UNIVERSITÉ SORBONNE PARIS NORD

ÉCOLE DOCTORALE GALILÉE

UNITÉ DE RECHERCHE
Laboratoire d'Éthologie Expérimentale et Comparée

Thèse pour obtenir le grade de
Docteur de l'Université Sorbonne Paris Nord
Discipline **Éthologie**

Présentée et soutenue publiquement par
Pooja RADHAKRISHNAN
Le **16 décembre 2024**

Titre:

Shaped by Society
Reaction Norm Plasticity in Growth and Sex Allocation in Ophryotrocha puerilis

Directrice de thèse :
Prof. Maria Cristina LORENZI

Composition du jury

Janet LEONARD	Rapporteure, Institute of Marine Sciences, UC Santa Cruz
Chiara BENVENUTO	Rapporteure, School of Science, Engineering & Environment, University of Salford
Heiko RÖDEL	Examinateur, LEEC, Université Sorbonne Paris Nord
Joris KOENE	Examinateur, A-LIFE, Vrije Universiteit
Sergi TABOADA	Examinateur, MNCN-CSIC & Universidad Complutense de Madrid
Maria Cristina LORENZI	Directrice de thèse, LEEC, Université Sorbonne Paris Nord

“Science may be described as the art of systematic oversimplification.”

— *Karl Popper*

“If you can approach the world's complexities, both its glories and its horrors, with an attitude of humble curiosity, acknowledging that however deeply you have seen, you have only scratched the surface, you will find worlds within worlds, beauties you could not heretofore imagine, and your own mundane preoccupations will shrink to proper size, not all that important in the greater scheme of things.”

— *Daniel C. Dennett*

Abstract

Sex allocation theory predicts that the optimal timing of sex change in sequential hermaphrodites depends on both individual condition and the social environment. While research has traditionally focused on adults, the influence of early-life social interactions on sex allocation and growth dynamics remains poorly understood. This thesis investigates how social cues and size relationships among juveniles shape the timing of sex change and the emergence of size hierarchies in the marine annelid *Ophryotrocha puerilis*.

I found evidence of socially mediated sex change: juveniles exposed to adults delayed sex change, whereas isolated individuals changed sex early. In the absence of adults, size-matched juveniles negotiated sex roles and developed directly into opposite sexes, forming stable breeding pairs, supporting what we propose as the *matchmaking hypothesis*. I also found that juveniles did not follow a predetermined growth trajectory but instead exhibited strategic growth modulation, an adaptive response previously documented only in vertebrates, in which individuals adjust their growth relative to close competitors. The resulting size hierarchies resolved reproductive conflict; consequently, the onset of reproduction either weakened or stabilized the hierarchy depending on the intensity of competition. These findings demonstrate that both sex allocation and growth are socially mediated processes sensitive to cues early in development, and I discuss the adaptive value of such early-life social sensitivity within the ecological context and the opportunistic life history of *O. puerilis*.

In addition to the empirical work, I review the literature on *O. puerilis*, synthesizing nearly a century of research on the environmental, genetic, pheromonal, and social factors influencing sex change and reversal, and highlighting that the species has received little attention over the past three decades, despite its historical reputation as the ‘*Drosophila*’ of polychaetes.

Key Words: phenotypic plasticity, strategic growth, developmental plasticity, *Ophryotrocha puerilis*, sequential hermaphroditism, sex change

Résumé

La théorie de l'allocation sexuelle prédit que le moment optimal du changement de sexe chez les hermaphrodites séquentiels dépend à la fois de l'état individuel et de l'environnement social. Alors que la recherche s'est traditionnellement concentrée sur les adultes, l'influence des interactions sociales précoces sur l'allocation sexuelle et la dynamique de croissance reste mal comprise. Cette thèse étudie comment les signaux sociaux et les relations de taille entre les juvéniles influencent le moment du changement de sexe et l'émergence de hiérarchies de taille chez l'annélide marin *Ophryotrocha puerilis*.

J'ai trouvé des preuves d'un changement de sexe médié socialement : les juvéniles exposés à des adultes retardaient leur changement de sexe, tandis que les individus isolés changeaient de sexe précocement. En l'absence d'adultes, des juvéniles de taille similaire ont négocié leurs rôles sexuels et se sont développés directement en sexes opposés, formant des couples reproducteurs stables, ce qui soutient ce que nous proposons comme l'« hypothèse du matchmaking ». J'ai également constaté que les juvéniles ne suivaient pas une trajectoire de croissance prédéterminée, mais manifestaient plutôt une modulation stratégique de la croissance, une réponse adaptative documentée jusqu'ici uniquement chez les vertébrés, dans laquelle les individus ajustent leur croissance en fonction de celle de leurs concurrents proches. Les hiérarchies de taille qui en résultent ont permis de résoudre les conflits reproducteurs ; par conséquent, le début de la reproduction affaiblissait ou stabilisait la hiérarchie selon l'intensité de la compétition. Ces résultats montrent que l'allocation sexuelle et la croissance sont des processus médiés socialement et sensibles aux signaux précoces, et je discute de la valeur adaptative de cette sensibilité sociale précoce dans le contexte écologique et le mode de vie opportuniste de *O. puerilis*.

En complément du travail empirique, je passe en revue la littérature sur *O. puerilis*, en synthétisant près d'un siècle de recherches sur les facteurs environnementaux, génétiques, phéromonaux et sociaux influençant le changement et l'inversion de sexe, tout en soulignant que l'espèce a reçu peu d'attention au cours des trois dernières décennies, malgré sa réputation historique de « *Drosophile* » des polychètes.

Mots-clés : plasticité phénotypique, croissance stratégique, plasticité développementale, *Ophryotrocha puerilis*, hermaphrodisme séquentiel, changement de sexe

Acknowledgements

I am grateful to my advisor, Prof. Maria Cristina Lorenzi. Our scientific discussions were always an enthusiastic tennis match of counter-arguments, and she had the singular ability to frame every new question as a murder mystery—two detectives staring at a graph, asking, “But what, precisely, is the adaptive value of this behaviour?” Thank you for the independent thought and the patience as I took my first steps in academia. My favourite memory is still a small gathering at my apartment with Franco, Kenzy, Shweta, and Cristina, where, in true biologist fashion, we launched into an in-depth, matter-of-fact conversation about the diversity of cockroaches from our respective countries. It felt like an appropriate summary of the last four years.

I thank my entire defence committee: Prof. Janet Leonard, Dr. Chiara Benvenuto, Dr. Joris Koene, Dr. Sergi Taboada, and Prof. Heiko Rödel. I owe particular thanks to my rapporteurs, Prof. Leonard and Dr. Benvenuto, whose careful reading and valuable commentary significantly improved the final text of this dissertation. I remain indebted to Prof. David Hosken and Prof. Nina Wedell, my MSc advisors, whose encouragement was the exact catalyst required to pursue this PhD.

It is a distinct honour to have built, even in this small way, upon the foundational work on *Ophryotrocha puerilis*. The realization that struck me, looking to reach out to those pioneering scientists, was the simple fact that some of them are no longer with us. It is a quiet bitterness to know I will never get to discuss the questions that arose from their original work on this remarkable little worm, but the contributions stand, a testament to the enduring, indifferent legacy of science.

To my friends in Bombay, Cornwall, and Paris (Kamakshi, Pranjali, Saumya, Hiro, Désiré, and Stéphane), for the invaluable perspective you offered me whenever I found myself catastrophizing. The many glasses of wine, the long drives through Paris, the homey, endless evenings spent cooking and talking: these were not sources of comfort, but necessary life support.

And, most importantly, my family. They remain my biggest cheerleaders and my rescue from the heartbreaks of academic life, though their understanding of my research remains fluid (some days I am a microbiologist; others, a conservation biologist). This kind of support is nothing new: my mother shopped with me for band T-shirts during my goth phase. Thank you, Mom, for the interest in the research, however complex, and for the consistently fitting Malayalam proverb for every situation, however niche. To my dad, the most reliable person in my life, who has the sense of humour and the perfect anecdote for everything. To my brother, whose influence remains total: from insisting on wearing a boy’s uniform to school, to quitting Bharatnatyam and pretending to like Slipknot just because he did. The appreciation, I note, is already becoming excessive for an (adopted) sibling.

Finally, to the mentors I've never met whose books left their indelible mark: Christopher Hitchens, Richard Dawkins, Sam Harris, David Deutsch, and Joseph Goldstein. I would often imagine Hitch shaking his head as I Googled, at 3 a.m., "Do giraffes sleep lying down?" instead of writing. It didn't work, though yes, they do.

My final thanks go to the more unexpected sources of grace: Ann-Marie, the cashier at the Leclerc, who provided inexplicable comfort on days I needed it most; the bakery with the perfect *entremets*; and the feral cats in the student accommodation who kept me company on long writing nights.

This journey was not perfect. If I could do it again, I would, and would likely change almost everything. Perhaps that is the point of it all. As the poet David Whyte once said, "If you have no regrets, where have you been all your life?"

Serment relatif à l'intégrité scientifique

En présence de mes pairs,

Parvenue à l’issue de mon doctorat en éthologie et ayant ainsi pratiqué, dans ma quête du savoir, une recherche scientifique exigeante fondée sur la rigueur intellectuelle, la réflexivité éthique et le respect des principes de l’intégrité scientifique, je m’engage, pour ce qui dépendra de moi, à maintenir, tout au long de ma carrière professionnelle, quel qu’en soit le secteur ou le domaine d’activité, une conduite intègre dans mon rapport au savoir, dans mes méthodes et dans mes résultats.

Oath of Scientific Integrity

In the presence of my peers,

Having completed my Doctorate in Ethology, and having thus engaged, in my pursuit of knowledge, in rigorous scientific research founded on intellectual integrity, ethical reflection, and respect for the principles of scientific integrity, I pledge, insofar as it depends on me, to maintain an honest and ethical approach to knowledge, to my methods, and to my results throughout my professional career, regardless of the sector or field of activity.

Pooja Radhakrishnan

Lu et Approuvé,
À Villetaneuse, le 16 décembre 2024

Table of Contents

CHAPTER 1

INTRODUCTION

Separate Sexes, Separate Strategies

The dynamics of competitive males and choosy females

Males of the nursery spider *Pisaura mirabilis* present potential mates with silk-wrapped insects, hoping they may be spared from cannibalism (Hansen et al., 2008). Even when spared, females often abscond, stealing the nuptial gift. Luckily for the male, that's not where this story ends. Males have evolved an adaptive counter-strategy known as thanatosis, or playing dead. When a female attempts to flee mid-copulation, the male feigns death, and only after she settles does he 'come back to life' and resume copulation (Hansen et al., 2008).

This dynamic of competitive males and choosy females recurs widely across the animal kingdom. The peacock *Pavo cristatus* tirelessly displays its flamboyant tail in an orchestrated dance to impress nearby peahens, while the females nonchalantly evade his eager advances. Male pufferfish *Torquigener sp.* spend days meticulously sculpting intricate geometric sand patterns as nests, presumably to attract mates (Kawase et al., 2013). Aggressive fights between males for access to harems occur across species, from animals as large as the northern elephant seal *Mirounga angustirostris* (Le Boeuf, 1974) to the minute watermite *Unionicola formosa* (Enquist & Leimar, 1990). Such striking asymmetries in courtship effort form a consistent pattern across species, raising a key question: why are males typically so competitive while females remain selective?

The answer lies in a fundamental biological asymmetry: the difference in gamete size and number between the sexes, known as anisogamy (Schärer et al., 2012). Anisogamy is thought to have arisen from isogamy through disruptive selection favouring two distinct strategies: the production of numerous, small gametes (to maximize fertilization rate) and the production of large, well-provisioned gametes (to maximize zygote survival) (Parker et al., 1972). The underlying theoretical models for the origin of anisogamy and the consequent evolution of sex roles remain an active and evolving area of research (Janicke et al., 2016; Gorelick et al., 2017; Lehtonen & Parker, 2024).

The unequal investment inherent to anisogamous reproduction often extends beyond fertilization. Females frequently invest disproportionately in reproduction and offspring care, incurring higher overall costs of mating (Janicke et al., 2016; Lehtonen & Parker, 2024). This differential investment of resources drives females to be more selective, while males experience intense intrasexual competition for access to females, a dynamic that forms the basis of sexual selection.

Sexual selection is the evolutionary process that favours traits enhancing reproductive success, increasing their frequency in a population even when they incur survival costs (Andersson, 1994). It ultimately drives the evolution of sophisticated courtship behaviours and tactics such as the spider's nuptial gifting and thanatosis, flamboyant secondary sexual characteristics like the peacock's tail, extended phenotypes like the pufferfish's laborious nest building, and traits that enhance physical dominance as observed in the haremic elephant seals and watermites, even extending to post-copulatory mechanisms such as sperm competition and female cryptic choice. Therefore, sexual selection, originating from anisogamy and often resulting in the dynamics of males outcompeting rivals for access to females, and choosy females exerting strong preferences on eager males, shapes the evolution of reproductive strategies that amplify male competitiveness and female selectivity, ultimately reinforcing sex-specific traits and behaviours (Schärer et al., 2012). Importantly, the fundamental conflicts arising from anisogamy, such as competition for mates and selective mate choice, also occur in hermaphroditic animals.

Hermaphrodites: One Body, Two Strategies?

Optimizing both sexual roles is a balancing act

If male reproductive strategies generally evolve to compete for mating opportunities by either outcompeting rivals or attracting females, and female strategies generally evolve to maximize reproductive success by being selective, how do these seemingly conflicting strategies manifest in hermaphrodites, which can function as both sexes? Hermaphroditic animals, like the gonochoristic (separate-sex) species mentioned above, are also generally anisogamous, although rare instances of isogamy occur in basal invertebrate lineages (Parker et al., 1972; Lehtonen et al., 2016). Hermaphrodites have the unique ability to fulfil both sexual roles by producing both gamete types, either simultaneously (simultaneous hermaphrodites) or at different life stages (sequential hermaphrodites).

Nevertheless, the potential for sexual selection to give rise to sex-specific behaviours still applies (Leonard, 2006, 2013; Lorenzi & Sella, 2008; Schärer et al., 2012). Hermaphrodites, like individuals in gonochoristic species, experience variance in reproductive success (Lorenzi & Sella, 2008). As a result, they may display both male-typical competitive behaviours and female-typical selective behaviours depending on their current sex allocation, which is influenced primarily by the social environment, as the relative reproductive advantage of adopting either male or female roles shifts with changes in group composition (Lorenzi & Sella, 2008).

For instance, simultaneous hermaphrodites, even without dramatic sexual dimorphism, may exhibit sex-specific traits and mating behaviours based on their sex allocation in a given mating encounter (Lorenzi & Sella, 2008; Schärer et al., 2012). In the simultaneous hermaphrodite *Ophryotrocha diadema*, individuals in larger social groups exhibit a more male-biased sex allocation, engaging in significantly more male-typical behaviours such as mate-searching (Picchi & Lorenzi, 2019) and even experiencing higher levels of oxidative stress

(Lorenzi et al., 2023). In contrast, individuals in monogamous pairs exhibit more female-biased sex allocation, investing significantly more in female-typical behaviours such as parental care (Picchi & Lorenzi, 2019). In sequential hermaphrodites, where sexual dimorphism is generally more evident, these sex-specific behaviours may be more pronounced. For instance, in the haremic bluebanded goby *Lythrypnus dalli*, an alpha female that delays changing sex in the presence of a dominant male shows a dramatic increase in male-typical aggressive behaviours toward other females in the harem within minutes of achieving dominant male status, days before gonadal transformation is complete (Black et al., 2011). Therefore, sexual selection should drive the evolution of adaptive strategies that allow hermaphrodites to not only compete for mates and exert mate choice, but also to maximize fitness through both male and female reproductive functions (Leonard, 2006, 2013; Lorenzi & Sella, 2008; Schärer et al., 2012).

Thesis Overview

In this thesis, I explore the reproductive strategies of *Ophryotrocha puerilis*, a marine annelid worm that is a protandrous sequential hermaphrodite capable of bidirectional sex change. **Chapter 2** provides an overview of hermaphroditism, with a focus on sex allocation theory and the evolutionary conditions that favour different sexual systems. **Chapter 3** examines the role of phenotypic plasticity in sex allocation and discusses how environmental and social cues can influence the timing and direction of sex change.

In **Chapter 4**, I review the extensive literature on *O. puerilis*, summarizing nearly a century of research on the environmental, genetic, pheromonal, and social factors that influence sex change and reversal in this species. Despite this long research history, studies on sex allocation in *O. puerilis* have been largely absent over the past three decades, leaving important gaps in our understanding of how social interactions shape reproductive strategies.

The empirical core of this thesis, presented in **Chapters 5** and **6**, aims to reignite scientific interest in this fascinating species by addressing these gaps through new experiments on the social mediation of sex change and strategic growth dynamics. In **Chapter 5**, we investigate whether, and by what mechanisms, initially age- and size-matched juveniles establish size differences and form size hierarchies that may influence their position within social groups. Because relative size is a likely determinant of phenotypic sex in this species, we analyse and compare growth curve parameters of fast- and slow-growing individuals to assess how growth trajectories are modulated by social competition for the female role.

In **Chapter 6**, we test whether sex change in *O. puerilis* is socially mediated. Age- and size-matched juveniles were exposed to a range of social environments to determine how the presence, absence, and number of conspecifics influence the timing and body size at which sex change occurs.

