute, and even the model of the cubic box used to perform these tests can influence the behaviour of the couple.

In the end, the reproductive behaviour of *A. facetus* revealed to be typical of the substrate brooder neotropical cichlids. Characteristically, this group of cichlids perform relatively long parental care periods (Keenleyside, 1991a), resulting in a high investment devoted by the parents (Rogers, 2010). Throughout all the behaviour assessment work, males and females presented the expected reproductive and parental behaviour in each of the stages of the experiment. Biparental cichlids are extremely aggressive at the beginning of pair formation during territory establishment and extend their parental care of the offspring until the free-swimming larvae stage (Teresa and Gonçalves-de-Freitas, 2011). In this species, all the parental care is a cooperative activity between male and female individuals, so each member of the pair performs similar behaviours and alternates between rearing offspring and territorial defence during post-spawning stages (Teresa and Gonçalves-de-Freitas, 2011). However, this share of parental duties was not equal during the evaluation done in the present work. As in other biparental substrate-brooding cichlids (see Teresa and Gonçalves-de-Freitas, 2011), females were more invested in caring for the offspring, while males were more engaged with territory defence and protection of the young (Itzkowitz, 1984; Keenleyside, 1991b; Lavery and Reeb, 1994). This division of parental care behaviour between sexes may predispose females to remain with the young if males desert (Keenleyside, 1991b).

Notwithstanding, it is important to keep in mind that the endocrine system plays an important role in the control of reproductive and parental behaviour in vertebrates (Gans, 1996; Knapp et al., 1999; Reburn and Wynne-Edwards, 1999; Slater and Milinski, 1996). So, it remains to be seen what the observed physiological basis for the differential roles and variability between couples is. Baduy et al., (2017) showed that, during hierarchy

formation, there was a tendency for fish with low cortisol to become dominant. These fish were even capable of reducing further their cortisol when this status was achieved. Additionally, sexual steroids such as testosterone, estradiol, and 11-keto-testosterone were also involved in modulating aggression and in predicting territoriality in *A. facetus*. In the *Chiclosoma dimerus* species, males exhibiting pre-spawning activity showed 8.4 times higher 11-keto-testosterone and 5.63 times higher testosterone levels than those involved in guarding newly hatched larvae of free-swimming larvae, but no differences were observed in estradiol and cortisol levels among the different phases (Birba et al., 2015).

As said above, in most animals, hormones control several of the innate behaviours performed by each sex (Balshine and Sloman, 2011; Algera et al., 2017) and in many cases, the levels or rations of these hormones can trigger the activity of the brain areas and neuroamines related to rewarding. Hormones such as prolactin or arginine-vasotocin have been shown to increase in parental stages and appear to be involved in the onset and regulation of such behaviours (Balshine and Sloman, 2011). On the other hand, hormones related to stress such as cortisol may be deleterious and disrupt parental care. In this study, it was decided not to disrupt parental care by removing the parents from the tanks and collecting blood under anaesthesia. These procedures have been reported to affect pair bonding and parental behaviours in previous experiments and, thus, the choice was to follow as many couples and their offspring through as many stages as possible. However, in future experiments, it would be of extreme interest to have mirror couples that could be sampled at each stage while others are allowed to continue. This strategy may render relevant results but is also highly affected by couple variability and implies that a very large number of reproductive couples must be formed.

The evolution of parental behaviour and behavioural transitions between sexes are very important topics that contribute to fully understanding the influence of these characteristics in the invasive behaviour of cichlids. It was hypothesized by Gittleman (1981) and supported by Gross and Sargent (1985) that the direction of parental care in teleost fishes is from “no care” to “male only” to “biparental” to “female-only” to “no care”. In this sense, knowing that currently *A. facetus* presents typical biparental care of the offspring and it is common for males to desert in advanced parental stages, it is possible to predict that this species may evolve to a “female-only” parental strategy in the next decades. Since the high level of parental care in *A. facetus* is a key factor to the

invasive success of this species (Ribeiro et al., 2013; Baduy et al., 2020), it is expected to find shifts in the parental behaviour of this fish over time, which will highly influence its invasive character and change the way we deal with its spreading.

5. Conclusions

At the end of this work, it is certain to conclude that the parental care of *Australoheros facetus* must have a great influence on the recruitment, establishment and spread of this species, given the high investment of both males and females in promoting the survival of their young. Similar behaviours have already been reported in the wild and the high local abundance and dominance of this species clearly indicate this (Baduy et al, 2020). However, more complex, and intensive studies with a focus on “chanchito” behaviour evolution and endocrine regulation are necessary to fully understand the direction and influence of the parental behaviour of this fish in its evolutionary process as an invasive species. Since the endocrine system plays an important role in the control of reproductive and parental behaviour in vertebrates (Gans, 1996; Rosenblatt and Snowdon, 1996; Reburn and Wynne-Edwards, 1999) it is essential to include and describe the hormonal profiles of the animals during each reproductive stage and evaluate the influence of other abiotic variables like water temperature and photoperiod duration in the behaviour of this fish.

6. References

- Abate, M. E., Noakes, D. L. G., & Balshine, S. (2021). Parental Care in Cichlid Fishes. *The Behavior, Ecology and Evolution of Cichlid Fishes* (Vol. 40, pp. 541–586). Essay, Springer.
- Algera, D. A., Gutowsky, L. F., Zolderdo, A. J., & Cooke, S. J. (2017). Parental care in a stressful world: Experimentally elevated cortisol and brood size manipulation influence nest success probability and nest-tending behavior in a wild teleost fish. *Physiological and Biochemical Zoology*, 90(1), 85–95. <https://doi.org/10.1086/689678>
- Altmann, J. (1974). Observational study of behaviour: sampling methods. *Behaviour*, 49(3/4), 227-267.
- Alzamora, A., & Lobos, G. (2021). Assessing the threat of a South American cichlid on anurans in the Chilean Mediterranean Region. *BioInvasions Records*, 10(3), 669–682. <https://doi.org/10.3391/bir.2021.10.3.17>
- Annett, C. A., Pierotti, R., & Baylis, J. R. (1999). Male and female parental roles in the monogamous cichlid, *Tilapia mariae*, introduced in Florida. *Environmental Biology of Fishes*, 54(3), 283–293. <https://doi.org/10.1023/a:1007567028017>
- Arthington, A. H., & Mitchell, D. S. (1986). Aquatic invading species. *Cambridge University Press*, 34– 53.

- Baduy, F., Guerreiro, P. M., Canário, A. V., & Saraiva, J. L. (2017). Social organization and endocrine profiles of *Australoheros facetus*, an exotic freshwater fish in southern Portugal. *Acta Ethologica*, 20(3), 263–277. <https://doi.org/10.1007/s10211-017-0271-6>
- Baduy, F., Saraiva, J. L., Ribeiro, F., Canário, A. V., & Guerreiro, P. M. (2020). Distribution and risk assessment of potential invasiveness of *Australoheros Facetus* (Jenyns, 1842) in Portugal. *Fishes*, 5(1), 3. <https://doi.org/10.3390/fishes5010003>
- Balshine, S., & Buston, P. (2008). Cooperative behaviour in fishes. *Fish Behaviour*, 437–484. <https://doi.org/10.1201/b10757-16>
- Balshine, S., & Sloman, K. (2011). Parental care in fishes. *Academic Press*, San Diego, pp 670–677.
- Barbasch, T. A., Rueger, T., Srinivasan, M., Wong, M. Y., Jones, G. P., & Buston, P. M. (2020). Substantial plasticity of reproduction and parental care in response to local resource availability in a wild clownfish population. *Oikos*, 129(12), 1844–1855. <https://doi.org/10.1111/oik.07674>
- Barlow, G. W. (1974). Contrasts in social behaviour between Central American cichlid fishes and coral-reef surgeon fishes. *American Zoologist*, 14(1), 9–34. <https://doi.org/10.1093/icb/14.1.9>
- Barlow, G. W. (2000). The cichlid fishes: nature's grand experiment in evolution. *Perseus Publishing*, 335p.
- Barlow, G. W. (2002). The cichlid fishes: Nature's grand experiment in evolution. *Basic Books*.
- Beacham, J. L., & Newman, J. A. (1987). Social experience and the formation of dominance relationships in the pumpkinseed sunfish, *Lepomis gibbosus*. *Animal Behaviour*, 35(5), 1560–1563. [https://doi.org/10.1016/s0003-3472\(87\)80029-5](https://doi.org/10.1016/s0003-3472(87)80029-5)
- Bergmann, G. T., & Motta, P. J. (2005). Diet and morphology through ontogeny of the nonindigenous Mayan cichlid '*Cichlasoma* (Nandopsis)' *urophthalmus* (Gunther, 1862) in southern Florida. *Environmental Biology of Fishes*, 72(2), 205–211. <https://doi.org/10.1007/s10641-004-1480-1>
- Birba, A., Ramallo, M. R., Lo Nostro, F., Guimarães Moreira, R., & Pandolfi, M. (2015). Reproductive and parental care physiology of *Cichlasoma dimerus* males. *General and Comparative Endocrinology*, 221, 193–200. <https://doi.org/10.1016/j.ygcen.2015.02.004>
- Blumer, L. S. (1979). Male parental care in the bony fishes. *The Quarterly Review of Biology*, 54(2), 149–161. <https://doi.org/10.1086/411154>
- Blumer, L. S. (1982). A bibliography and categorization of bony fishes exhibiting parental care. *Zoological Journal of the Linnean Society*, 75(1), 1–22. <https://doi.org/10.1111/j.1096-3642.1982.tb01939.x>
- Breder, C. M. & Rosen, D. E. (1966). Modes of reproduction in fishes. *Natural History Press*, Garden City. 931pp.
- Briffa, M., & Weiss, A. (2010). Animal personality. *Current Biology*, 20(21). <https://doi.org/10.1016/j.cub.2010.09.019>
- Carecho, J., Baduy, F., Guerreiro, P. M., Saraiva, J. L., Ribeiro, F., & Veríssimo, A. (2018). Taxonomic re-evaluation of the non-native cichlid in Portuguese drainages. *Fishes in Mediterranean Environments*. <https://doi.org/10.29094/fishmed.2018.001>
- Casal, C. M. (2006). Global documentation of fish introductions: The growing crisis and recommendations for action. *Biological Invasions*, 8(1), 3–11. <https://doi.org/10.1007/s10530-005-0231-3>