CHAPTER 2

HERMAPHRODITISM

Excluding insects, approximately 30% of metazoan species are hermaphroditic (Leonard, 2018). Among vertebrates, however, hermaphroditism is rare, occurring in only about 1% of species, most of which are fish (Pla et al., 2022). As described earlier, there are two principal forms of hermaphroditism: *simultaneous hermaphrodites*, which produce both types of gametes at the same time, and *sequential hermaphrodites*, which produce one type of gamete when they are young and small, typically switching to the other as they grow older and larger, when social or environmental conditions permit (West, 2009).

2.1. **Sex Allocation**

Sex allocation refers to the relative partitioning of reproductive investment between male and female functions (Charnov, 1982; West, 2009). In simultaneous hermaphrodites, sex allocation can be skewed towards one sexual function or the other, whereas in sequential hermaphrodites, it is reflected in the timing of sex change, i.e., the proportion of life spent as a male versus a female (Fig. 2.1).

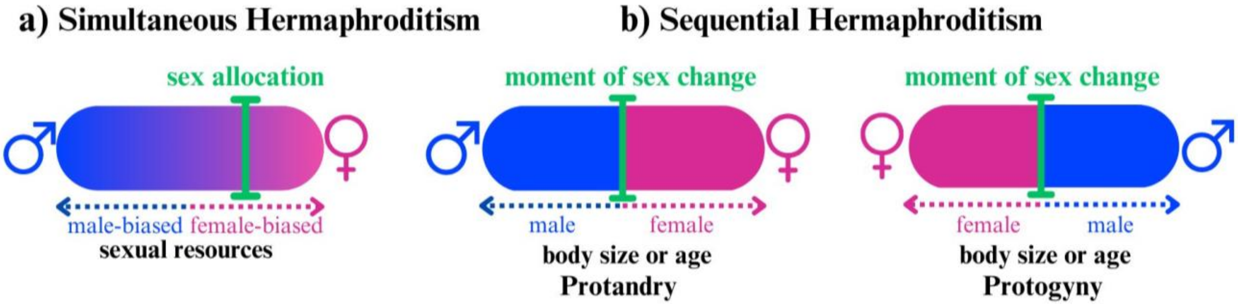

Figure 2.1. Schematic representation of sex allocation. (a) In simultaneous hermaphrodites, sex allocation is skewed towards one sex over the other. (b) In sequential hermaphrodites, it is expressed as the time spent in the first sex before switching to the second.

An individual's sex allocation is not determined solely by resource availability, which may constrain investment in one sexual function over the other; it is also shaped and constrained by environmental and social conditions (Schärer, 2009). Charnov (1982) proposed the sex allocation theory to explain and predict how individuals should make optimal sex allocation decisions while accounting for factors that skew allocation toward one sex or the other, such as an individual's condition (e.g., body size, age, social status) and the social environment.

2.2. **Conditions Favouring the Evolution of Different Sexual Systems**

The two forms of hermaphroditism have generally evolved in response to distinct evolutionary challenges and are therefore shaped by distinct selection pressures (Table 2.1). Optimality theory predicts which form of hermaphroditism individuals within a species are likely to exhibit under specific selection forces (Charnov, 1982; Schärer; 2009), although exceptions and intermediate forms can occur.

In the following sections, I discuss each form in detail, outlining the predictions of sex allocation theory, the selection pressures driving their evolution, and their respective adaptive advantages.

Table 2.1. Conditions Favouring the Evolution of Different Sexual Systems and Associated Sex Allocation Strategies

Sexual System	Selection Pressures	Pattern of Sex Allocation	Examples
I) Simultaneous Hermaphroditism	Scarcity of mates; diminishing fitness gains from investing exclusively in one sex (Schärer, 2009)	Optimal allocation skewed towards either the male or female function, depending primarily on the social environment (Schärer, 2009).	*Ophryotrocha diadema* (Picchi & Lorenzi, 2019); *Macrostomum* sp. (Schärer & Ladurner, 2003)
a) Self-fertilizing Simultaneous Hermaphroditism	Extreme scarcity of mating opportunities (Avise & Mank, 2009); strong selection for reproductive assurance (Jarne & Auld, 2006)	Allocation strongly favours the production of eggs, with enough resources reserved for the male function to ensure self-fertilization (Charnov, 1982; Jarne & Auld, 2006)	*Kryptolebias marmoratus* (Gresham et al., 2021); *Bulinus truncatus* (Doums et al., 1996)
II) Sequential Hermaphroditism	Fecundity increases more strongly with size in one sex; relative fitness of male vs female changes with body size, age, and social environment (Munday et al., 2006; Avise & Mank, 2009; Vega-Frutis et al., 2014)	Optimal timing of sex change occurs when the expected reproductive success in the second sex exceeds that in the first (Munday et al., 2006); allocation expressed as time, effort, and resources invested in each sex (effort in the first role may limit growth and the chance to change sex) (Charnov, 1982).	
a) Protandry ♂ → ♀	Female fecundity increases with size; small males retain mating opportunities under random or non-size-assortative mating; sperm competition generally low, except in broadcast spawners	Change sex to female after reaching threshold size or age, or in response to intrinsic and extrinsic factors (Collin, 2000; Collin et al., 2005)	*Amphiprion percula* (Wong et al., 2016); *Patella ferruginea* (Rivera-Ingraham et al., 2011); *Crepidula coquimbensis* (Brante et al., 2011); *Coralliophila neritoidea* (Richter & Luque, 2004)

Table 2.1. Conditions Favouring the Evolution of Different Sexual Systems and Associated Sex Allocation Strategies *(Contd.)*

Sexual System	**Selection Pressures**	**Pattern of Sex Allocation**	**Examples**
b) Protogyny ♀ → ♂	Generally haremic, territorial, or group-spawners where large males monopolize females; harem defence limits sperm competition by restricting rival access	Change sex to male when the opportunity to take on a harem arises (Kadota, 2022); or under limited mating opportunities to gain a growth advantage and improve future harem takeover (bachelor sex change, Sakanoue & Sakai, 2022)	*Labroides dimidiatus* (Sakai et al., 2001); *Trimma okinawae* (Manabe et al., 2007); *Dascyllus reticulatus* (Sakanoue & Sakai, 2022)
c) Bidirectional ♂ → ♀ → ♂ ♀ → ♂ → ♀	Scarcity of mating opportunities or mating in pairs (Todd et al., 2016); risk of movement or predation (Munday, 2002); low population density (Kuwamura et al., 2002); growth-rate advantage (Berglund, 1986); disruption of social structure Todd et al., 2016; Fukuda et al., 2017)	Change sex to complement the sex of the individual(s) in the environment (Munday et al., 2010); if haremic, revert to female if social structure is disrupted and a larger male takes over the harem (Todd et al., 2016)	*Lythrypnus dalli* (Solomon-Lane et al., 2019); *Ctenactis echinata* & *Saccostrea mordax* (Yasuoka & Yusa, 2017); *Serpulorbis arenarius* (Calvo & Templado, 2005); *Patella ferruginea* (Guallart et al., 2013); *Crassostrea gigas* (Yasuoka & Yusa, 2016)
Social Sex Determination* juv. + ♀ present → ♂ juv. + ♀ absent → ♀ *SSD is a form of epigenetic sex determination, i.e., a form of sexual lability expressed during the juvenile stage, which facilitates pair formation	Generally occurs in species that are sessile or have low-mobility or low density; relative benefit of expressing either sex depends on the social environment; triggered by social cues during early development (Beukeboom & Perrin, 2014); strong selection for reproductive assurance when individuals are sessile and isolated (Yusa et al., 2012)	Development is steered to complement the sex of the individual(s) in the environment (Beukeboom & Perrin, 2014); occurrence of dwarf, mobile males to overcome distance constraints (complemental males, Yusa et al., 2012)	*Bonellia viridis* (Jaccarini et al., 1983); *Crepidula* spp. (Collin et al., 2005); *Quoyula monodonta* (Soong & Chen, 2003); *Chelonibia testudinaria* (Zardus & Hadfield, 2004)

2.3. **Simultaneous hermaphroditism**

It is often suggested that if sexual systems were distributed along a continuum of phenotypic plasticity, species with separate sexes would be positioned at the “fixed” end, while simultaneous hermaphrodites would be at the “flexible” end (Avise & Mank, 2009; Leonard, 2013, 2018). This is because simultaneous hermaphrodites maintain both male and female sexual functions within the same individual, with sex allocation expressed as a bias towards one sex or the other (Fig. 2.1) (Charnov, 1982; Leonard, 2018; Santi et al., 2018). Given the fundamental difference in investment cost, simultaneous hermaphrodites face the core challenge of determining the optimal allocation of resources to maximize fitness through each sexual function.

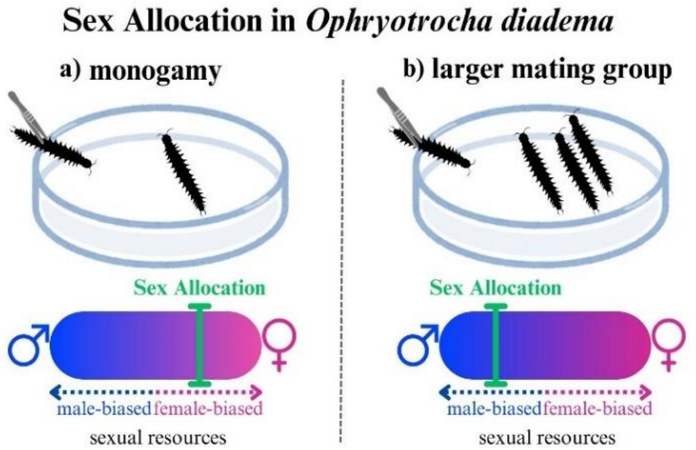

Figure 2.2. Sex allocation in the simultaneous hermaphrodite polychaete *Ophryotrocha diadema*. a) Under monogamous conditions, individuals exhibit a female-biased sex allocation, whereas b) in larger groups, they shift towards a male-biased allocation.

Since the relative benefit of investing in either sexual function depends on the social environment, optimal sex allocation varies with mating group size (West, 2009). According to sex allocation theory, individuals should allocate more resources to the female function when in smaller mating groups because increased investment in the male function would lead to greater competition between related sperm for fertilization (local sperm competition), resulting in diminishing fitness returns, which is offset by shifting allocation towards the female function, a key tenet of sex allocation theory (Schärer, 2009; Janicke et al., 2013). Such socially mediated shifts in sex allocation have been documented in the simultaneously hermaphroditic polychaete worm *Ophryotrocha diadema* (Fig. 2.2) (Lorenzi et al., 2005, 2006; Santi et al., 2018) and the flatworm *Macrostomum* sp. (Schärer & Ladurner, 2003).

2.3.1. ***Low-density hypothesis***

The low-density hypothesis for the evolution of simultaneous hermaphroditism, first formulated by Tomlinson (1966) and later developed by Ghiselin (1969), posits that simultaneous hermaphroditism may have evolved as a reproductive assurance strategy in environments where encounter rates are low. Under these conditions, any conspecific can become a potential mate (Schärer, 2009; Avise & Mank, 2009; Beukeboom & Perrin, 2014; Vega-Frutis et al., 2014). Consequently, simultaneous hermaphroditism is often found in species characterized by low population densities, poor mate-search efficiency, or limited mobility, such as in sluggish or sessile organisms, although exceptions to this pattern exist (Eppley & Jesson, 2008; Schärer, 2009; Leonard, 2013).

In sessile or low-mobility organisms, simultaneous hermaphroditism enables any proximate individual to act as both sperm donor and recipient, thereby ensuring cross-fertilization without the need for mate searching (Ghiselin 1969; Charnov, 1982). Puurtinen and Kaitala (2002) also demonstrated that high costs of mate-searching can stabilize simultaneous hermaphroditism. For instance, simultaneous hermaphroditism is thought to occur in the deep-sea anglerfishes *Gigantura chuni* and *G. indica* due to extremely low population densities. Recent observations that these fish are often found in pairs support this (Kupchik et al., 2018; Sawada, 2022). Therefore, this form of hermaphroditism is adaptive in species where mate-encounter rates are low.

Interestingly, in the *Ceratioidei* genus of deep-sea anglerfishes, in which sex is genetically determined, a different strategy to cope with low-density conditions called sexual parasitism has evolved. In this system, small males permanently fuse with a female and gradually degenerate until they exist solely as a reproductive organ (Pietsch, 2005).

Some simultaneous hermaphrodites, such as the freshwater snail *Physa acuta*, are also capable of self-fertilization under conditions of extreme isolation (Avise & Mank, 2009; see Jarne & Auld, 2006 for a comprehensive review). In *P. acuta*, selfing is facultative and often delayed. Isolated individuals postpone self-fertilization in anticipation of finding a mate, thereby reducing inbreeding costs while reallocating resources to future growth and fecundity (Tsitrone et al., 2003). This plasticity represents an adaptive balance between reproductive assurance and the fitness costs of inbreeding, and may facilitate the persistence of small or spatially isolated populations.

2.4. **Sequential Hermaphroditism**

In the continuum of phenotypic plasticity in sexual systems, sequential hermaphroditism would be positioned towards the middle (Leonard, 2018). Sequential hermaphrodites also modulate their relative investment in either sex primarily as a function of their social environment, although this partitioning of sexual resources occurs temporally through sex change, the timing of which depends on the relative increase in fitness expected from reproduction in the second sex (reproductive value; Munday et al., 2006). As a result, individuals in the first sex tend to be smaller and younger, while those in the second sex are generally larger and older. This size-specific sex expression can be explained the size-advantage hypothesis, originally introduced by Ghiselin (1969) and later expanded by Warner

(1975, 1988) and Charnov (1982), which explains the adaptive value of sequential hermaphroditism.

The marine environment appears to be especially conducive to the evolution of sequential hermaphroditism (Casas & Saborido-Rey, 2021). It occurs in three forms: protandrous (or male first) sequential hermaphroditism, protogynous (or female first) sequential hermaphroditism, and bidirectional sequential hermaphroditism. The direction of sex change, i.e., whether a species is protandrous or protogynous, is closely tied to its mating system (Beukeboom & Perrin, 2014; Vega-Frutis et al., 2014) (Table 2.1). Generally, protogyny occurs predominantly in vertebrates, specifically fish, with very few instances also found in crustaceans (Benvenuto & Weeks, 2020), while protandry is more common in invertebrates, and is notably prevalent in crustaceans (Allsop & West, 2004).

2.4.1. *Size Advantage Hypothesis*

In 1969, Ghiselin proposed the Size Advantage Hypothesis (SAH) to explain the adaptive significance of sequential hermaphroditism, positing that reproductive success is optimized by expressing the sex with size-independent fecundity when small and switching to the sex with size-dependent fecundity when large. Since female fecundity generally increases with size (Charnov, 1982), particularly in invertebrates and fish, where sequential hermaphroditism is primarily observed (West, 2009), the direction of sex change (whether protandrous or protogynous) is shaped by the male size-fitness curve (Charnov, 1982; Warner, 1988), which in turn is determined by the species' mating system (Guallart et al., 2013; Beukeboom & Perrin, 2014; Kuwamura et al., 2020), as male fitness is subject to sexual selection (Vega-Frutis et al., 2014).

If male reproductive success increases more slowly with size than that of females, protandry is favoured; conversely, if female reproductive success increases more slowly with size than that of males, protogyny is favoured (Warner, 1975, Munday et al., 2006; Cadet et al., 2004; West, 2009; Henshaw, 2018). Sequential hermaphroditism, therefore, bridges the sex-specific disparities in size-dependent fitness outcomes by adopting sexual functions in a size-specific manner (Avise & Mank, 2009; Todd et al., 2016). The robustness of this theoretical prediction is supported by large-scale comparative analyses in fish, which indicate that the evolution of sex change is influenced by phylogenetic history and ecological context, leading to recurring and predictable patterns across lineages (Kuwamura et al., 2020; Pla et al., 2022).

While the size-advantage hypothesis has been successful at explaining the occurrence of sex change, subsequent research has also incorporated the role of life-history parameters, such as mortality and growth (Warner, 1988), as well as the potential for sperm competition and reproductive skew (Muñoz & Warner, 2003, 2004), especially in species where the traditional size-advantage model alone does not sufficiently explain patterns of sex change.

2.4.2. **Protandrous Hermaphroditism**

After a brief sexually immature juvenile phase, protandrous hermaphrodites mature as males and may change sex to female later in life (Avise & Mank, 2009). Protandry typically occurs in small, stable groups that exhibit monogamy, such as in the clown anemonefish *Amphiprion percula* (Fitzgerald et al., 2022), or in species that exhibit random mating through temporary pair formations, such as in the platycephalid fish *Thysanophrys celebica* (Sunobe et al., 2016), which limits the potential for sexual selection since males cannot monopolize females and females do not exert preferences for any particular male body size (Warner, 1984; Avise & Mank, 2009; Smiley et al., 2024). Therefore, large body size does not confer additional reproductive benefits to males and even small males can access females and successfully fertilize eggs, therefore, male mating success is not strongly influenced by body size (Avise &

Mank, 2009). However, in some species, larger males may dominate mating opportunities, creating a male size advantage, as has been observed in the pandalid shrimp *Pandalus latirostris* (Chiba, 2007) and the snapping shrimp *Athanas kominatoensis* (Nakashima, 1987).