- Chase, I. D., Tovey, C., Spangler-Martin, D., & Manfredonia, M. (2002). Individual differences versus social dynamics in the formation of animal dominance hierarchies. *Proceedings of the National Academy of Sciences*, 99(8), 5744–5749. <https://doi.org/10.1073/pnas.082104199>
- Clavero, M., & García-Berthou, E. (2005). Invasive species are a leading cause of animal extinctions. *Trends in Ecology & Evolution*, 20(3), 110–110. <https://doi.org/10.1016/j.tree.2005.01.003>
- Clutton-Brock, T. H., Albon, S. D., Gibson, R. M., & Guinness, F. E. (1979). The Logical Stag: Adaptive aspects of fighting in red deer (*Cervus elaphus L.*). *Animal Behaviour*, 27, 211–225. [https://doi.org/10.1016/0003-3472\(79\)90141-6](https://doi.org/10.1016/0003-3472(79)90141-6)
- Colléter, M., & Brown, C. (2011). Personality traits predict hierarchy rank in male rainbowfish social groups. *Animal Behaviour*, 81(6), 1231–1237. <https://doi.org/10.1016/j.anbehav.2011.03.011>
- Dewsbury, D. A. (1982). Dominance rank, copulatory behavior, and differential reproduction. *The Quarterly Review of Biology*, 57(2), 135–159. <https://doi.org/10.1086/412672>
- Ehrlich, P. R., Sodhi, N. S., & Simberloff, D. (2011). Invasive species. *Conservation biology for all*, 131–135. Oxford University Press.
- Fiszbein, A., Cánepa, M., Vázquez, G. R., Maggese, C., & Pandolfi, M. (2010). Photoperiodic modulation of reproductive physiology and behaviour in the cichlid fish *Cichlasoma dimerus*. *Physiology & Behaviour*, 99(4), 425–432. <https://doi.org/10.1016/j.physbeh.2009.11.017>
- Fitzgerald, G. J., & Keenleyside, M. H. (1978). The effects of numerical density of adult fish on reproduction and parental behavior in the convict cichlid fish *Cichlasoma nigrofasciatum* (Günther). *Canadian Journal of Zoology*, 56(6), 1367–1371. <https://doi.org/10.1139/z78-188>
- Fryer, G. & Iles, T. D. (1972). The cichlid fishes of the Great Lakes of Africa: their biology and evolution. *TFH Publications*, Neptune City. 16+641pp.
- Galis, F., & Metz, J. A. J. (1998). Why are there so many cichlid species? *Trends in Ecology & Evolution*, 13(1), 1–2. [https://doi.org/10.1016/s0169-5347\(97\)01239-1](https://doi.org/10.1016/s0169-5347(97)01239-1)
- Gallardo, B., Clavero, M., Sánchez, M. I., & Vilà, M. (2015). Global ecological impacts of invasive species in aquatic ecosystems. *Global Change Biology*, 22(1), 151–163. <https://doi.org/10.1111/gcb.13004>
- Gans, C. (1996). An overview of parental care among the Reptilia. *Advances in the Study of Behavior*, 145–157. [https://doi.org/10.1016/s0065-3454\(08\)60332-0](https://doi.org/10.1016/s0065-3454(08)60332-0)
- Gasith, A., & Resh, V. H. (1999). Streams in Mediterranean climate regions: Abiotic influences and biotic responses to predictable seasonal events. *Annual Review of Ecology and Systematics*, 30(1), 51–81. <https://doi.org/10.1146/annurev.ecolsys.30.1.51>
- Gittleman, J. L. (1981). The phylogeny of parental care in fishes. *Animal Behaviour*, 29(3), 936–941. [https://doi.org/10.1016/s0003-3472\(81\)80031-0](https://doi.org/10.1016/s0003-3472(81)80031-0)
- Goldberg, R. L., Downing, P. A., Griffin, A. S., & Green, J. P. (2020). The costs and benefits of paternal care in fish: A meta-analysis. *Proceedings of the Royal Society B: Biological Sciences*, 287(1935), 20201759. <https://doi.org/10.1098/rspb.2020.1759>
- Gomes-Ferreira, A., Ribeiro, F., Moreira da Costa, L., Cowx, I. G. & Collares-Pereira, M. J., (2005). Variability in diet and foraging behaviour between sexes and ploidy forms of the hybridogenetic *Squalius alburnoides* complex (Cyprinidae) in the Guadiana River basin, Portugal. *Journal of Fish Biology*, 66, 454–467.

- Goodwin, N. B., Balshine-Earn, S., & Reynolds, J. D. (1998). Evolutionary transitions in parental care in cichlid fish. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 265(1412), 2265–2272. <https://doi.org/10.1098/rspb.1998.0569>
- Greenwood, P. H. (1984). African cichlids and evolutionary theories. A. A. Echelle & I. Kornfield (eds.). *Evolution of fish species flocks*, 141-154. Univ. of Maine at Orono Press.
- Gross, M. R., & Sargent, R. C. (1985). The evolution of male and female parental care in fishes. *American Zoologist*, 25(3), 807–822. <https://doi.org/10.1093/icb/25.3.807>
- Hara, T. J. (1994). The diversity of chemical stimulation in fish olfaction and gustation. *Reviews in Fish Biology and Fisheries*, 4(1), 1–35. <https://doi.org/10.1007/bf00043259>
- Hildemann, W. H. (1959). A cichlid fish, *Symphysodon discus*, with unique nurture habits. *The American Naturalist*, 93(868), 27–34. <https://doi.org/10.1086/282054>
- Hubbard, P. C., Baduy, F., Saraiva, J. L., Guerreiro, P. M., & Canário, A. V. (2017). High olfactory sensitivity to conspecific intestinal fluid in the chameleon Cichlid *Australoheros facetus*: Could faeces signal dominance? *Journal of Fish Biology*, 90(5), 2148–2156. <https://doi.org/10.1111/jfb.13297>
- Huxley, J. S. (1938). Darwin's theory of sexual selection and the data subsumed by it, in the light of recent research. *The American Naturalist*, 72(742), 416–433. <https://doi.org/10.1086/280795>
- IBM Corp (2021). Online software released in 2021. IBM SPSS Statistics for Windows, Version 28.0. Armonk, NY: IBM Corp.
- Iles, T. D., & Holden, M. J. (1969). Bi-parental mouth brooding in *Tilapia galilaea* (Pisces, Cichlidae). *Journal of Zoology*, 158(3), 327–333. <https://doi.org/10.1111/j.1469-7998.1969.tb02151.x>
- Iriarte, J. A., Lobos, G. A., & Jaksic, F. M. (2005). Invasive vertebrate species in Chile and their control and monitoring by governmental agencies. *Revista Chilena De Historia Natural*, 78(1). <https://doi.org/10.4067/s0716-078x2005000100010>
- Itzkowitz, M. (2010). Sexual differences in offspring defense in a monogamous cichlid fish. *Zeitschrift Für Tierpsychologie*, 70(3), 247–255. <https://doi.org/10.1111/j.1439-0310.1985.tb00516.x>
- Itzkowitz, M., & Nyby, J. (1982). Field observations of parental behavior of the texas Cichlid *Cichlasoma cyanoguttatum*. *American Midland Naturalist*, 108(2), 364. <https://doi.org/10.2307/2425497>
- Itzkowitz, M., Santangelo, N., Cleveland, A., Bockelman, A., & Richter, M. (2005). Is the selection of sex-typical parental roles based on an assessment process? A test in the monogamous convict cichlid fish. *Animal Behaviour*, 69(1), 95–105. <https://doi.org/10.1016/j.anbehav.2003.12.027>
- Keenleyside, M. H. (1979). Diversity and adaptation in fish behaviour. *Zoophysiology*. <https://doi.org/10.1007/978-3-642-81374-0>
- Keenleyside, M. H. A. (1991a). *Cichlid fishes: behaviour, ecology and evolution*. Great Britain, Chapman & Hall, 376p.
- Keenleyside, M. H. A. (1991b). Parental care. *Cichlid fishes - behaviour, ecology and evolution* (ed. M. H. A. Keenleyside), pp. 192-208. London: Chapman & Hall.
- Keenleyside, M. H. A., & Courtenay, S. C. (1983). Wiggler-hanging: A response to hypoxia by brood-rearing *Herotilapia multispinosa* (Teleostei, Cichlidae). *Behaviour*, 85(3-4), 183–196. <https://doi.org/10.1163/156853983x00219>