Additionally, protandrous species exhibit a male-biased sex ratio, creating an excess of sperm, which may explain why protandry is less common compared to the prevalence of protogyny (Avise & Mank, 2009). Since increased male size does not significantly improve reproductive success, due to limitations imposed by female fecundity in pair-bonded or monogamous species, and by access to females in others, the male reproductive fitness curve grows more slowly. At this point, it becomes more advantageous to switch to female, as female fecundity increases with size, often exponentially. For instance, in the aforementioned snapping shrimp *A. kominatoensis,* females smaller than 4.5 mm in carapace length laid approximately 26 eggs per oviposition, whereas females larger than 5.5 mm laid approximately 294 eggs (Nakashima, 1987). Therefore, males should change sex when their reproductive output as a small female would exceed that of remaining a large male.

2.4.3. **Protogynous Hermaphroditism**

After a brief sexually immature juvenile phase, protogynous sequential hermaphrodites mature as females and may change sex to male later in life (Avise & Mank, 2009). According to sex allocation theory, protogyny is favoured in species characterized by haremic polygyny, male-territory-visiting polygyny (Avise & Mank, 2009; Casas & Saborido-Rey, 2021), and in a few species with size-assortative monogamy (Kuwamura et al., 2020). The adaptive significance of starting life as a female and changing sex to male at a larger body size can also be explained by the direct effects of size on reproductive success (Klinkhamer et al., 1997; Henshaw, 2018). In polygynous systems, larger males gain disproportionate reproductive success by monopolizing females within a harem or defended territory (Sakai, 2022). Consequently, small males have little to no reproductive success, making it advantageous to begin life as a female, when even smaller sizes can confer some reproductive success.

As an individual grows, remaining female yields a steady, size-dependent increase in fecundity, whereas becoming a dominant male can dramatically enhance reproductive success. Consequently, it would be adaptive for females to change sex to male when the opportunity to assume the dominant role arises, often termed “takeover sex change” (Hamaguchi et al., 2002; Sakanoue & Sakai, 2022; Kadota, 2022). Therefore, sex change is favoured when the direct effects of size on male fitness greatly outweigh the steady, budget-based fitness gains of remaining a large female (Klinkhamer et al., 1997).

Protogyny is the prevalent form of sequential hermaphroditism in fishes (Pla et al., 2022) but is extremely rare among invertebrates. In crustaceans, where protandry is favoured, protogyny has been documented in only about 20 of approximately 45,000 species, all within the peracarid orders Isopoda and Tanaidacea (Benvenuto & Weeks, 2020). One example is the miniscule the miniscule Tanaidacean species *Nesotanais* sp. aff. r*yukyuensis*, a tube-dwelling species in which the male size advantage inherent to protogyny is thought to arise from larger males being more effective at defending and competing for females within their tubes (Kakui & Hiruta, 2022).

2.4.4. **Bidirectional Hermaphroditism**

Bidirectional hermaphrodites are sequential hermaphrodites capable of reverting to their initial sex multiple times. In fish, it is thought to have evolved from either protogynous or gonochoristic ancestors (Kuwamura et al., 2020). The adaptive significance and selection pressures driving bidirectional sex change parallel those underlying simultaneous hermaphroditism, as both facilitate breeding pair formation among conspecifics regardless of initial sex, particularly under conditions of low density, limited mobility, and costly mate searching.

Increasingly, species previously thought to change sex irreversibly are being discovered to be capable of sex reversal. However, the phenomenon has been known for decades in several small, socially controlled coral reef fishes, such as the gobies *Gobiodon histrio* and *Paragobiodon echinocephalus* (Munday et al., 1998; Kuwamura et al., 2014), and the dottyback *Pseudochromis flavivertex* (Wittenrich & Munday, 2005), which can rapidly switch sex to ensure breeding pair formation. Among protandrous invertebrates, examples include *Patella ferruginea* (Rivera-Ingraham et al. 2011; Guallart et al., 2013), *P. vulgata* (Le Quesne & Hawkins, 2006), and oysters like *Crassostrea gigas* (Yasuoka & Yusa, 2016; Broquard et al., 2020), *Saccostrea glomerata* and *S. cucullate* (Yasuoka & Yusa, 2017). Additionally, in the coral *Montastraea cavernosa*, sex reversal from female to male can be experimentally induced by food reduction (Laforest et al., 2024), while in the noble scallop *Chlamys nobilis*, sex reversal is triggered by temperature (Feng et al., 2023).

In protandrous or protogynous species, sex change is expected to occur when the reproductive value from changing sex exceeds that of staying in the current sex (Munday et al., 2006). In contrast, bidirectional sex change is favoured when the relative reproductive value of males and females fluctuates multiple times during an individual's life (Manabe et al., 2007). For instance, in haremic species, if a male loses all females in his harem and a larger male assumes the dominant role, reverting to female would improve its reproductive value (low density hypothesis; Kuwamura et al., 2011, 2014). Likewise, in monogamous coral-dwelling fishes, if a male loses his mate and encounters a widowed larger male, reverting to female would improve his reproductive value by facilitating new pair formation (risk-of-movement hypothesis; Munday et al., 2010). Alternatively, in species with unstable social structures, a male may revert to the female phase after losing his social status to a larger rival (Todd et al., 2016).

2.4.4.1. ***Risk of movement hypothesis***

The risk of movement hypothesis proposes that bidirectional sex change evolved as an adaptation to reduce the costs of mate searching when population densities are low and risk of predation is high, as it allows any two conspecifics to form a breeding pair irrespective of initial sex (Munday et al., 2010; Vegafrutis et al., 2014). Gobiids, small and colourful coral-dwelling fish, experience high levels of predation (Munday et al., 1998). Since their populations are sparse and spatially scattered, monogamy is the predominant mating system in these species (Kuwamura et al., 2014). When a partner in a pair-bonded couple dies, the surviving individual need only find another conspecific, regardless of sex, to form a new pair (Todd et al., 2016). If the new partner is of the same sex, individuals make subsequent sex changes based on their relative size and social status (see Section 3.3) to form a functional breeding pair (Kuwamura et al., 2014; Kadota, 2022). This has been demonstrated through field experiments in *Paragobiodon echinocephalus* (Nakashima et al., 1995), *Gobiodon histrio* (Munday et al., 1998), and *Priolepis akihitoi* (Manabe et al., 2013). Therefore, the capacity for bidirectional sex change allows widowed individuals to rapidly re-establish breeding pairs while minimizing the energetic and ecological costs of mate searching (Munday et al., 2010).

2.4.4.2. ***Low-density hypothesis***

In polygynous haremic fishes, bidirectional sex reversal can be induced under low-density conditions or when females are scarce (Kuwamura et al., 2011; 2014). In haremic species such as the cleaner wrasse *Labroides dimidiatus* and the rusty angelfish *Centropyge ferrugata*, experimental removal of all females resulted in the widowed males pairing with one another, followed by sex reversal to female in the smaller male (Kuwamura et al., 2011).

The primary difference between the risk-of-movement and the low-density hypotheses likely lies in their relevance to different mating systems. In monogamous coral-dwelling species where predation risk is high, mate-loss is a common and natural occurrence. In contrast, in polygynous and haremic systems, sex reversal is observed predominantly in the complete absence of females and juveniles, which is less likely to occur naturally, as it is rare for a dominant male to lose all females simultaneously, even under intense predation (Kuwamura et al., 2014, 2015). Nevertheless, in very low-density populations, even typically polygynous fish may adopt facultative monogamy, making bidirectional sex change advantageous (Kuwamura et al., 2015). Therefore, while bidirectional sex change facilitates pair formation among widowed individuals in monogamous species, in polygynous species it appears to be a conditional “if-necessary” strategy when females are entirely absent.

The low-density hypothesis has also been invoked to explain bidirectional hermaphroditism among invertebrates (Calvo & Templado, 2005). Among gastropods and bivalves, it is thought to occur primarily in free-spawning species or those lacking copulatory structures (Collin, 2013). For instance, the protandrous vermetid gastropod *Serpulorbis arenarius*, a sessile and solitary species, is often found in particularly low-density conditions where conspecifics can be meters apart (Calvo & Templado, 2005). The authors hypothesize that males may release spermatophores into the water, which are then captured by another male. The receiving individual stores the sperm and subsequently changes sex to female, using the stored sperm to fertilize eggs before reverting back to male after oviposition (Calvo & Templado, 2005). Although direct experimental evidence remains limited, this mechanism provides a plausible explanation for how bidirectional sex change facilitates reproduction under conditions of extreme isolation.

CHAPTER 3

PHENOTYPIC PLASTICITY IN SEX CHANGE

More than a flip of a Switch

3.1. Is the timing of (or body size at) sex change constant?

In sequential hermaphrodites, sex allocation is expressed in terms of the duration of the male- versus female-phase, making the timing of sex change crucial. It was once thought that sex change was a fixed process occurring upon reaching a critical age or body size, independent of social influence (Allsop and West, 2003). The critical size is defined as the evolutionarily optimal size where the expected fitness gain of switching sex decisively outweighs the costs of remaining in the current sex (Mariani et al., 2013). This appears true for some species, such as the protandrous black porgy *Acanthopagrus schlegeli* (Wu et al., 2010; 2016) and the gilthead seabream *Sparus aurata* (García Hernández et al., 2020), as well as some invertebrates like the limpet *Cymbula oculus* (Branch & Odendaal, 2003). Allsop and West (2003) proposed that sex change patterns can be predictable, typically occurring at 80% of body size or 2.5 times the age at maturity. This has been empirically supported in some species, primarily fish (Avise & Mank, 2009), such as the platycephalid fish *Thysanophrys celebica* (Sunobe et al., 2016).

However, studies have now widely demonstrated that the timing of sex change varies significantly among individuals, primarily in response to social cues (see below) and even to environmental conditions such as temperature (Athauda & Anderson, 2014), salinity (Powell et al., 2012), and pollution (Garcia Hernández et al., 2020). Additionally, an individual's physical condition can also influence sex allocation: for example, injured or starved individuals may prolong (or revert to) the male phase, as observed in the coral *Ctenactis echinata* (Loya & Sakai, 2008) and in the polychaetes *Ophryotrocha puerilis* (Hartmann & Huth, 1936), and *Exogone gemmifera* (Leonard, 2018). This shift likely occurs because individuals in poor condition or unfavourable environments may have fewer resources to invest in female reproduction (Vega-Frutis et al., 2014; Leonard, 2018; Gemmel et al., 2019). As Leonard (2018) explains, "maleness represents a type of 'physiological sink' that individuals may fall into under unfavourable conditions or when the energy loss associated with egg-laying is sufficiently great". Stress can also alter the timing of sex change through the mediation of cortisol (Gemmell et al., 2019).

3.2. **Sex & The City**

Social Mediation of Sex Change

The timing and body size at sex change in most sequential hermaphrodites is socially mediated because the relative benefit of being male or female is closely tied to the social environment (e.g., absence of females, Hoch & Cahill, 2012; presence of smaller conspecifics, Cole & Shapiro, 1995; sex ratio, Shapiro & Lubbock, 1980; social status, Warner & Swearer, 1991; size-ratio among conspecifics, Ross et al., 1983). For instance, in the monogamous and protandrous Red Sea clownfish *Amphiprion bicinctus*, breeding males change sex to female upon the disappearance of the dominant female, but only if a smaller subordinate is present (Sunobe, 2022). Isolated males, even upon reaching the size threshold for sex change (100 mm; Sunobe, 2022), do not change sex in the absence of a subordinate. This may be a form of reproductive assurance, as the smaller subordinate will likely become the male breeding partner. Changing sex in isolation would be costly because sex change in this species is irreversible. Moreover, since clownfish are obligately monogamous and site-attached, finding another anemone to assume the female role is unlikely. In fact, forcibly pairing two females led to severe injury or death of the subdominant individual, likely due to failure to establish a hierarchy as females cannot revert to male (Fricke and Fricke, 1977). Thus, the fitness of the former male (now a female) is maximized by sex change only when a subordinate is immediately available to assume the male breeding role. Changing sex without a subordinate or male in the environment would be maladaptive, while remaining male could be advantageous, as males can either: (i) change sex to female, (ii) remain male, or (iii) revert to being a subordinate juvenile depending on the social context.

3.2.1. ***Social cues triggering sex change in vertebrates***

In the protogynous goby *Coryphopterus glaucofraenum*, isolated females rarely change sex, however, the presence of a competitor, whether male or female, triggers sex change (Cole & Shapiro, 1995). Interestingly, in groups of females, even visual cues of smaller females can induce sex change in the largest female of the social group (Cole & Shapiro, 1995). Comparable findings have been reported in the haremic protogynous angelfish *Centropyge ferrugata,* where males not only revert to the female phase when paired with a larger male, but being sexually dichromatic, even change back to female-specific fin coloration (Sakai et al., 2003).

In the protogynous *Thalassoma duperrey*, removal of the dominant male is insufficient to trigger sex change in the female, as sex change is induced by the presence of smaller females (size-ratio induction; Ross et al., 1983, 1990; Lutnesky, 1994). Another form of social mediation of sex change occurs when deviations from the local sex ratio trigger sex change, as observed in the serranid reef fish *Anthias squamipinnis* (Shapiro & Lubbock, 1980). In this species, the removal of females can induce the largest males to revert to female, thereby restoring the threshold sex ratio (sex-ratio induction; Shapiro & Lubbock, 1980). In the protogynous blackspot tuskfish *Choerodon schoenleinii¸* tactile contact from males inhibits sex change in females. However, in the absence of a male, tactile contact among females induces sex change in the largest female (Sato et al., 2018), as if the presence of female competitors for the male role triggers sex change.

Social status also predicts phenotypic sex in many haremic species, such as the bluebanded goby *Lythrypnus dalli* (Solomon-Lane et al., 2016). Consequently, the disruption of social order through the loss of dominant status, due to territory loss or takeover, may lead to sex reversal in the relatively smaller dominant male, as observed in the hawkfish

Cirrhitichthys falco (Kadota et al., 2012) and the magenta dottyback *Pictichromis porphyrea* (Kuwamura et al., 2015), while sex change in subordinates may be triggered by ascending the social ranks following the loss of the dominant individual, as seen in the cleaner wrasse *Labroides dimidiatus* (Munday et al., 2010).

The protandrous *Amphiprion* typically lives in social groups consisting of a monogamous breeding pair and several small non-breeders, forming a dominance hierarchy in which larger individuals hold higher social rank (Casadevall et al., 2009). When the dominant female disappears or dies, the breeding male partner may change sex to become female, the largest non-breeder may mature to take on the male breeding role, and other individuals may shift upward in the social hierarchy. This female-disappearance-induced sex change, similar to the take-over sex change documented in haremic species, has been documented in *A. akallopisos* (Casadevall et al., 2009), *A. frenatus* (Hattori 1991, 2005), *A. ocellaris* (Madhu et al., 2010; Hattori 2012), *A. melanopus* (Godwin, 1994), *A. percula* (Buston, 2003) and *A. bicinctus* (Fricke, 1983). Fascinatingly, in *A. clarkii*, where social status is distinguishable by distinct caudal fin coloration, individuals ascending in the breeding queue also undergo corresponding colour phase changes, suggesting that social rank is closely linked to both behaviour and phenotype (Hattori & Yanagisawa, 1991).

3.2.2. ***Social cues triggering sex change in invertebrates***

In protandrous gastropods, social mediation of sex change was long thought to only occur in species with a planktonic larval phase, as free-swimming larvae encounter unpredictable social environments. By contrast, sex change in direct-developing species was assumed to not be as strongly influenced by the social environment (Coe, 1938; Hoagland, 1978). Recent studies, however, show that social mediation of sex change also occurs in direct-developing protandrous gastropods (Collin et al., 2005; Brante et al., 2012; Henry and Lyons, 2016), demonstrating that the potential for social interactions, rather than developmental mode, is the key factor for the evolution of socially-mediated sex change (Brante et al., 2012).

For example, in the protandrous tropical slipper limpets *Crepidula norrisiarum, C. incurva, C.* cf. *onyx* and *Crepidula* cf. *marginalis*, solitary males change sex earlier and at a smaller body size than males paired with a female. Interestingly, pairing a male with a smaller individual triggers an even earlier sex change than isolation, whereas pairing with a female delays sex change (Warner et al., 1996; Collin et al., 2005; Carrillo-Baltodano & Collin, 2015). This tendency for males to delay sex change when females are present is common across protandrous invertebrates (Richter & Luque, 2004) such as the sea snails *Corallophilia neritoidea* (Chen et al., 1998; Richter & Luque, 2004), *C. lingulata* (Collin, 2000), *C. violacea* (Soong & Chen, 1991), *C. norrisiarum* (Warner et al., 1996). Delaying sex change in the presence of a larger female is adaptive, as size-dependent female fecundity enhances reproductive success for both individuals in a pair. Similarly, in the protandrous ferruginous limpet *Patella ferruginea*, males change sex earlier when fewer females are present (Rivera-Ingraham et al., 2011).

In the territorial, protandrous giant limpet *Lottia gigantea*, dominance is determined by body size and reinforced through aggressive interactions, with larger individuals securing priority access to territory and mating opportunities; dominant limpets are more likely to change sex to female, while subordinates delay sex change (Wright, 1989). Social mediation of sex change has also been observed in protogynous crustaceans such as in the leptocheliid species *Heterotanais oerstedii* and *Chondrochelia dubia*, where females delay sex change in the presence of males but accelerate sex change when housed with other females (Kakui & Hiruta, 2022).