- Klopfer, P. H. (1981). Origins of parental care. *Parental Care in Mammals*, 1–12. https://doi.org/10.1007/978-1-4613-3150-6_1
- Kohda, M., & Awata, S. (2004). Parental roles and the amount of care in a bi-parental substrate brooding cichlid: The effect of size differences within pairs. *Behaviour*, 141(9), 1135–1149. <https://doi.org/10.1163/1568539042664623>
- Kohda, M., Heg, D., Makino, Y., Takeyama, T., Shibata, J.-ya, Watanabe, K., Munehara, H., Hori, M., & Awata, S. (2009). Living on the wedge: Female control of paternity in a cooperatively polyandrous cichlid. *Proceedings of the Royal Society B: Biological Sciences*, 276(1676), 4207–4214. <https://doi.org/10.1098/rspb.2009.1175>
- Kolar, C. S., & Lodge, D. M. (2000). Freshwater nonindigenous species: interactions with other global changes. *Invasive species in a changing world*, 3-30.
- Kuwamura, T. (1986). Parental care and mating systems of cichlid fishes in Lake Tanganyika: A preliminary field survey. *Journal of Ethology*, 4(2), 129–146. <https://doi.org/10.1007/bf02348115>
- Lavery, R. J., & Reeb, S. G. (2010). Effect of mate removal on current and subsequent parental care in the convict cichlid (*Pisces: Cichlidae*). *Ethology*, 97(4), 265-277. <https://doi.org/10.1111/j.1439-0301.1994.tb01046.x>
- Linton, J. R., & Soloff, B. L. (1964). The physiology of the brood pouch of the male sea horse *Hippocampus erectus*. *Bulletin of Marine Science*, 14(1), 45-61.
- Loiselle, P. V. & Barlow, G. W. (1978). Do fishes lek like birds? E. S. Reese & F. J. Lighter (eds.). *Contrasts in behaviour*, 31-75. Wiley-Interscience, New York.
- Lowe S., Browne M., Boudjelas S., & De Poorter M. (2000). 100 of the World's Worst Invasive Alien Species. A selection from the Global Invasive Species Database. Published by The Invasive Species Specialist Group (ISSG) a specialist group of the Species Survival Commission (SSC) of the World Conservation Union (IUCN), 12pp. First published as a special lift-out in *Aliens* 12, December 2000. Updated and reprinted version: November 2004.
- Lowe-McConnell, R. H. (1959). Breeding behaviour patterns and ecological differences between *Tilapia* species and their significance for evolution within the genus *Tilapia* (*Pisces: Cichlidae*). *Proceedings of the Zoological Society of London*, 132(1), 1–30. <https://doi.org/10.1111/j.1469-7998.1959.tb05510.x>
- Mack, R. N., & Erneberg, M. (2002). The United States naturalized flora: Largely the product of deliberate introductions. *Annals of the Missouri Botanical Garden*, 89(2), 176. <https://doi.org/10.2307/3298562>
- Magalhães, M. F., Beja, P., Canas, C., & Collares-Pereira, M. J. (2002). Functional heterogeneity of dry-season fish refugia across a Mediterranean catchment: The role of habitat and predation. *Freshwater Biology*, 47(10), 1919–1934. <https://doi.org/10.1046/j.1365-2427.2002.00941.x>
- Magalhães, M.F. (1993). Effects of season and body size on the distribution and diet of the Iberian chub *Leuciscus pyrenaicus* in a lowland catchment. *Journal of Fish Biology*, 42, 875– 888.
- Magurran, A. E., & Ramnarine, I. W. (2005). Evolution of mate discrimination in a fish. *Current Biology*, 15(21). <https://doi.org/10.1016/j.cub.2005.10.034>
- McKaye, K. R. (1984). Behavioural aspects of cichlid reproductive strategies: patterns of territoriality and brood defence in Central American substratum spawners and African mouthbrooders. G. W. Potts & R. J. Wootton (eds.). *Fish reproduction: strategies and tactics*, 245-273. Academic Press, London.
- McKenna, J. J. (1979). Aspects of infant socialization, attachment, and maternal caregiving patterns among primates: A cross-disciplinary review. *Yrbk. Phys. Anthropol.*, 22, 250-286.

- Morse, D. H. (1974). Niche breadth as a function of social dominance. *The American Naturalist*, 108(964), 818–830. <https://doi.org/10.1086/282957>
- Nagoshi, M. (1987). Survival of broods under parental care and parental roles of the cichlid fish, *Lamprologus toae*, in Lake Tanganyika. *Japanese Journal of Ichthyology*, 34(1). <https://doi.org/10.1007/bf02904146>
- Oliveira, R. F., & Almada, V. C. (1996). Dominance hierarchies and social structure in captive groups of the Mozambique Tilapia “*Oreochromis mossambicus*” (Teleostei: Cichlidae). *Ethology Ecology & Evolution*, 8(1), 39–55. <https://doi.org/10.1080/08927014.1996.9522934>
- Oliveira, R. F., & Canário, A. V. (2000). Hormones and social behavior of cichlid fishes: a case study in the Mozambique tilapia. *Journal of Aquaculture and Aquatic Sciences*, Cichlid Research: State of the Art, 109-129.
- Panikkar, N. K., & Tampi, P. R. S. (1954). Mouth-breeding cichlid, *Tilapia mossambica* Peters. *Indian Journal of Fisheries*, 1 (1&2). pp. 217-230.
- Peters, H. M., & Berns, S. (1982). Die maulbrutpflege der Cichliden: Untersuchungen zur evolution eines Verhaltensmusters1. *Journal of Zoological Systematics and Evolutionary Research*, 20(1), 18–52. <https://doi.org/10.1111/j.1439-0469.1983.tb00548.x>
- Pires, A. M., Cowx, I. G., & Coelho, M. M. (1999). Seasonal changes in fish community structure of intermittent streams in the middle reaches of the Guadiana basin, Portugal. *Journal of Fish Biology*, 54, 235– 249.
- Pires, A. M., Cowx, I. G., & Coelho, M. M. (2000). Benthic macroinvertebrate communities of intermittent streams in the middle reaches of the Guadiana Basin (Portugal). *Hydrobiologia*, 435, 167– 175.
- Ramallo, M. R., Birba, A., Honji, R. M., Morandini, L., Moreira, R. G., Somoza, G. M., & Pandolfi, M. (2015). A multidisciplinary study on social status and the relationship between inter-individual variation in hormone levels and agonistic behavior in a neotropical cichlid fish. *Hormones and Behavior*, 69, 139–151. <https://doi.org/10.1016/j.yhbeh.2015.01.008>
- Ramallo, M. R., Morandini, L., Alonso, F., Birba, A., Tubert, C., Fiszbein, A., & Pandolfi, M. (2014). The endocrine regulation of cichlids’ social and reproductive behaviour through the eyes of the chanchita, *Cichlasoma dimerus* (Percomorpha; Cichlidae). *Journal of Physiology - Paris*, 108(2-3), 194–202. <https://doi.org/10.1016/j.jphysparis.2014.08.004>
- Reburn, C. J., & Wynne-Edwards, K. E. (1999). Hormonal changes in males of a naturally biparental and a uniparental mammal. *Hormones and Behavior*, 35(2), 163–176. <https://doi.org/10.1006/hbeh.1998.1509>
- Ribeiro, F. (2008). Patterns and Processes of Fish Invasions in Iberian Rivers: The Lower Guadiana Drainage as a Case Study. *Doctoral dissertation*, University of Lisbon, 216 pp.
- Ribeiro, F., Magalhães, M. F., & Collares-Pereira, M. J. (2013). Spatial and temporal variation in assemblage structure of fish larvae in Mediterranean-type streams: Contrasts between native and non-native species. *Environmental Biology of Fishes*, 96(4), 467–480. <https://doi.org/10.1007/s10641-012-0030-5>
- Ribeiro, F., Orjuela, R. L., Magalhães, M. F., & Collares-Pereira, M. J. (2007). Variability in feeding ecology of a South American cichlid: A reason for successful invasion in Mediterranean-type rivers? *Ecology of Freshwater Fish*, 16(4), 559–569. <https://doi.org/10.1111/j.1600-0633.2007.00252.x>