3.3. **Size Matters**

Relative size for discerning sex roles or position in the hierarchy

A common theme emerges: body size relative to conspecifics emerges as an important (and sometimes predominant) cue triggering sex change. There are two main explanations for how relative size determines sex. First, among size-unmatched individuals, in line with the size-advantage hypothesis, protandrous species will follow the large female/small male pattern, whereas protogynous species will follow the large male/small female pattern. Second, when two unfamiliar individuals of similar size are paired, sex may also be determined by social status (Muñoz-Arroyo et al., 2019). This was neatly demonstrated in the protogynous, bidirectional, haremic gorgeous goby *Lythrypnus pulchellus*. The authors created pairs of fish: size-unmatched males, size-matched males, size-unmatched females, and size-matched females. In size-unmatched male pairs, the smaller individual reverted to female; and in size-unmatched female pairs, the larger female changed sex to male, as evidenced by the onset of male-typical courtship behaviors and subsequent gonadal transformation. In size-matched male pairs, the dominant remained male while the subordinate reverted to female. Similarly, in size-matched female pairs, the dominant changed sex to male while the subordinate remained female. In essence, all pairs reverted to the typical large dominant male/small subordinate female arrangement (Muñoz-Arroyo et al., 2019).

Consequently, conspecific influences on the decision to change sex may be simplified using conditional if/then principles that accurately predict whether an individual should change sex or remain in its current sex (see Fig. 3.1). While these principles provide a useful framework for understanding social mediation of sex change, there are notable exceptions, such as early-maturing females (EMFs) in the protandrous pink shrimp *Pandalus borealis* (Bergström, 1997; Subramoniam, 2018), or late reversing males (LRMs) in the protandrous *P.*

latirostris (Chiba et al., 2003), or the occurrence of dwarf males in some protandrous species (Collin, 2013), which may deviate from these rules and require additional conditions to explain.

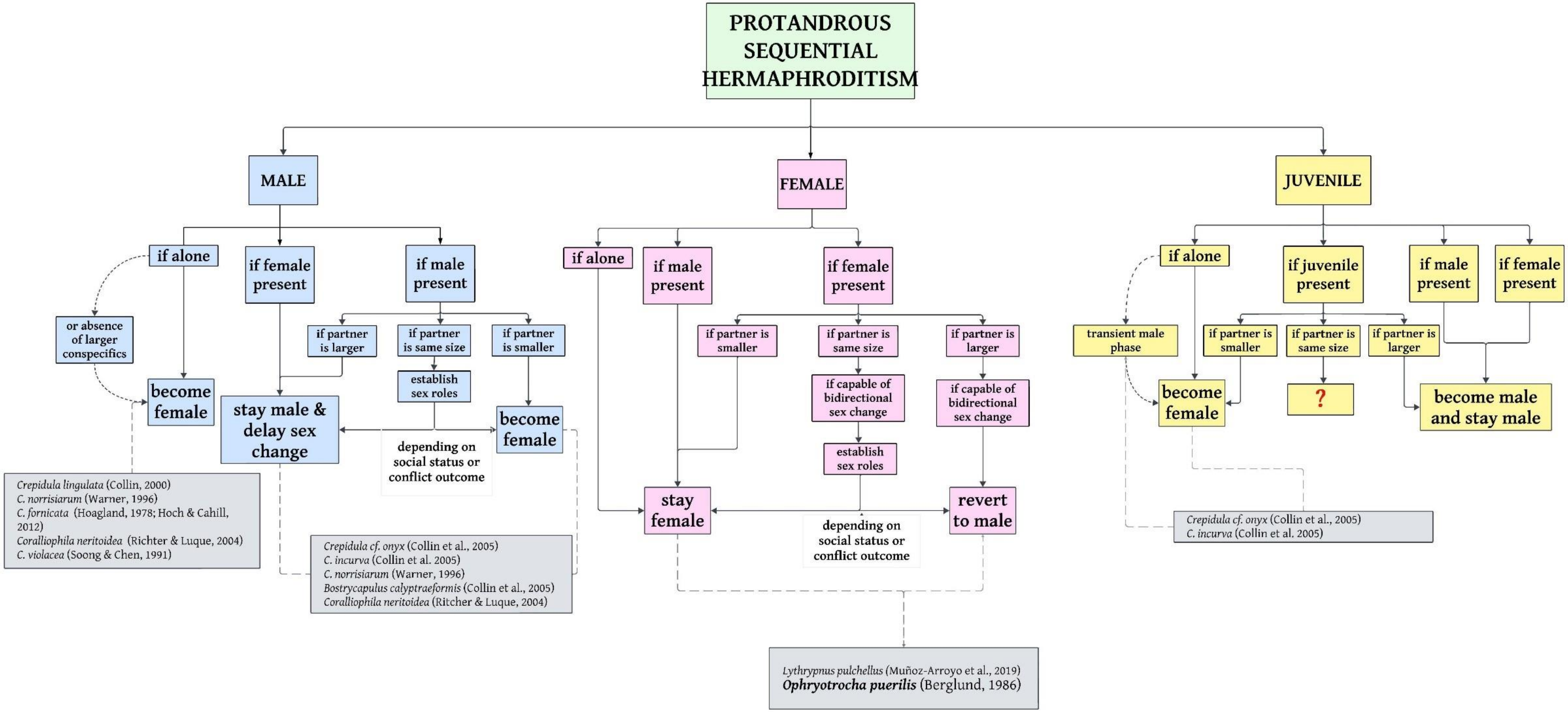

Figure 3.1. Conditional sex allocation decisions in protandrous sequential hermaphrodites. This flowchart illustrates how the timing (or body size) of sex change is influenced by social cues such as relative size, social status, and the presence and sex of conspecifics among males, females, and juveniles. While this provides a generalized framework for understanding social mediation of sex change, exceptions to these rules exist.

3.4. **Why do subordinates delay sex change?**

Social control of sex change

In species characterized by social hierarchies, typically exhibiting haremic polygyny, where a single dominant individual expresses the second sex, individuals of the opposite sex are thought to be suppressed from changing sex through aggression, not only from the dominant individual, but also from their immediate competitor. This form of social mediation of sex change is recognized as social control (Robertson, 1972), and assumes a top-down mechanism where dominants inhibit sex change in subordinates (Sakai, 2022).

Consequently, experiments involving the removal of dominant individuals are often used to diagnose social control in a species (Sakai, 2022). If, following this removal, the largest subordinate changes sex (often preceded by a growth spurt known as sex-change-induced growth spurt, Walker & McCormick 2009; Munday et al., 2009) and takes over the harem, the species is said to be under social control (Sakai, 2022).

Such male removal triggering sex change in the largest female has been reported in many species, such as the haremic and protogynous *Anthias squamipinnis* (Beukeboom & Perrin, 2014) and several species of the haremic and protogynous *Centropyge* angelfishes (*C. bicolor*, Ang & Manica, 2010; *C. interruptus & C.* tibicen, Moyer & Zaiser, 1984), as well as the bidirectional protogynous and polygynous Okinawa rubble gobiid fish, *Trimma okinawae* (Kobayashi et al., 2012).

3.5. **Why do subordinates delay sex change**?

Autonomous decision making

Benvenuto & Lorenzi (2023) argue that subordinates delay sex change not due to dominant control but rather because they make independent decisions based on their own condition relative to that of interacting individuals, as well as social cues such as relative size and status within the group. Indeed, the dominant-imposed delays in sex change assumed by social control would be easily disrupted if a "mutant" subordinate emerged in the population that resisted this control (Benvenuto & Lorenzi, 2023). Furthermore, subordinates under such sex change inhibition would likely evolve counter-strategies that help them bypass this control and fulfil their own reproductive interests, leading to an evolutionary arms race where dominants would need to continually enhance their manipulation. Therefore, the persistence of such a top-down mechanism is evolutionarily unstable (Benvenuto & Lorenzi, 2023).

In the cleaner wrasse, *Labroides dimidiatus* and the gobiid fish *Trimma okinawae*, both haremic and protogynous, some subordinate females will "tour" neighbouring harems to assess social conditions, presumably observing the number and body size of females, and may subsequently intrude to take over the dominant male role (Sakai et al., 2001; Manabe et al., 2007). In some cases, however, migrating females may not be large enough to take on the harem but will still move to the new group simply to improve their rank among subordinate females, thereby hastening their eventual takeover of the harem (Manabe et al., 2007). Similarly, in the haremic damselfish *Dascyllus reticulatus*, where the largest female takes over the harem after the disappearance of the dominant male, smaller females with limited mating opportunities may sometimes change sex to male, even in the presence of the dominant male and larger females. This "bachelor sex change" provides a growth advantage and allows these males to grow faster and potentially take over a harem sooner than if they had remained in the breeding queue as females (Sakanoue & Sakai, 2022).

These examples support the argument made by Benvenuto & Lorenzi (2023) that subordinates are not simply passive in their reproductive strategies but instead actively assess their position in the social environment, making independent decisions to improve their reproductive value, such as intruding on other harems for improving their rank in the breeding queue or by bachelor sex change to grow faster and improve future fitness, even at the short-term cost of current fitness.

Group size is also crucial for the maintenance of such a system, as it determines the stability of dominance hierarchies, the level of competition for the male role, and the costs associated with territorial defence or mate finding (Benvenuto & Lorenzi, 2023). As Nakashima (1987) proposed, in small and stable groups, the loss of a dominant individual typically triggers a one-to-one sex change. However, as group size increases, the cue for changing sex may shift to a sensitivity to unbalanced sex ratios, and as group size grows even larger, individuals may use relative body size as the predominant cue for sex change (Nakashima, 1987). Indeed, relative size emerges as a predominant cue triggering sex change across many sequential hermaphrodites (See Section 3.3).

3.6. **Read the Room**

Primary and secondary developmental trajectories

Sex allocation strategies can diverge even at the juvenile stage, resulting in alternative developmental trajectories that are sensitive to the social environment. In the protogynous bluehead wrasse *Thalassoma bifasciatum*, most individuals recruit to the reef as juveniles, mature first as females, and later change sex to males once they are large enough to defend territories (Warner & Robertson, 1978; Munday et al., 2006). However, some juveniles bypass the female phase entirely and mature directly as males. These “primary males” differ from “secondary males” (sex-changed females) in both behaviour and morphology, often adopting sneaker or group-spawning tactics rather than defending harems (Warner, 1988). The frequency of primary males in a population is density-dependent: in low-density populations, dominant secondary males can effectively monopolize females, leaving little opportunity for small males to reproduce, making the primary male pathway maladaptive. By contrast, in dense populations where harems are more difficult to defend, primary males may obtain reproductive success through sneaking or joining group spawns (Munday et al., 2006). Thus, the developmental decision to follow the primary or secondary male trajectory is socially mediated at the juvenile stage, reflecting an adaptive response to variation in local population structure.

Similarly, in the protandrous pandalid shrimps *Pandalus borealis* and *P. jordani*, while most individuals in the population reproduce first as males and later change sex to female (secondary females), a small proportion of juveniles bypass the male phase entirely, developing directly as females (primary females) (Charnov et al. 1978; Charnov, 1982, Bergström, 1997). Like diandry in fish, this binary developmental switch is an adaptive response to the social environment, specifically the occurrence of primary females was found to increase when the proportion of large females in the population decreased due to fishing pressure (Charnov et al. 1978; Charnov, 1982). Therefore, unlike the reaction-norm pattern of phenotypic plasticity in the timing of sex change (early, delayed, inhibited), primary males and females reflect a developmental switch at the juvenile stage in response to social cues, functioning as an alternative mating strategy.

Developmentally plastic responses at the juvenile stage appear to be a convergent solution to variable social environments. However, research on juvenile sex allocation remains comparatively sparse, with most studies focusing on adult sex change (Fig. 3.1). This gap motivates the present study, which investigates how juvenile sequential hermaphrodites modulate their sex allocation in response to varying group size, and how the presence of size-matched conspecifics, the presence of adults, and relative size influence the timing and body size at which juveniles change sex. It further explores the mechanisms through which individuals exert preferences for sexual roles. *Ophryotrocha puerilis* is a protandrous sequential hermaphrodite polychaete worm capable of bidirectional sex change. This species offers a particularly powerful model for investigating social mediation of sex allocation because of its plastic responses to environmental and social conditions, its tractability in laboratory culture, and its long history as a subject of sex change research. In the following chapter, I provide a detailed overview of the biology, reproductive system, and research legacy of *O. puerilis*, which sets the stage for my empirical investigations in Chapters 5 and 6.

References

Allsop, D. J., & West, S. A. (2003). Constant relative age and size at sex change for sequentially hermaphroditic fish. *Journal of Evolutionary Biology*, *16*(5), 921-929.

Allsop, D. J., & West, S. A. (2004). Sex-ratio evolution in sex changing animals. *Evolution*, *58*(5), 1019-1027.

Andersson, M. (1994). *Sexual selection* (Vol. 72). Princeton University Press.

Athauda, S., & Anderson, T. (2014). Effect of temperature and salinity on sex inversion in Asian Seabass (*Lates calcarifer*): relationship with plasma sex steroids concentration and aromatase activity of gonad and brain. *Aquaculture Research*, *45*(5), 787-797.

Avise, J. C., & Mank, J. E. (2009). Evolutionary perspectives on hermaphroditism in fishes. *Sexual Development*, *3*(2-3), 152-163.

Benvenuto, C., & Weeks, S. C. (2020). Hermaphroditism and gonochorism. *The natural history of the Crustacea: Reproductive Biology*, *6*, 197-241.

Berglund, A. (1986). Sex change by a polychaete: effects of social and reproductive costs. *Ecology*, *67*(4), 837-845.

Bergström, B. I. (1997). Do protandric pandalid shrimp have environmental sex determination?. *Marine Biology*, *128*(3), 397-407.

Beukeboom, L. W., & Perrin, N. (2014). The diversity of sexual cycles. In *The evolution of sex determination*, pp. 18–36. Oxford, UK: Oxford University Press.

Black, M. P., Balthazart, J., Baillien, M., & Grober, M. S. (2011). Rapid increase in aggressive behavior precedes the decrease in brain aromatase activity during socially mediated sex change in *Lythrypnus dalli*. *General and Comparative Endocrinology*, *170*(1), 119-124.

Branch, G. M., & Odendaal, F. (2003). The effects of marine protected areas on the population dynamics of a South African limpet, *Cymbula oculus*, relative to the influence of wave action. *Biological Conservation*, *114*(2), 255-269.

Brante, A., Vilches, C., & Quiñones, A. (2012). Role of population traits and local social interactions in sex-change plasticity of the protandric marine gastropod *Crepidula coquimbensis*. *Marine Ecology Progress Series*, *458*, 123-132.

Broquard, C., Martinez, A. S., Maurouard, E., Lamy, J. B., & Dégremont, L. (2020). Sex determination in the oyster *Crassostrea gigas*-A large longitudinal study of population sex ratios and individual sex changes. *Aquaculture*, *515*, 734555.

Buston, P. (2003). Size and growth modification in clownfish. *Nature*, *424*(6945), 145-146.

Cadet, C., Metz, J. A., & Klinkhamer, P. G. (2004). Size and the not-so-single sex: disentangling the effects of size and budget on sex allocation in hermaphrodites. *The American Naturalist*, *164*(6), 779-792.

Calvo, M., & Templado, J. (2005). Reproduction and sex reversal of the solitary vermetid gastropod *Serpulorbis arenarius*. *Marine Biology*, *146*, 963-973.

Carrillo-Baltodano, A., & Collin, R. (2015). *Crepidula* slipper limpets alter sex change in response to physical contact with conspecifics. *The Biological Bulletin*, *229*(3), 232-242.

Casadevall, M., Delgado Sureda, E., Colleye, O., Ber Monserrat, S., & Parmentier, E. (2009). Histological study of the sex-change in the skunk clownfish *Amphiprion akallopisos*. *The Open Fish Science Journal, 2009, vol. 2, p. 55-58.*

Casas, L., & Saborido-Rey, F. (2021). Environmental cues and mechanisms underpinning sex change in fish. *Sexual Development*, *15*(1-3), 108-121.

Charnov, E. L., Gotshall, D. W., & Robinson, J. G. (1978). Sex ratio: adaptive response to population fluctuations in pandalid shrimp. *Science*, *200*(4338), 204-206.

Charnov, E. L. (1982). *The Theory of Sex Allocation* (Vol. 18). Princeton University Press.d

Chen, M. H., Yang, Y. W., & Soong, K. (1998). Preliminary observations on change of sex by the coral-inhabiting snails *Coralliophila violacea* (Lamarck) (Gastropoda: Coralliophilidae). *Journal of Experimental Marine Biology and Ecology*, *230*(2), 207-212.

Chiba, S. (2007). A review of ecological and evolutionary studies on hermaphroditic decapod crustaceans. *Plankton and Benthos Research*, *2*(3), 107-119.

Coe, W. R. (1938). Influence of association on the sexual phases of gastropods having protandric consecutive sexuality. *The Biological Bulletin*, *75*(2), 274-285.

Cole, K. S., & Shapiro, D. Y. (1995). Social faciliation and sensory mediation of adult sex change in a cryptic, benthic marine goby. *Journal of Experimental Marine Biology and Ecology*, *186*(1), 65-75.

Collin, R. (2000). Sex change, reproduction and development of *Crepidula adunca* and *C. lingulata* (Gastropoda: Calyptraeidae). *Veliger,* 43: 24–33.

Collin, R., McLellan, M., Gruber, K., & Bailey-Jourdain, C. (2005). Effects of conspecific associations on size at sex change in three species of calyptraeid gastropods. *Marine Ecology Progress Series*, *293*, 89-97.

Collin, R. (2013). Phylogenetic patterns and phenotypic plasticity of molluscan sexual systems. *Integrative and Comparative Biology*, *53*(4), 723-735.

Doums, C., Viard, F., Pernot, A. F., Delay, B., & Jarne, P. (1996). Inbreeding depression, neutral polymorphism, and copulatory behavior in freshwater snails: a self-fertilization syndrome. *Evolution*, *50*(5), 1908-1918.

Enquist, M., & Leimar, O. (1990). The evolution of fatal fighting. *Animal Behaviour*, *39*(1), 1-9.

Eppley, S. M., & Jesson, L. K. (2008). Moving to mate: the evolution of separate and combined sexes in multicellular organisms. *Journal of Evolutionary Biology*, *21*(3), 727-736.

Feng, M., Tan, K., Zhang, H., & Zheng, H. (2023). Factors affecting sex reversal in bivalves: Case study on the effects of sex reversal on growth of offspring employing the noble scallop *Chlamys nobilis*. *Aquaculture*, *566*, 739192.

Fitzgerald, L. M., Harrison, H. B., Coker, D. J., Saenz-Agudelo, P., Srinivasan, M., Majoris, J. E., Boström Einarsson, L., Pujol, B., Bennett-Smith, M., Thorrold, S. R., & Planes, S. (2022). Rank change and growth within social hierarchies of the orange clownfish, *Amphiprion percula*. *Marine Biology, 169*(10), 128.

Fricke, H., & Fricke, S. (1977). Monogamy and sex change by aggressive dominance in coral reef fish. *Nature*, *266*(5605), 830-832.

Fricke, H. W. (1983). Social control of sex: field experiments with the anemonefish *Amphiprion bicinctus*. *Zeitschrift für Tierpsychologie*, *61*(1), 71-77.

Fukuda, K., Tanazawa, T., & Sunobe, T. (2017). Polygynous mating system and field evidence for bidirectional sex change in the gobiid fish *Trimma grammistes*. *Int J Pure Appl Zool*, *5*, 92-99.

García Hernández, M. P., Cabas, I., Rodenas, M. C., Arizcun, M., Chaves-Pozo, E., Power, D. M., & García Ayala, A. (2020). 17α-ethynylestradiol prevents the natural male-to-female sex change in gilthead seabream (*Sparus aurata* L.). *Scientific Reports*, *10*(1), 20067.

Gemmell, N. J., Todd, E. V., Goikoetxea, A., Ortega-Recalde, O., & Hore, T. A. (2019). Natural sex change in fish. *Current Topics in Developmental Biology*, *134*, 71-117.

Ghiselin, M. T. (1969). The evolution of hermaphroditism among animals. *The Quarterly Review of Biology*, *44*(2), 189-208.

Godwin, J. (1994). Behavioural aspects of protandrous sex change in the anemonefish, *Amphiprion melanopus*, and endocrine correlates. *Animal Behaviour*, *48*(3), 551-567.

Gorelick, R., Carpinone, J., & Derraugh, L. J. (2017). No universal differences between female and male eukaryotes: anisogamy and asymmetrical female meiosis. *Biological Journal of the Linnean Society*, *120*(1), 1-21.

Gresham, J. D., Lewis, K. A., Summers, S. P., Gresham, P. E., & Earley, R. L. (2021). Outbreeding depression as a selective force on mixed mating in the mangrove rivulus fish, *Kryptolebias marmoratus*. *bioRxiv*, 2021-02.

Guallart, J., Calvo, M., Acevedo, I., & Templado, J. (2013). Two-way sex change in the endangered limpet *Patella ferruginea* (Mollusca, Gastropoda). *Invertebrate Reproduction & Development*, *57*(3), 247-253.

Hamaguchi, Y., Sakai, Y., Takasu, F., & Shigesada, N. (2002). Modeling spawning strategy for sex change under social control in haremic angelfishes. *Behavioral Ecology*, *13*(1), 75-82.

Hansen, L. S., Gonzales, S. F., Toft, S., & Bilde, T. (2008). Thanatosis as an adaptive male mating strategy in the nuptial gift–giving spider *Pisaura mirabilis*. *Behavioral Ecology*, *19*(3), 546-551.

Hattori, A. (1991). Socially controlled growth and size-dependent sex change in the anemonefish *Amphiprion frenatus* in Okinawa, Japan. *Japanese Journal of Ichthyology*, *38*(2), 165-177.

Hattori, A., & Yanagisawa, Y. (1991). Life-history pathways in relation to gonadal sex differentiation in the anemonefish, *Amphiprion clarkii*, in temperate waters of Japan. *Environmental Biology of Fishes*, *31*, 139-155.

Hattori, A. (2005). High mobility of the protandrous anemonefish *Amphiprion frenatus*: nonrandom pair formation in limited shelter space. *Ichthyological Research*, *52*, 57-63.

Hattori, A. (2012). Determinants of body size composition in limited shelter space: why are anemonefishes protandrous?. *Behavioral Ecology*, *23*(3), 512-520.

Henry, J. Q., & Lyons, D. C. (2016). Molluscan models: *Crepidula fornicata*. *Current Opinion in Genetics & Development*, *39*, 138-148.

Henshaw, J. M. (2018). Protandrous hermaphroditism. In: Vonk J, Shackelford T (Eds.) *Encyclopedia of animal cognition and behaviour* (pp. 1-6). Springer International Publishing.

Hoagland, K. E. (1978). Protandry and the evolution of environmentally mediated sex change: a study of the Mollusca. *Malacologia* 17: 365–391.

Hoch, J. M., & Cahill, A. E. (2012). Variation in size at sex-change among natural populations of the protandrous hermaphrodite, *Crepidula fornicata* (Gastropoda, Calyptraeidae). *Marine Biology*, *159*, 897-905.

Jaccarini, V., AGius, L., Schembri, P. J., & Rizzo, M. (1983). Sex determination and larval sexual interaction in *Bonellia viridis* Rolando (Echiura: Bonelliidae). *Journal of Experimental Marine Biology and Ecology*, *66*(1), 25-40.

Janicke, T., Häderer, I. K., Lajeunesse, M. J., & Anthes, N. (2016). Darwinian sex roles confirmed across the animal kingdom. *Science Advances*, *2*(2), e1500983.

Janicke, T., Marie-Orleach, L., De Mulder, K., Berezikov, E., Ladurner, P., Vizoso, D. B., & Schärer, L. (2013). Sex allocation adjustment to mating group size in a simultaneous hermaphrodite. *Evolution*, *67*(11), 3233-3242.

Jarne, P., & Auld, J. R. (2006). Animals mix it up too: the distribution of self-fertilization among hermaphroditic animals. *Evolution*, *60*(9), 1816-1824.

Kadota, T., Osato, J., Nagata, K., & Sakai, Y. (2012). Reversed sex change in the haremic protogynous hawkfish *Cirrhitichthys falco* in natural conditions. *Ethology*, *118*(3), 226-234

Kadota, T. (2022). Bidirectional sex change in fishes. In T. Kuwamura, K. Sawada, T. Sunobe, Y. Sakai, & T. Kadota (Eds.), *Hermaphroditism and Mating Systems in Fish* (pp. 145–180). New York, NY: Springer.

Kakui, K., & Hiruta, C. (2022). Protogynous hermaphroditism in Crustacea: a new example from Tanaidacea. *Canadian Journal of Zoology*, *100*(8), 481-487.

Kawase, H., Okata, Y., & Ito, K. (2013). Role of huge geometric circular structures in the reproduction of a marine pufferfish. *Scientific Reports*, *3*(1), 1-5.

Klinkhamer, P. G., De Jong, T. J., & Metz, H. (1997). Sex and size in cosexual plants. *Trends in Ecology & Evolution*, *12*(7), 260-265.

Kobayashi, Y., Usami, T., Sunobe, T., Manabe, H., Nagahama, Y., & Nakamura, M. (2012). Histological observation of the urogenital papillae in the Bi-directional sex-changing Gobiid fish, *Trimma okinawae*. *Zoological Science*, *29*(2), 121-126.

Kupchik, M. J., Benfield, M. C., & Sutton, T. T. (2018). The first in situ encounter of *Gigantura chuni* (Giganturidae: Giganturoidei: Aulopiformes: Cyclosquamata: Teleostei), with a preliminary investigation of pair-bonding. *Copeia*, *106*(4), 641-645.

Kuwamura, T., Tanaka, N., Nakashima, Y., Karino, K., & Sakai, Y. (2002). Reversed sex-change in the protogynous reef fish *Labroides dimidiatus*. *Ethology*, *108*(5), 443-450.

Kuwamura, T., Suzuki, S., & Kadota, T. (2011). Reversed sex change by widowed males in polygynous and protogynous fishes: female removal experiments in the field. *Naturwissenschaften*, *98*, 1041-1048.

Kuwamura, T., Kadota, T., & Suzuki, S. (2014). Testing the low-density hypothesis for reversed sex change in polygynous fish: experiments in *Labroides dimidiatus*. *Scientific Reports*, *4*(1), 4369.

Kuwamura, T., Kadota, T., & Suzuki, S. (2015). Bidirectional sex change in the magenta dottyback *Pictichromis porphyrea*: first evidence from the field in Pseudochromidae. *Environmental Biology of Fishes*, *98*, 201-207.

Kuwamura, T., Sunobe, T., Sakai, Y., Kadota, T., & Sawada, K. (2020). Hermaphroditism in fishes: an annotated list of species, phylogeny, and mating system. *Ichthyological Research*, *67*, 341-360.

Laforest, K. V., Petrik, C. G., Hylton, A. A., Ionata, R. L., McDonald, E. M., Short, M. L., & Figueiredo, J. (2024). Induced sexual reproduction ex situ reveals bidirectional sex change of the coral *Montastraea cavernosa*. *Coral Reefs*, 1-12.

Le Boeuf, B. J. (1974). Male-male competition and reproductive success in elephant seals. *American Zoologist*, *14*(1), 163-176.

Le Quesne, W. J. F., & Hawkins, S. J. (2006). Direct observations of protandrous sex change in the patellid limpet *Patella vulgata*. *Journal of the Marine Biological Association of the United Kingdom*, *86*(1), 161-162.

Lehtonen, J., Parker, G. A., & Schärer, L. (2016). Why anisogamy drives ancestral sex roles. *Evolution*, *70*(5), 1129-1135.

Lehtonen, J., & Parker, G. A. (2024). The correlation between anisogamy and sexual selection intensity—the broad theoretical predictions. *Evolution Letters*, *8*(6), 749-755.

Leonard, J. L. (2006). Sexual selection: lessons from hermaphrodite mating systems. *Integrative and Comparative Biology*, *46*(4), 349-367.

Leonard, J. L. (2013). Sexual selection and hermaphroditic organisms: testing theory. *Current Zoology*, *59*(4), 579-588.

Leonard, J. L. (2018). The evolution of sexual systems in animals. *Transitions between sexual systems: understanding the mechanisms of, and pathways between, dioecy, hermaphroditism and other sexual systems*, 1-58.

Lorenzi, M. C., Sella, G., Schleicherova, D., & Ramella, L. (2005). Outcrossing hermaphroditic polychaete worms adjust their sex allocation to social conditions. *Journal of Evolutionary Biology*, *18*(5), 1341-1347.

Lorenzi, M. C., Schleicherová, D., & Sella, G. (2006). Life history and sex allocation in the simultaneously hermaphroditic polychaete worm *Ophryotrocha diadema*: the role of sperm competition. *Integrative and Comparative Biology*, *46*(4), 381-389.

Lorenzi, M. C., & Sella, G. (2008). A measure of sexual selection in hermaphroditic animals: parentage skew and the opportunity for selection. *Journal of Evolutionary Biology*, *21*(3), 827-833.

Lorenzi, M. C., Robles-Guerrero, F. G., & Costantini, D. (2023). The oxidative cost of competing for egg fertilization exceeds the cost of egg production. *Evolution*, *77*(1), 199-209.

Loya, Y., & Sakai, K. (2008). Bidirectional sex change in mushroom stony corals. *Proceedings of the Royal Society B: Biological Sciences*, *275*(1649), 2335-2343.

Lutnesky, M. M. (1994). Density-dependent protogynous sex change in territorial-haremic fishes: models and evidence. *Behavioral Ecology*, *5*(4), 375-383.

Madhu, R., Madhu, K., & Venugopal, K. M. (2010). Sex change of hatchery produced *Amphiprion ocellaris*: Influence of mating system removal on gonad maturation and nesting success. *Journal of the Marine Biological Association of India*, *52*(1), 62-69.

Manabe, H., Ishimura, M., Shinomiya, A., & Sunobe, T. (2007). Inter-group movement of females of the polygynous gobiid fish *Trimma okinawae* in relation to timing of protogynous sex change. *Journal of Ethology*, *25*, 133-137.

Manabe, H., Toyoda, K., Nagamoto, K., Dewa, S. I., Sakurai, M., Hagiwara, K., Shinomiya, A., & Sunobe, T. (2013). Bidirectional sex change in seven species of *Priolepis* (Actinopterygii: Gobiidae). *Bulletin of Marine Science*, *89*(2), 635-642.

Mariani, S., Sala-Bozano, M., Chopelet, J., & Benvenuto, C. (2013). Spatial and temporal patterns of size-at-sex-change in two exploited coastal fish. *Environmental biology of fishes*, *96*(4), 535-541.

Moyer, J. T., & Zaiser, M. J. (1984). Early sex change: a possible mating strategy of *Centropyge* angelfishes (Pisces: Pomacanthidae). *Journal of Ethology*, *2*(1), 63-67.

Munday, P. L. (2002). Bi-directional sex change: testing the growth-rate advantage model. *Behavioral Ecology and Sociobiology*, *52*, 247-254.

Munday, P. L., Buston, P. M., & Warner, R. R. (2006). Diversity and flexibility of sex-change strategies in animals. *Trends in Ecology & Evolution*, *21*(2), 89-95.

Munday, P. L., Caley, M. J., & Jones, G. P. (1998). Bi-directional sex change in a coral-dwelling goby. *Behavioral Ecology and Sociobiology*, *43*, 371-377.

Munday, P. L., Kuwamura, T., & Kroon, F. J. (2010). Bidirectional sex change in marine fishes. In: Cole KS (ed) *Reproduction and sexuality in marine fishes: patterns and processes*. University of California Press, Berkeley, California, pp 241–271.

Munday, P. L., Ryen, C. A., McCormick, M. I., & Walker, S. P. W. (2009). Growth acceleration, behaviour and otolith check marks associated with sex change in the wrasse *Halichoeres miniatus*. *Coral Reefs*, *28*, 623-634.

Munoz, R. C., & Warner, R. R. (2003). A new version of the size-advantage hypothesis for sex change: incorporating sperm competition and size-fecundity skew. *The American Naturalist*, *161*(5), 749-761.

Munoz, R. C., & Warner, R. R. (2004). Testing a new version of the size-advantage hypothesis for sex change: sperm competition and size-skew effects in the bucktooth parrotfish, *Sparisoma radians*. *Behavioral Ecology*, *15*(1), 129-136.

Muñoz-Arroyo, S., Rodríguez-Jaramillo, C., & Balart, E. F. (2019). The goby *Lythrypnus pulchellus* is a bi-directional sex changer. *Environmental Biology of Fishes*, *102*, 1377-1391.

Nakashima, Y. (1987). Reproductive strategies in a partially protandrous shrimp, *Athanas kominatoensis* (Decapoda: Alpheidae): sex change as the best of a bad situation for subordinates. *Journal of Ethology*, *5*(2), 145-159.

Nakashima, Y., Kuwamura, T., & Yogo, Y. (1995). Why be a both-ways sex changer?. *Ethology*, *101*(4), 301-307.

Parker, G. A., Baker, R. R., & Smith, V. G. F. (1972). The origin and evolution of gamete dimorphism and the male-female phenomenon. *Journal of theoretical biology*, *36*(3), 529-553.

Picchi, L., & Lorenzi, M. C. (2019). Gender-related behaviors: evidence for a trade-off between sexual functions in a hermaphrodite. *Behavioral Ecology*, *30*(3), 770-784.

Pietsch, T. W. (2005). Dimorphism, parasitism, and sex revisited: modes of reproduction among deep-sea ceratioid anglerfishes (Teleostei: Lophiiformes). *Ichthyological Research*, *52*, 207-236.

Pla, S., Benvenuto, C., Capellini, I., & Piferrer, F. (2022). Switches, stability and reversals in the evolutionary history of sexual systems in fish. *Nature Communications*, *13*(1), 3029.

Powell, E. N., Morson, J. M., Ashton-Alcox, K. A., & Kim, Y. (2013). Accommodation of the sex-ratio in eastern oysters *Crassostrea virginica* to variation in growth and mortality across the estuarine salinity gradient. *Journal of the Marine Biological Association of the United Kingdom*, *93*(2), 533-555.

Puurtinen, M., & Kaitala, V. (2002). Mate-search efficiency can determine the evolution of separate sexes and the stability of hermaphroditism in animals. *The American Naturalist*, *160*(5), 645-660.

Richter, A., & Luque, Á. A. (2004). Sex change in two mediterranean species of Coralliophilidae (Mollusca: Gastropoda: Neogastropoda). *Journal of the Marine Biological Association of the United Kingdom*, *84*(2), 383-392.

Rivera-Ingraham, G. A., Espinosa, F., & García-Gómez, J. C. (2011). Environmentally mediated sex change in the endangered limpet *Patella ferruginea* (Gastropoda: Patellidae). *Journal of Molluscan Studies*, *77*(3), 226-231.