- Rican, O., & Kullander, S. O. (2006). Character- and tree-based delimitation of species in the 'Cichlasoma' facetum Group (*Teleostei*, Cichlidae) with the description of a new genus. *Journal of Zoological Systematics and Evolutionary Research*, 44(2), 136–152. <https://doi.org/10.1111/j.1439-0469.2005.00347.x>
- Ripley, J. L., & Lobel, P. S. (2005). Reproductive behaviour of the Lake Malawi cichlid fish, *Tramitichromis intermedius*. *Environmental Biology of Fishes*, 73(2), 171–180. <https://doi.org/10.1007/s10641-004-5567-5>
- Rogers, W. (2010). Parental Investment and division of labor in the Midas cichlid (*Cichlasoma citrinellum*). *Ethology*, 79(2), 126–142. <https://doi.org/10.1111/j.1439-0310.1988.tb00706.x>
- Ros, A. F. H., Becker, K., & Oliveira, R. F. (2006). Aggressive behaviour and energy metabolism in a cichlid fish, *Oreochromis Mossambicus*. *Physiology & Behavior*, 89(2), 164–170. <https://doi.org/10.1016/j.physbeh.2006.05.043>
- Rosenblatt, J. S., & Snowdon, C. T. (1996). Parental care: Evolution, mechanisms and adaptive significance. *Academic Press*.
- Royle, N. J., Smiseth, P. T., & Kölliker Mathias. (2012a). Preface. In *The evolution of parental care*. Oxford University Press.
- Royle, N. J., Smiseth, P. T., Kölliker Mathias, Klug, H., Alonzo, S. H., & Bonsall, M. B. (2012b). Theoretical foundations of parental care. In *The evolution of parental care* (pp. 21–39). essay, Oxford University Press.
- Ruiz, V., & Figueroa, R. (2004). Fecundidad de *Cichlasoma facetum* (Jenyns, 1842) (*Pisces*, Cichlidae) en la Laguna Grande de San Pedro, Concepción – Chile. *Boletín de la Sociedad de Biología de Concepción*, 75, 103– 105.
- Ruiz, V., Moyano, H., & Marchant, M. (1992). Aspectos biológicos del pez exótico *Cichlasoma facetum* (Jenyns, 1842) (*Pisces*, Cichlidae) en aguas dulces de Concepción. *Boletín de la Sociedad de Biología de Concepción*, 63, 193– 201.
- Schütz, M., & Barlow, G. W. (1997). Young of the Midas cichlid get biologically active nonnutrients by eating mucus from the surface of their parents. *Fish Physiology and Biochemistry*, 16(1), 11–18. <https://doi.org/10.1007/bf00004536>
- Schwanck, E. J. (1989). Parental care of *Tilapia mariae* in the field and in aquaria. *Environmental Biology of Fishes*, 24(4), 251–265. <https://doi.org/10.1007/bf00001399>
- Sefc, K. M. (2011). Mating and parental care in Lake Tanganyika's cichlids. *International Journal of Evolutionary Biology*, 2011, 1–20. <https://doi.org/10.4061/2011/470875>
- Shuker, D. M., & Simmons, L. W. (2014). Parental care. In *The evolution of Insect Mating Systems*. essay, OUP.
- Sopinka, N. M., Fitzpatrick, J. L., Desjardins, J. K., Stiver, K. A., Marsh-Rollo, S. E., & Balshine, S. (2009). Liver size reveals social status in the African cichlid *Neolamprologus pulcher*. *Journal of Fish Biology*, 75(1), 1–16. <https://doi.org/10.1111/j.1095-8649.2009.02234.x>
- Sternecker, K., Cowley, D. E., & Geist, J. (2012). Factors influencing the success of Salmonid egg development in river substratum. *Ecology of Freshwater Fish*, 22(2), 322–333. <https://doi.org/10.1111/eff.12020>
- Sylvain, F., & Derome, N. (2017). Vertically and horizontally transmitted microbial symbionts shape the gut microbiota ontogenesis of a skin-mucus feeding discus fish progeny. *Scientific Reports*, 7(1). <https://doi.org/10.1038/s41598-017-05662-w>
- Székely, T., & Reynolds, J. D. (1995). Evolutionary transitions in parental care in shorebirds. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 262(1363), 57-64.