Robertson, D. R. (1972). Social control of sex reversal in a coral-reef fish. *Science*, *177*(4053), 1007-1009.

Ross, R. M. (1990). The evolution of sex-change mechanisms in fishes. *Environmental Biology of Fishes*, *29*, 81-93.

Ross, R. M., Losey, G. S., & Diamond, M. (1983). Sex change in a coral-reef fish: dependence of stimulation and inhibition on relative size. *Science*, *221*(4610), 574-575.

Sakai, Y. (2022) Protogyny in Fishes. In: Kuwamura T, Sawada K, Sunobe T, Sakai Y, Kadota T (eds), *Hermaphroditism and Mating Systems in Fish*. Springer Nature, Singapore, pp 87–143.

Sakai, Y., Karino, K., Kuwamura, T., Nakashima, Y., & Maruo, Y. (2003). Sexually dichromatic protogynous angelfish *Centropyge ferrugata* (Pomacanthidae) males can change back to females. *Zoological science*, *20*(5), 627-633.

Sakai, Y., Kohda, M., & Kuwamura, T. (2001). Effect of changing harem on timing of sex change in female cleaner fish *Labroides dimidiatus*. *Animal behaviour*, *62*(2), 251-257.

Sakanoue, R., & Sakai, Y. (2022). Cryptic bachelor sex change in haremic colonial groups of the coral-dwelling damselfish *Dascyllus reticulatus*. *Journal of Ethology*, *40*(2), 181-192.

Santi, M., Picchi, L., & Lorenzi, M. C. (2018). Dynamic modulation of reproductive strategies in a simultaneous hermaphrodite and preference for the male role. *Animal Behaviour*, *146*, 87-96.

Sato, T., Kobayashi, M., Takebe, T., Hirai, N., Okuzawa, K., Sawaguchi, S., Matsubara, T., Yamaguchi, T., Shinoda, R., Koiso, M. & Teruya, K. (2018). Induction of female-to-male sex change in a large protogynous fish, *Choerodon schoenleinii*. *Marine Ecology*, *39*(1), e12484.

Sawada, K. (2022). Simultaneous hermaphroditism in fishes. In T. Kuwamura, K. Sawada, T. Sunobe, Y. Sakai, & T. Kadota (eds.), *Hermaphroditism and Mating Systems in Fish.* Springer Nature, Singapore, pp 31–62.

Schärer, L., & Ladurner, P. (2003). Phenotypically plastic adjustment of sex allocation in a simultaneous hermaphrodite. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *270*(1518), 935-941.

Schärer, L. (2009). Tests of sex allocation theory in simultaneously hermaphroditic animals. *Evolution*, *63*(6), 1377-1405.

Schärer, L., Rowe, L., & Arnqvist, G. (2012). Anisogamy, chance and the evolution of sex roles. *Trends in Ecology & Evolution*, *27*(5), 260-264.

Shapiro, D. Y., & Lubbock, R. (1980). Group sex ratio and sex reversal. *Journal of Theoretical Biology*, *83*(3), 411-426.

Smiley, K. O., Munley, K. M., Aghi, K., Lipshutz, S. E., Patton, T. M., Pradhan, D. S., & Solomon-Lane, T. K. (2024). Sex diversity in the 21st century: Concepts, frameworks, and approaches for the future of neuroendocrinology. *Hormones and Behavior*, *157*, 105445.

Soong, K., & Chen, J. L. (1991). Population structure and sex-change in the coral-inhabiting snail *Coralliophila violacea* at Hsiao-Liuchiu, Taiwan. *Marine Biology*, *111*, 81-86.

Soong, K., & Chen, M. H. (2003). Sex expression of an immobile coral-inhabiting snail, *Quoyula monodonta*. *Marine Biology*, *143*, 351-358.

Subramoniam, T. (2018). Mode of reproduction: Invertebrate animals. *Encyclopedia of reproduction*, *6*, 32-40.

Sunobe, T., Sakaida, S., & Kuwamura, T. (2016). Random mating and protandrous sex change of the platycephalid fish *Thysanophrys celebica* (Platycephalidae). *Journal of Ethology*, *34*, 15-21.

Todd, E. V., Liu, H., Muncaster, S., & Gemmell, N. J. (2016). Bending genders: the biology of natural sex change in fish. *Sexual Development*, *10*(5-6), 223-241.

Tomlinson, J. (1966). The advantages of hermaphroditism and parthenogenesis. *Journal of Theoretical Biology*, *11*(1), 54-58.

Tsitrone, A., Jarne, P., & David, P. (2003). Delayed selfing and resource reallocations in relation to mate availability in the freshwater snail *Physa acuta*. *The American Naturalist*, *162*(4), 474-488.

Vega-Frutis, R., Macías-Ordóñez, R., Guevara, R., & Fromhage, L. (2014). Sex change in plants and animals: A unified perspective. *Journal of Evolutionary Biology, 27*(4), 667–675.

Walker, S. P., & McCormick, M. I. (2009). Sexual selection explains sex-specific growth plasticity and positive allometry for sexual size dimorphism in a reef fish. *Proceedings of the Royal Society B: Biological Sciences*, *276*(1671), 3335-3343.

Warner, R. R. (1975). The adaptive significance of sequential hermaphroditism in animals. *The American Naturalist*, *109*(965), 61-82.

Warner, R. R. (1988). Sex change and the size-advantage model. *Trends in Ecology & Evolution*, *3*(6), 133-136.

Warner, R. R., & Robertson, D. R. (1978). *Sexual patterns in the labroid fishes of the western Caribbean, I, the wrasses (Labridae)* (Vol. 254). Washington: Smithsonian Institution Press.

Warner, R. R., & Swearer, S. E. (1991). Social control of sex change in the bluehead wrasse, *Thalassoma bifasciatum* (Pisces: Labridae). *The Biological Bulletin*, *181*(2), 199-204.

Warner, R. R., Fitch, D. L., & Standish, J. D. (1996). Social control of sex change in the shelf limpet, *Crepidula norrisiarum*: size-specific responses to local group composition. *Journal of Experimental Marine Biology and Ecology*, *204*(1-2), 155-167.

West, S. (2009). *Sex allocation*. Princeton University Press.

Wittenrich, M. L., & Munday, P. L. (2005). Bi-directional sex change in coral reef fishes from the family Pseudochromidae: an experimental evaluation. *Zoological science*, *22*(7), 797-803.

Wong, M. Y., Uppaluri, C., Medina, A., Seymour, J., & Buston, P. M. (2016). The four elements of within-group conflict in animal societies: an experimental test using the clown anemonefish, *Amphiprion percula*. *Behavioral Ecology and Sociobiology*, *70*, 1467-1475.

Wright, W. G. (1989). Intraspecific density mediates sex-change in the territorial patellacean limpet *Lottia gigantea*. *Marine Biology*, *100*, 353-364.

Wu, G. C., Tomy, S., Lee, M. F., Lee, Y. H., Yueh, W. S., Lin, C. J., Lau, E.L. & Chang, C. F. (2010). Sex differentiation and sex change in the protandrous black porgy, *Acanthopagrus schlegeli*. *General and Comparative Endocrinology*, *167*(3), 417-421.

Wu, G. C., Li, H. W., Huang, C. H., Lin, H. J., Lin, C. J., & Chang, C. F. (2016). The testis is a primary factor that contributes to epigenetic modifications in the ovaries of the protandrous black porgy, *Acanthopagrus schlegelii*. *Biology of Reproduction*, *94*(6), 132-1.

Yasuoka, N., & Yusa, Y. (2016). Effects of size and gregariousness on individual sex in a natural population of the Pacific oyster *Crassostrea gigas*. *Journal of Molluscan Studies*, *82*(4), 485-491.

Yasuoka, N., & Yusa, Y. (2017). Direct evidence of bi-directional sex change in natural populations of the oysters *Saccostrea kegaki* and *S. mordax*. *Plankton and Benthos Research*, *12*(1), 78-81.

Yusa, Y., Yoshikawa, M., Kitaura, J., Kawane, M., Ozaki, Y., Yamato, S., & Høeg, J. T. (2012). Adaptive evolution of sexual systems in pedunculate barnacles. *Proceedings of the Royal Society B: Biological Sciences*, *279*(1730), 959-966.

Zardus, J. D., & Hadfield, M. G. (2004). Larval development and complemental males in *Chelonibia testudinaria*, a barnacle commensal with sea turtles. *Journal of Crustacean Biology*, *24*(3), 409-421.

CHAPTER 4

ANTHING BUT PUERILE:

A Review of Sex Allocation Research in O. puerilis

Pooja Radhakrishnan[1]

[1]Laboratoire d’Éthologie Expérimentale et Comparée,
Université Sorbonne Paris Nord, Villetaneuse (France)

In preparation for submission to *Biological Reviews*
(expected Dec. 2025)

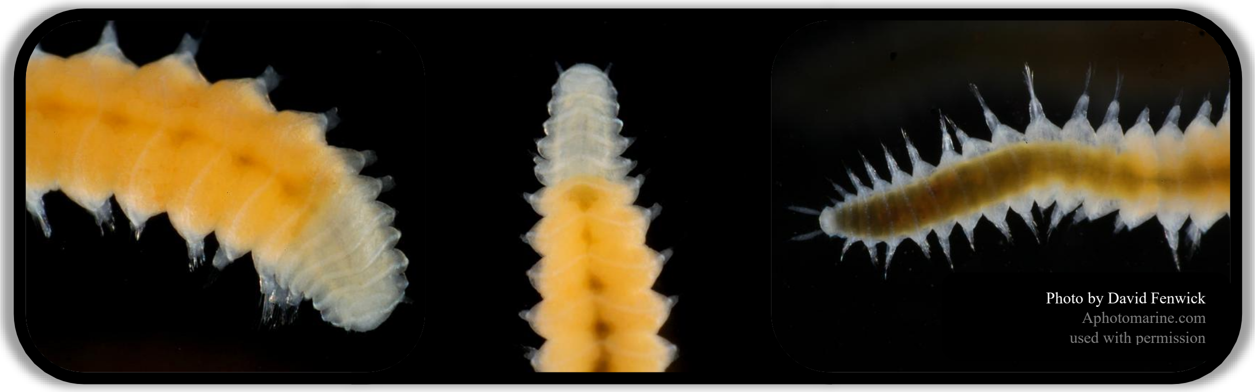

Figure 4.1. ***Ophryotrocha puerilis***: Specimen collected at Newlyn Marina, Newlyn, Cornwall.

Ophryotrocha puerilis is a marine annelid first discovered in 1869 at a marine station in Naples by Claparède and Metschnikow. The species' name *puerilis*—Latin for "childlike"—refers to its relatively "youthful" appearance (Paxton & Åkesson, 2007). *Ophryotrocha* worms belong to the class Polychaeta, so named for their characteristic bristle-bearing segments (chaetigers), and belong to the superfamily Eunicea and the family Dorvillidae (Åkesson, 1967).

4.1. **Evolutionary and Phylogenetic Context of *Ophryotrocha***

The genus *Ophryotrocha* currently contains over 80 described species, with new species being discovered constantly (Zhang et al., 2023). This genus is particularly notable for its diversity of sexual systems, even among closely-related and often sympatric species, ranging from gonochorism to simultaneous and sequential hermaphroditism and even occasional asexuality (Premoli & Sella, 1995; Dahlgren et al., 2001; Tempestini et al., 2020) (Table 4.1). The remarkable variation in reproductive strategies makes *Ophryotrocha* a model genus for evolutionary studies, as noted by Charnov (1982).

Table 4.1. Sexual Systems in *Ophryotrocha*

Species Name	Describing Author (Year)	Sexual System	Reference
Ophryotrocha adherens	Paavo et al., 2000	**Simultaneous Hermaphrodite**	Paavo et al., 2000
Ophryotrocha akessoni	Blake, 1985	Unknown	
Ophryotrocha alborana	Paxton & Åkesson, 2011	**Simultaneous Hermaphrodite**	Paxton & Åkesson, 2011
Ophryotrocha atlantica	Hilbig & Blake, 1991	Unknown	
Ophryotrocha beringiana	Alalykina & Polyakova, 2022	Unknown	
Ophryotrocha bifida	Hilbig & Blake, 1991	Unknown	
Ophryotrocha birgittae	Paxton & Åkesson, 2011	**Simultaneous Hermaphrodite**	Paxton & Åkesson, 2011
Ophryotrocha bohnorum	Zhang et al., 2023	Unknown	
Ophryotrocha cantabrica	Núñez et al., 2014	Unknown	
Ophryotrocha charlottae	Zhang et al., 2023	Unknown	
Ophryotrocha chemecoli	Ravara et al., 2021	Unknown	
Ophryotrocha clava	Taboada et al., 2013	Unknown	
Ophryotrocha cosmetandra	Oug, 1990	Unknown	
Ophryotrocha costlowi	Paxton & Åkesson, 2010	**Gonochoric**	Paxton & Åkesson, 2010
Ophryotrocha craigsmithi	Wiklund et al., 2009	**Gonochoric**	Svensson et al., 2023
Ophryotrocha cyclops	Salvo et al., 2014	Unknown	
Ophryotrocha dahlgreni	Georgieva et al., 2023	Unknown	
Ophryotrocha diadema	Åkesson, 1976	**Simultaneous Hermaphrodite**	Paxton & Åkesson, 2011
Ophryotrocha dimorphica	Zavarzina & Tsetlin, 1986	**Simultaneous Hermaphrodite**	Paxton & Åkesson, 2010
Ophryotrocha dubia	Hartmann-Schröder, 1974	Unknown	

Ophryotrocha eutrophila	Wiklund et al., 2009	**Gonochoric**	Wiklund et al., 2009
Ophryotrocha fabriae	Paxton & Morineaux, 2009		
Ophryotrocha flabella	Wiklund et al., 2012	Unknown	
Ophryotrocha geoffreadi	Ravara et al., 2021	Unknown	
Ophryotrocha gerlachi	Hartmann-Schröder, 1974	Unknown	
Ophryotrocha geryonicola	Esmark, 1874	**Gonochoric**	Pfannenstiel et al., 1982
Ophryotrocha globopalpata	Blake & Hilbig, 1990	**Gonochoric**	
Ophryotrocha gracilis	Huth, 1933	**Simultaneous Hermaphrodite**	Parenti, 1964
Ophryotrocha hadalis	Jumars, 1974	Unknown	
Ophryotrocha hanneloreae	Georgieva et al., 2023	Unknown	
Ophryotrocha hartmanni	Huth, 1933	**Simultaneous Hermaphrodite**	Prevedelli et al., 2005
Ophryotrocha irinae	Tsetlin, 1980	Unknown	
Ophryotrocha japonica	Paxton & Åkesson, 2010	**Gonochoric**	Simonini, 2002; Prevedelli et al., 2005; Paxton & Åkesson, 2010;
Ophryotrocha jiaolongi	Zhang et al., 2017	Unknown	Zhang et al., 2017
Ophryotrocha kagoshimaensis	Miura, 1997	Unknown	
Ophryotrocha kailae	Zhang et al., 2023	Unknown	
Ophryotrocha labidion	Hilbig & Blake, 1991	Unknown	
Ophryotrocha labronica	La Greca & Bacci, 1962	**Gonochoric**	Paxton & Åkesson, 2007, 2010
Ophryotrocha langstrumpae	Wiklund et al., 2012	Unknown	
Ophryotrocha lipovskyae	(Paxton, 2009)	Unknown	
Ophryotrocha lipscombae	Lu & Fauchald, 2000	Unknown	
Ophryotrocha littoralis	(Levinsen, 1879)	Unknown	

Ophryotrocha lobifera	Oug, 1978	Unknown	
Ophryotrocha longicollaris	Wiklund et al., 2012	Unknown	
Ophryotrocha longidentata	Josefson, 1975	Unknown	
Ophryotrocha lusa	Ravara et al., 2015	Unknown	
Ophryotrocha maciolekae	Hilbig & Blake, 1991	Unknown	
Ophryotrocha macrovifera	Paxton & Åkesson, 2010	**Gonochoric**	Paxton & Åkesson, 2010
Ophryotrocha maculata	Åkesson, 1973	**Simultaneous Hermaphrodite**	Åkesson, 1973
Ophryotrocha magnadentata	Wiklund et al., 2012	Unknown	
Ophryotrocha mammillata	Ravara et al., 2015	Unknown	
Ophryotrocha mandibulata	Hilbig & Blake, 1991	Unknown	
Ophryotrocha marinae	Zhang et al., 2023	Unknown	
Ophryotrocha mediterranea	Martin et al., 1991	**Gonochoric**	Martin et al., 1991
Ophryotrocha natans	Pfannenstiel, 1975	Unknown	
Ophryotrocha nauarchus	Wiklund et al., 2012	Unknown	
Ophryotrocha notialis	(Ehlers, 1908)	Unknown	
Ophryotrocha notoglandulata	Pfannenstiel, 1972	**Gonochoric**	Paxton & Åkesson, 2010
Ophryotrocha nunezi	Ravara et al., 2021	Unknown	
Ophryotrocha obtusa	Hilbig & Blake, 1991	Unknown	
Ophryotrocha orensanzi	Taboada et al., 2013	Unknown	
Ophryotrocha pachysoma	Hilbig & Blake, 1991	Unknown	
Ophryotrocha paragerlachi	Brito & Núñez, 2003	Unknown	
Ophryotrocha paralabidion	Hilbig & Blake, 1991	Unknown	
Ophryotrocha permanae	Paxton & Åkesson, 2010	**Mixed**	Paxton & Åkesson, 2010

Ophryotrocha platykephale	Blake, 1985	Unknown	
Ophryotrocha pruittae	Zhang et al., 2023	Unknown	
Ophryotrocha puerilis	Claparède & Mecznikow, 1869	**Sequential Hermaphrodite**	Paxton & Åkesson, 2007
Ophryotrocha ravarae	Georgieva et al., 2023	Unknown	
Ophryotrocha robusta	Paxton & Åkesson, 2010	**Gonochoric**	Paxton & Åkesson, 2010
Ophryotrocha rubra	Paxton & Åkesson, 2010	**Gonochoric**	Paxton & Åkesson, 2010
Ophryotrocha sadina	Ravara et al., 2015	Unknown	
Ophryotrocha scarlatoi	Averincev, 1989	Unknown	
Ophryotrocha schubravyi	Tsetlin, 1980	**Gonochoric**	Paxton & Åkesson, 2010
Ophryotrocha scutellus	Wiklund et al., 2009	Unknown	
Ophryotrocha seepens	Alalykina & Polyakova, 2022	Unknown	
Ophryotrocha shieldsi	Paxton & Davey, 2010	Unknown	
Ophryotrocha socialis	Ockelmann & Akesson, 1990	**Simultaneous Hermaphrodite**	Ockelmann & Åkesson, 1990
Ophryotrocha spatula	Fournier & Conlan, 1994	Unknown	
Ophryotrocha splendida	Brito & Núñez, 2003	Unknown	
Ophryotrocha tridentata	(Orensanz, 1990)	Unknown	
Ophryotrocha urbis	Jimi et al., 2019	Unknown	
Ophryotrocha vellae	Paxton & Åkesson, 2010	**Gonochoric**	Paxton & Åkesson, 2010
Ophryotrocha vivipara	Banse, 1963	**Gonochoric**	Banse, 1963; Åkesson, 1994
Ophryotrocha wubaolingi	Miura, 1997	Unknown	
Ophryotrocha zitae	Miranda et al., 2020	Unknown	

Phylogenetic analyses indicate that reproductive modes are distributed non-randomly across the genus (Dahlgren et al., 2001; Tempestini et al., 2020) (Fig. 4.2). The ancestral reproductive mode is debated: Dahlgren et al. (2001) suggested sequential hermaphroditism evolved from a simultaneous hermaphroditic ancestor, whereas more recent analyses using mitochondrial (COI, 16S) and nuclear (H3) markers support gonochorism as the most parsimonious ancestral state (Tempestini et al., 2020).

Regardless of the ancestral condition, sequential hermaphroditism in *O. puerilis* represents a derived trait. Phylogenetic reconstructions (Fig. 4.2) show that sequential hermaphroditism is largely confined to the *O. puerilis* species complex (Table 4.2), which is nested within a clade of other hermaphroditic species exhibiting simultaneous hermaphroditism (Tempestini et al., 2020). Therefore, while the evolution of sequential hermaphroditism is highly significant for understanding sex change, it appears to be a specialized strategy that arose within a limited clade rather than a basal characteristic of the genus.

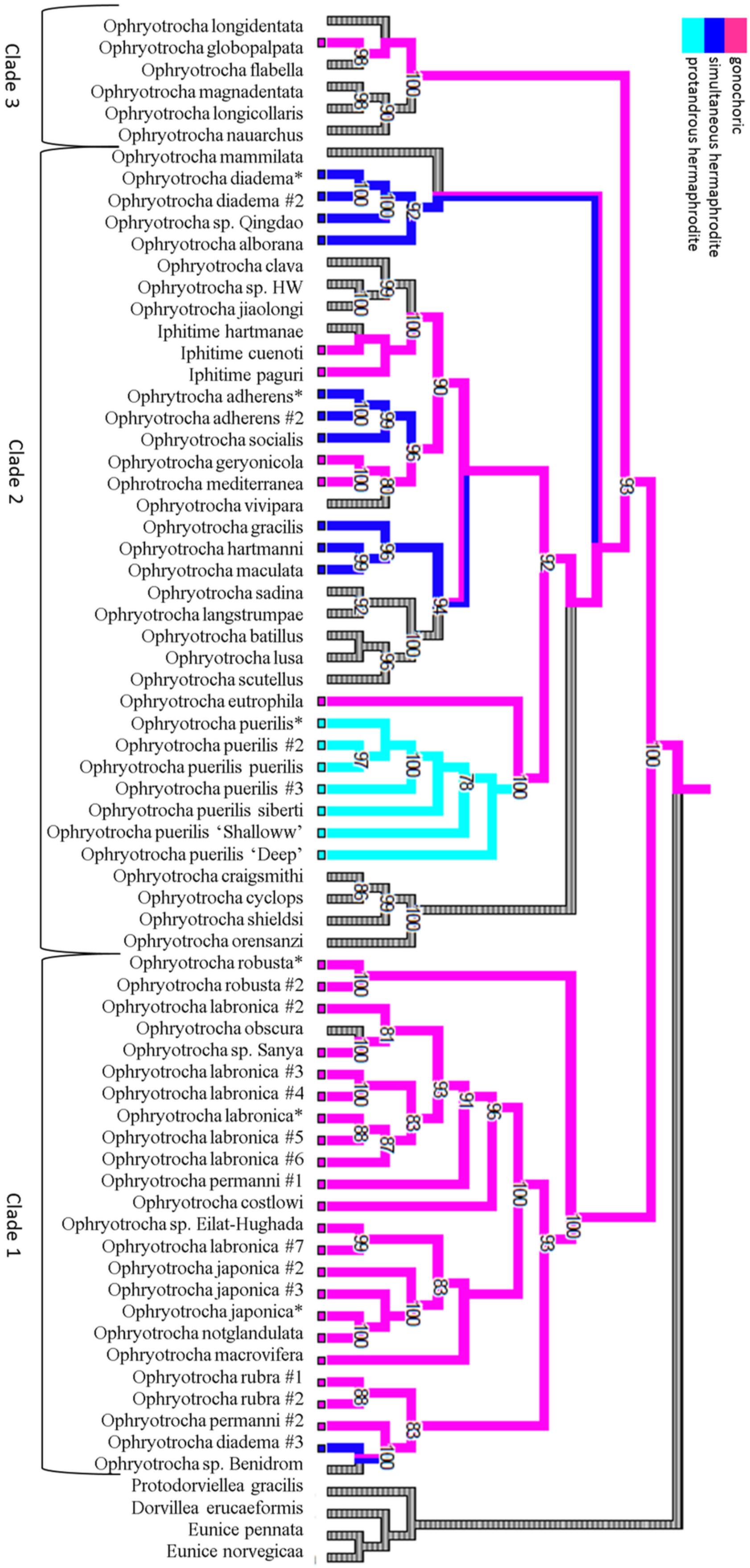

Figure 4.2. Phylogeny of *Ophryotrocha* with Ancestral State Reconstruction of Sexual Systems (reproduced from Tempestini et al., 2020). Node and branch coloration indicate the most parsimonious reconstructed ancestral state: Gonochorism (Pink), Simultaneous Hermaphroditism (Blue), and Sequential Hermaphroditism (Cyan). The phylogeny supports the derived nature of sequential hermaphroditism, which is confined to the *O. puerilis* clade and nested within a larger hermaphroditic lineage.

The phylogenetic tree was constructed using Maximum Likelihood based on concatenated mitochondrial (COXI, 16S) and nuclear (H3) gene markers. Numbers adjacent to the nodes indicate statistical support (Bootstrap/Posterior Probability).

The evolutionary and taxonomic history of *O. puerilis* is complex, primarily due to the high morphological similarity yet significant genetic divergence within its species complex (Table 4.2). Historically, the two major recognized forms were the subspecies *O. puerilis puerilis* from the Mediterranean Sea (Claparède & Metschnikow, 1869) and *O. puerilis siberti* from the Atlantic Ocean (McIntosh, 1885). Bacci & La Greca (1953) initially distinguished these two populations based on morphological differences (mandible shape) and evidence of reproductive isolation (crossbreeding failure). However, recent molecular phylogenetic evidence (COI gene analysis), however, confirms significant genetic divergence between the lineages, leading to the current consensus that *O. puerilis puerilis* and *O. puerilis siberti* should be treated as distinct species (Tilic & Rouse, 2024).

Table 4.2: Taxonomic Status of the *Ophryotrocha puerilis* Complex

Species	Taxonomic Authority	Geographic Origin	Taxonomic Status	Sexual System
Ophryotrocha puerilis puerilis	Claparède & Metschnikow (1869)	Mediterranean Sea (Naples)	Species (Tilic & Rouse, 2024)	Protandrous Sequential Hermaphrodite (Åkesson, 1974)
Ophryotrocha puerilis siberti	McIntosh (1885)	Atlantic Ocean (Plymouth)	Species (Tilic & Rouse, 2024)	Protandrous Sequential Hermaphrodite (Åkesson, 1974)
Ophryotrocha puerilis 'Deep'	Taboada et al. (2017)	Mediterranean Sea (Coastal)	Cryptic Lineage (Taboada et al., 2017)	Protandrous Sequential Hermaphrodite (Tempestini et al., 2020)
Ophryotrocha puerilis 'Shallow'	Taboada et al. (2017)	Mediterranean Sea (Submarine Canyon)	Cryptic Lineage (Taboada et al., 2017)	Protandrous Sequential Hermaphrodite (Tempestini et al., 2020)

Compounding this complexity, phylogenetic analysis of *O. puerilis* populations from the Mediterranean has also revealed the co-occurrence of at least two cryptic species (morphologically identical but genetically distant) temporarily referred to as *O. puerilis* 'Shallow' and *O. puerilis* 'Deep' (Taboada et al., 2017) (Table 4.2; Section 4.2).

4.2. **Habitat, colonization & colony persistence**

Ophryotrocha puerilis are highly opportunistic colonizers, frequently inhabiting soft sediments, wharf areas, and, most commonly, the interstitial spaces within mussel colonies (Taboada et al., 2016; Fig. 4.3).

Figure 4.3. Mussel colonies on anchor chain, a typical habitat for *O. puerilis*

Taboada et al. (2016) investigated the colonization process by placing several bones of minke whales *Balaenoptera acutorostrata*, cows *Bos primigenius*, and pigs *Sus domesticus* along the Mediterranean coast of Blanes at depths of 20 meters for one year. Additionally, whale bones were placed at a depth of 53 meters in the Blanes submarine canyon.

Quarterly analysis of the bones revealed that *O. puerilis* was consistently the first invertebrate to colonize the bones and was often the most abundant species, although population densities fluctuated during different times of the year (Taboada et al., 2016; Premoli & Sella, 1995; Sella & Ramella, 1999; Berglund, 1986; Prevedelli et al., 2005). During low-density periods, populations can drop to fewer than one individual per 150 grams of substrate, whereas in high-density conditions, individuals tend to form clusters, which allow for social interactions (Sella & Ramella, 1999). Similar group clusters are also observed in laboratory populations (personal observation), resembling the swarming behaviour observed in terrestrial worms.

These established communities included adults, juveniles, and even egg masses, suggesting that colonies, once established, exhibit a degree of stability and continuous reproduction. Body size of the individuals found on the bones ranged from 0.375mm – 4.125 mm (Taboada et al., 2016). Since the typical adult body size for *O. puerilis* ranges from 4 mm – 7 mm (Thornhill et al., 2009), I infer that these populations were comprised of relatively fewer females and a predominance of juveniles and males, which is characteristic of a protandrous species.

Haplotype analysis was performed on these individuals to better understand their colonization processes and taxonomic relationships (Taboada et al., 2017). The results suggested that the sampled populations comprise a species complex, identifying *O. puerilis* and two newly proposed sibling species: *O. puerilis* 'Shallow' and *O. puerilis* 'Deep'. The 'Shallow' population was found across the depth gradient (both 20 m and 53 m), while the 'Deep' population was primarily restricted to 53 m, suggesting a pattern of allopatric or parapatric distribution based on depth, though further research is required to clarify sympatry. Genetic analysis indicated that colonized communities tend to be relatively stable, with many individuals persisting over time, alongside potential new recruits and individuals that disperse after an initial phase (Taboada et al., 2017).

Closer analysis of faecal matter revealed that *O. puerilis* are both carnivores and omnivores, primarily feeding on *Beggiatoa*-like bacteria and algae growing on the bone substrate (Taboada et al., 2016). Interestingly, the authors also found remnants of chaetigerous segments and mandibles of *Ophryotrocha* spp. (the specific species is unknown, but it is possible that these remnants are from biting conspecifics, as same-sex violent fighting behaviour has been previously recorded by Berglund, 1986; 1990; 1991; or potentially even cannibalism, Taboada et al., 2016). In line with these omnivorous/carnivorous findings**,** other studies have reported that *O. puerilis* feeds mainly on diatoms and filamentous algae growing on stones and shells (Szaniawski, 1974) and has been observed scraping algae off aquarium walls with its jaws (Simonini et al., 2010).

4.3. **Reproductive biology of *Ophryotrocha puerilis***

Ophryotrocha puerilis is unique within its genus for being the only species to exhibit sequential hermaphroditism (Sella & Ramella, 1999; Tempestini et al., 2020) (Table 4.1). Worms live for four to five months on average under laboratory conditions (Prevedelli et al., 2006). Individuals typically mature first as males at 9-10 segments and then change sex to female at 16 - 18 segments (Åkesson, 1974; Berglund, 1986, 1990). Additionally, *O. puerilis* is also capable of sex reversal (changing from female back to male), which takes about 5 days (Berglund, 1986, 1990). However, the timing and body size at sex change and sex reversal are highly plastic, influenced by various factors such as individual condition, temperature, pheromonal cues, and social interactions (see Table 4.3 & Section 4.4 below).

These worms are continuous spawners, they do not have a defined reproductive season (Pfannenstiel & Grünig, 1982; Jacobson, 1999), and spawning occurs every 5 days in the pair-culture (Berglund, 1986, 1990). A reproductive cycle, from the time of laying a cocoon (a gelatinous mucous matrix) to the next spawning, lasts approximately 35 days at 20°C (Åkesson, 1967). Females can carry up to three generations of oocytes at any given time, distinguishable by size, in the coelom (Pfannenstiel, 1975), with mature oocytes measuring between 100-110 µm (Paxton & Åkesson, 2007). In isolated females, eggs reaching 120 µm are not shed but resorbed, as isolated females never lay eggs (Pfannenstiel, 1975).

Reproduction is thought to occur through pseudocopulation, where gametes are released simultaneously into the cocoon secreted by the female (Westheide, 1984; Jacobsohn, 1999; Paxton & Åkesson, 2007). Female fecundity is strongly size-dependent (Berglund, 1986; 1990): small females may produce only about 30 eggs in their first clutch, while larger females can lay up to 350 – 400 eggs per clutch (Åkesson; 1967). However, females also experience reproductive senescence, particularly if they invest in the female function from an early age (Berglund, 1986).

Both parents care for the cocoon by engaging in constant "housekeeping" behaviour, cleaning the jelly mucous, presumably to protect the eggs from bacteria or fungi, and moving through the cocoon, likely to aerate it (Åkesson, 1967, 1973). Åkesson (1967) observed that females typically do not forage in the first few days after laying a cocoon. Absence of parental care during this early brooding phase results in nearly 100% mortality (Åkesson, 1967, but see Berglund, 1986).

Larvae emerge from the cocoon after 7 – 8 days, and begin forming chaetigerous segments one at a time (Åkesson 1967). Unlike some polychaetes, such as *Osedax* spp., whose larvae have a planktonic dispersal phase, *O. puerilis* larvae undergo direct development. This may explain the persistence of colonies and the overlapping of generations in the colonization study (Taboada et al., 2017). Nevertheless, as a colonizing species, some dispersal capacity is still expected (Taboada et al., 2017).

4.4. **Sex Change in *Ophryotrocha puerilis*:**
Historical contributions

Ophryotrocha puerilis has been one of the most extensively studied polychaetes, famously referred to as the "*Drosophila* of polychaete literature" (Paxton & Åkesson, 2007; Charnov, 1982) due to a substantial body of research on sex change and sex reversal (Table 4.3). However, there has been a notable gap in research on this species over the past three decades. In the following sections, I outline the current understanding of the physiological, genetic, environmental, and social factors that influence sex change in *O. puerilis*.

4.4.1. **Physiological and Environmental Influences on Sex Reversal**

The earliest empirical research on sex determination and sex change in *O. puerilis* dates back almost a century to the significant contributions of Hartmann & Huth (1936) and Hartmann & von Lewinski (1938, 1940) (Table 4.3). Hartmann & Huth (1936) initially proposed that sex was phenotypically determined, suggesting that all young animals were males and older animals were females, with sex change occurring around 15 – 20 segments.

In this pre-Charnov era, before the widespread acceptance of phenotypic plasticity, experiments on the environmental and physiological influences on the timing of sex change or reversal were often framed with a somewhat rigid cause-and-effect approach. For instance, Hartmann & Huth (1936) discovered that bodily injury, specifically the amputation of posterior segments, or starvation, could induce females to revert to the male sex. Later, Hartmann & von Lewinski (1938) also demonstrated that elevated concentrations of copper, magnesium, and potassium ions in the environment induced sex reversal in females (as cited in Jaccarini et al., 1983).