- Teresa, F. B., & Gonçalves-de-Freitas, E. (2011). Reproductive behavior and parental roles of the cichlid fish *Laetacara Araguaiae*. *Neotropical Ichthyology*, 9(2), 355–362. <https://doi.org/10.1590/s1679-62252011005000018>
- Townshend, T. J., & Wootton, R. J. (1985). Adjusting parental investment to changing environmental conditions: The effect of food ration on parental behaviour of the convict cichlid, *Cichlasoma nigrofasciatum*. *Animal Behaviour*, 33(2), 494–501. [https://doi.org/10.1016/s0003-3472\(85\)80072-5](https://doi.org/10.1016/s0003-3472(85)80072-5)
- Trivers, R. L. (1972). Parental investment and sexual selection. *Sexual Selection & the Descent of Man*, Aldine de Gruyter, New York, 136-179.
- Trivers, R. L. (1974). Parent-offspring conflict. *American Zoologist*, 14(1), 249–264. <https://doi.org/10.1093/icb/14.1.249>
- Tubert, C., Lo Nostro, F., Villafañe, V., & Pandolfi, M. (2012). Aggressive behaviour and reproductive physiology in females of the social cichlid fish *Cichlasoma dimerus*. *Physiology & Behavior*, 106(2), 193–200. <https://doi.org/10.1016/j.physbeh.2012.02.002>
- Vieira, A. B., Salvador-Jr., L. F., Melo, R. M., Santos, G. B., & Bazzoli, N. (2009). Reproductive Biology of the peacock Bass *Cichla piquiti* (Perciformes: Cichlidae), an exotic species in a Neotropical Reservoir. *Neotropical Ichthyology*, 7(4), 745–750. <https://doi.org/10.1590/s1679-62252009000400024>
- Vincent, A., Ahnesjö, I., Berglund, A., & Rosenqvist, G. (1992). Pipefishes and seahorses: Are they all sex role reversed? *Trends in Ecology & Evolution*, 7(7), 237–241. [https://doi.org/10.1016/0169-5347\(92\)90052-d](https://doi.org/10.1016/0169-5347(92)90052-d)
- Wickler, W. (2010). Vergleich des ablaichverhaltens einiger paarbildender sowie nichtpaarbildender pomacentriden und Cichliden: (Pisces: Perciformes). *Zeitschrift Für Tierpsychologie*, 24(4), 457–470. <https://doi.org/10.1111/j.1439-0310.1967.tb01239.x>
- Wisenden, B. D., Lanfranconi-Izawa, T. L., & Keenleyside, M. H. (1995). Fin digging and leaf lifting by the convict cichlid, *Cichlasoma nigrofasciatum*: Examples of parental food provisioning. *Animal Behaviour*, 49, 623–631. [https://doi.org/10.1016/0003-3472\(95\)90037-3](https://doi.org/10.1016/0003-3472(95)90037-3)
- Wong, B. B., Keogh, J. S., & Jennions, M. D. (2004). Mate recognition in a freshwater fish: Geographical distance, genetic differentiation, and variation in female preference for local over foreign males. *Journal of Evolutionary Biology*, 17(3), 701–708. <https://doi.org/10.1046/j.1420-9101.2003.00651.x>
- Wootton, R. J., & Townshend, T. J. (1985). Variation in the mating system of a biparental cichlid fish, *Cichlasoma panamense*. *Behaviour*, 95(3-4), 181–197. <https://doi.org/10.1163/156853985x00118>