Table 4.3. Historical contributions on the physiological, genetic, and social influences on body size and timing of sex change in *Ophryotrocha puerilis*

Author	Year	Contribution	References
Hartmann & Huth	1936	Discovered the pair-culture effect Isolated individuals change sex to female early and never revert to male The presence of female induces sex reversal Amputation and starvation induce sex reversal	Hartmann & Huth, 1936; Pfannenstiel, 1975, 1977, 1978; Berruti, 1980; Jaccarini et al., 1983; Kegel & Pfannenstiel, 1983
Hartmann & von Lewinski	1938	Found that elevated concentrations of copper, magnesium, and potassium ions induce sex change At high densities, sex ratio fluctuations result in large males and sex reversed males Juveniles delay sex change when subjected to starvation or "egg extracts"	Hartmann & von Lewinski, 1938; Jaccarini et al., 1983
Hartmann & von Lewinski	1940	Demonstrated that extracts from ripe females induce sex reversal in females In pair-culture, the female with smaller oocytes reverts to male	Marchionni & Rolando, 1981; Jaccarini et al., 1983
Hartmann & von Lewinski	1942	Showed that Eiweißstoffe (water-soluble extracts from mature females) induce sex reversal to male in smaller females	Pfannenstiel, 1975; Charnov, 1982
Berruti	1980	Found that. starvation leads to slower growth rates and induces sex reversal in both isolated individuals and pairs of females (therefore, disrupting the pair-culture)	Berruti, 1980
Bacci	1951	Explored the role of genetic influences on variation in body size and age at sex change	Bacci, 1951
Bacci & La Greca	1953	Chromosome number (2n=8) Crossbreeding between *O. puerilis puerilis* (Naples) and *O. puerilis siberti* (Plymouth) resulted in embryo degeneration, supporting their classification as sibling species	Bacci & La Greca, 1953
Bacci & Bortesi	1961a	Demonstrated polygenic sex determination Artificial selection produced 'pure' males (remaining male to 23 segments) and 'pure' females (changing at 11 segments) through selection in male-biased and female-biased lines	Bacci & Bortesi, 1961a

Continued on next page

Table 4.3. Historical contributions on the physiological, genetic, and social influences on body size and timing of sex change in *Ophryotrocha puerilis (Continued)*

Author	Year	Contribution	References
Bacci & Bortesi	1961b	Extended prior findings on polygenic sex determination Produced 'pure' male and female lines through selective breeding of arrhenogenous (male-promoting) and thelygenous (female-promoting) lines in *Ophryotrocha puerilis siberti*	Bacci & Bortesi, 1961b
Bacci	1965	Proposed that genetic balance between male and female genes is crucial for the normal expression of either sex.	Bacci, 1965
Bacci & Bortesi	1967	Found that 'pure' males and females exhibit significantly reduced fertility Therefore, the exclusion of one sexual function jeopardizes the expression and fertility of the other Crossing individuals from the arrhenogenous and thelygenous lines reestablishes sex balance and improves fertility	Bacci & Bortesi, 1967; Jaccarini et al., 1983
Bacci & Voria	1970	Investigated the effect of temperature on timing and body size at sex change: at 10°C, 18 segments in ~40 days at 18°C, 17 segments in ~11 days at 21°C, 16 segments in ~12 days Suggested genetic differences could account for strain variations.	Bacci & Voria, 1970
Müller	1962	Direct contact is necessary to facilitate pair-culture effect	Marchionni & Rolando, 1981; Kegel & Pfannenstiel, 1983
Sella	1980	Suggested that body size at sex change may have been under stabilizing selection Confirmed Bacci’s (1965) & Bacci & Bortesi's (1967) assertion that body size at sex change responds to artificial selection, and that a biased expression of only one sex decreases fitness	Sella, 1980

Continued on next page

Table 4.3. Historical contributions on the physiological, genetic, and social influences on body size and timing of sex change in *Ophryotrocha puerilis (Continued)*

Author	Year	Contribution	References
Pfannenstiel	1975	Proposed the possibility of endocrinological control of sex change. Timeline of pair-culture: i. female sheds oocytes in 4 days ii. replaces p-jaw with K-jaw iii. produces sperm in 8 days Suggested that a pheromone facilitating pair-culture may be transmitted and received by the prostomium An ootropic hormone which is released from the prostomium differentiates undifferentiated gonia into oocytes, removal of prostomium results in sex reversal	Pfannenstiel, 1975, 1977, 1978; Kegel & Pfannenstiel, 1983
Pfannenstiel	1977	Suggested the possibility of a contact pheromone inhibiting the ootropic hormone in partners, thereby inducing sex reversal in pair-culture Located regions of the prostomium responsible for the release and receipt of the stimulus (however, see Kegel & Pfannenstiel, 1983 below)	Pfannenstiel, 1977
Pfannenstiel	1978	Confirmed role of an ootropic hormone by showing that decerebrated juveniles mature as males, but only individuals with intact female prostomia complete the female phase Hormonal influence is necessary for oocyte differentiation, maintaining the male phase in its absence	Pfannenstiel, 1978
Pfannenstiel & Grünig	1990	Demonstrated that both sperm and oocytes originate from undifferentiated gonia Proliferation of oocytes is triggered by an ootropic hormone	Pfannenstiel & Grünig, 1990
Marchionni & Rolando	1979	Tested whether 'bonellin', the masculinizing pigment in *Bonellia viridis*, influences sex change Treatment with 0.1 µg/ml of bonellin pigment induced sex change at a relatively smaller body size and higher fertility and reproductive rate	Marchionni & Rolando, 1979
Marchionni & Rolando	1981	Found evidence of a sexual pheromone Ether-soluble extracts triggered oocyte degeneration more frequently than water-soluble extracts	Marchionni & Rolando, 1981

Continued on next page

Table 4.3. Historical contributions on the physiological, genetic, and social influences on body size and timing of sex change in *Ophryotrocha puerilis (Continued)*

Author	Year	Contribution	References
Jaccarini et al.	1983	Comparison of *O. puerilis* to social sex determination in *Bonellia viridus*	Jaccarini et al., 1983
Kegel & Pfannenstiel	1983	Suggested that an unbalanced sex ratio may induce sex reversal at high densities, contradicting previous findings by Pfannenstiel (1975)	Kegel & Pfannenstiel, 1983
Berglund	1986	Explained the pair-culture effect through costs of reproduction, reporting a growth-rate advantage for males Observed that aggression was not sex-specific: both males and females injured each other in the presence of the opposite sex, leading to reproductive suppression in losing females Found that paired females had higher variance in growth than isolated females 4 out of 14 pairs in the pair-culture reportedly became simultaneous hermaphrodites, producing cocoons approx. every 3 days as opposed to approx. 5 days in the other pairs	Berglund, 1986
Berglund	1990	Tested the size-advantage hypothesis Large males generally win contests for access to females, but this advantage is often negated by female choice Females prefer males smaller than themselves to avoid conflicts over the female role Violent fighting occurs among females for the female role, with the loser reverting to the male phase The cost of sex change is approximately 5 days Suggested that jaw size may confer an advantage in fights, with larger jaws being more effective	Berglund, 1990; Sella & Ramella, 1999
Berglund	1991	Investigated the adaptive value of sex change in *O. puerilis* by making comparisons with the closely related gonochoristic *O. labronica* Proposed that female preference for smaller males maintains sequential hermaphroditism in *O. puerilis*, while male size advantage stabilizes gonochorism in *O. labronica*	Berglund, 1991

Hartmann & Huth (1936) were also the first to describe the "pair-culture effect", wherein pairing two females triggered sex reversal in the smaller female, thereby forming a functional breeding pair. Subsequent studies revealed that it was specifically the female with less ripe eggs that reversed to male (Hartmann & von Lewinski, 1940, however, see Kegel & Pfannenstiel, 1983). Hartmann & von Lewinski (1940) further found that extracts from ripe females, which they termed *Eiweißstoffe* (German for protein substances), were sufficient to induce sex reversal in the smaller female (as cited in Pfannenstiel, 1975; Marchionni & Rolando, 1981; Jaccarini et al., 1983). Marchionni & Rolando (1981) later tested for a sexual pheromone by exposing females to both water-soluble and ether-soluble extracts from females. They found that ether-soluble extracts induced oocyte degeneration and sex reversal more frequently than water-soluble extracts, suggesting a lipidic nature for the pheromone. However, Müller, (1962) and Pfannenstiel (1975, 1978) reported that sex reversal in the pair-culture is only triggered through direct contact, possibly involving a contact pheromone (see Section 4.3.3).

4.4.2. **Genetic Predisposition Towards Male- or Female-ness**

Bacci (1951) expanded upon Hartmann's work, suggesting that environmental influence on sex does not preclude the possibility of genetic sex determination. During his observations, he found that age or body size did not strictly coincide with phenotypic sex, documenting 'continuous variation' in the size at which sex change occurred (Bacci, 1951). For example, he documented instances of 11-segment females and 32-segment males (Bacci, 1951), whereas Hartmann (1936) had reported that sex change to female typically occurred at 15 – 20 segments.

Bacci (1951) also described how, while *O. puerilis* is a protandrous sequential hermaphrodite, some individuals expressed only one sex throughout their lives, either bypassing the male phase entirely to become females at a young age or prolonging the male phase indefinitely and never changing sex. He referred to these individuals as ‘pure’ or ‘primary’ males and females.

4.4.2.1. ***Heritability of body size at sex change***

Evidence for the heritability of body size at sex change comes from comparisons between geographically separated populations (Bacci & La Greca, 1953). In laboratory experiments, worms originating from the Bay of Naples changed sex at an average of 18 segments, while those from Plymouth changed sex at an average of 20 segments (Bacci & La Greca, 1953). This difference may be partly attributable to the influence of temperature on sex change; Bacci & Voria (1970) demonstrated that higher temperatures triggered sex change at younger ages and smaller body sizes (e.g., ~ 40 days at 10°C compared to just ~ 11 days at 18°C).

The authors further suggested that the difference in body size at sex change between these two strains likely arose from genetic differences, as these trends persisted even when they were reared at the same temperature (Bacci & Voria, 1970). Furthermore, crosses between individuals obtained from Naples and Plymouth resulted in degenerated embryos that were consumed by the parents (Bacci & La Greca, 1953). Based on this evidence of reproductive isolation as well as morphological differences in the mandibles, Bacci & La Greca (1953) suggested these two populations might represent subspecies—the Mediterranean *O. puerilis puerilis* Claparède & Metschnikow, 1869, and the Atlantic *O. puerilis siberti* McIntosh, 1885.

Charnov (1982) questioned this subspecies classification, due to the clear morphological differences in jaw shape as well as the failure of crossing experiments. This distinction has now been phylogenetically confirmed by Tilic & Rouse (2024), who, based on significant COI gene divergence, propose that the two populations should indeed be treated as distinct species (Table 4.2).

4.4.2.2. ***Artificial selection of early and late sex changers***

Bacci & Bortesi (1961a, b, 1967) successfully conducted several artificial selection experiments to test the genetic basis of sex change. Bacci & Bortesi (1961b) demonstrated the heritability of body size at sex change by selecting worms that either delayed or accelerated the process. They paired two worms from the Atlantic population, which typically change sex at around 20 segments (Bacci & La Greca, 1953). In this study, the F0 generation changed sex at approximately 18.8 segments. The experiment consisted of two selection lines: the 'arrhenogenous' line, which selected for late sex change (and extended 'maleness'), and the 'thelygenous' line, which selected for early sex change (promoting 'femaleness') (Bacci & Bortesi, 1961b).

Over successive generations, a clear heritable trend emerged. In the late sex change line, the average size at which sex change occurred increased incrementally, from 20.9 segments in F1 to 22 segments in F2, and 23 segments in F3. Conversely, in the early sex change line, the average size at which sex change occurred gradually decreased, from 18 segments in F1 to 17 segments in F2, and 16.44 segments in F3. By F4, Bacci & Bortesi (1961a) identified individuals that changed sex at 11 segments, presumably skipping the male phase entirely, as well as individuals that never changed sex despite reaching sizes of 26 segments or even 42 segments. Crosses between early sex changers (changing at 15 segments) and never-changers (which did not change sex despite being 23 segments) resulted in a nearly mid-point size at sex change of 18.69 segments (Bacci & Bortesi, 1961b). The authors concluded that *O. puerilis* has a polygenic sex determination system, suggesting that 'pure' males and females can be derived through selection experiments (Bacci & Bortesi 1961a; Bacci, 1965).

4.4.2.3. *Fertility implications of extreme bias in sex expression*

There was a marked decrease in fertility among the early sex changers and never-changers, as if selection for biased sex expression through complete exclusion of the opposite sex compromised fitness in both sexual functions (Bacci, 1965). This is consistent with the convex fitness curves predicted by Charnov's Size-Advantage Model, in which deviations from the optimal size or timing of sex change reduce total reproductive success (Charnov, 1979, 1982).

The alternative explanation of high inbreeding levels jeopardizing fertility was rejected, as crosses between pure male and pure females restored fertility (Bacci & Bortesi, 1967). It was hypothesized that this restoration of fertility upon interaction with individuals of the opposite sex was due to a pheromone produced by one sex that activates the sex genes of the opposite sex, similar to the pheromonal influence by females on sex reversal in smaller females facilitating the pair-culture (Pfannenstiel, 1975), thereby mitigating the lack of sex genes in inbred lines (Bacci & Bortesi, 1967; Jaccarini et al., 1983). Therefore, while *O. puerilis* exhibits genetic sexual bipotency, any extreme deviation towards pure male or female expression is unstable (Bacci, 1965; Bacci & Bortesi, 1967).

Sella (1980), building on Bacci's research with her own genetic model, suggested that body size at sex change in *O. puerilis* may have been under stabilizing selection in the past and perhaps continues to be, despite being an important component of fitness. Therefore, while body size influences fitness, the relative balance in germ cell production may play an even more critical role, as demonstrated by the infertility observed in the pure males and females (Bacci & Bortesi, 1967; Sella, 1980).

4.4.3. **Social influence on sex change in *O. puerilis***

In *O. puerilis*, isolation of adults promotes sex change to, or maintenance of, the female phase, while the presence of a larger female, or one with more mature eggs, triggers sex reversal to male, as observed in the pair-culture effect (Hartmann & Huth, 1936; Pfannenstiel, 1975).

Specifically, Müller (1962) found that direct physical interaction was necessary to induce this sex reversal; worms separated by a mesh that allowed water flow between them remained female (Pfannenstiel, 1977; Kegel & Pfannenstiel, 1983). Therefore, Hartmann & von Lewinski's (1942) finding that water-soluble extracts "Eiweißstoffe" from mature females were sufficient to induce sex reversal was ultimately not upheld. The need for physical contact to trigger the pair-culture effect prompted Pfannenstiel (1975, 1977) to investigate whether the source might be a contact pheromone.

4.4.3.1. ***Possibility of a contact pheromone facilitating sex change***

Ophryotrocha puerilis lacks specialized reproductive organs, both sperm and oocytes originating from the same stem cells (Pfannenstiel & Grünig, 1990). So how exactly does sex change occur at the cellular level?

The differentiation of undifferentiated gonia into oocytes is triggered by the production of an ootropic (meaning feminizing) hormone from the prostomium (the anterior segment of an annelid worm, essentially the 'head'), which is released into the coelomic cavity (Pfannenstiel, 1975; Pfannenstiel & Grünig, 1982). Interestingly, surgical removal of the prostomium in males prevents sex change, while in females, it causes a sex reversal to male (Pfannenstiel, 1975). This was not merely a consequence of injury due to the operation, because transplanting a female's prostomium into the body of prostomium-less worms immediately triggered the proliferation of oocytes (Pfannenstiel, 1975). Therefore, instead of a gonadal transformation, as is typical during sex change in most sequential hermaphrodites, in *O. puerilis*, the early stages of gametogenesis are identical. The eventual differentiation of this 'bi-potential gamete' into either oocytes or sperm is guided by the feminizing ootropic hormone.

Pfannenstiel (1978) observed that decerebrated (i.e., with the prostomium, or head, removed) juveniles of *O. puerilis* matured as males but were unable to produce eggs; decerebrated males extended their male phase, while decerebrated females reverted to males. Additionally, if a prostomium from a male was implanted into a prostomium-less individual (whether juvenile, male, or female), the individual continued to extend its male phase. In contrast, when a prostomium from a female was implanted, decerebrated males underwent a normal sex change after reaching 16 segments, and decerebrated females resumed normal oocyte production. Therefore, the implantation of the female's prostomia had a feminizing effect, confirming earlier findings (Pfannenstiel, 1975) that a sex-specific gonadotropic (ootropic) hormone guides the differentiation of undifferentiated gonia into oocytes, and that, in the absence of this hormone, the male phase is maintained (Pfannenstiel, 1978).

4.4.3.2. *Social control of sex reversal?*

If sex change to female and the maintenance of the female phase are mediated by the production of the ootropic hormone in the prostomium, how then is sex reversal to male triggered in pairs of females, who both have their prostomia intact and therefore are capable of producing this hormone? Pfannenstiel (1975) suggested that this sex reversal must contradict the conditions that initially led to the change in sex. He hypothesized that females might transmit a pheromone that is released upon direct physical contact, potentially influencing the endocrine system of the recipient and inhibiting the production of the ootropic hormone. It is possible that a larger female produces higher concentrations of this pheromone, or that the strength of the stimulus correlates with the maturity of the oocytes, resulting in the smaller female or the female with relatively smaller oocytes reverting to male (Pfannenstiel, 1977). Such hormonal control of sex change imposed by females on smaller females and on males results in a male-biased sex ratio and therefore stronger male-male competition (Sella & Ramella, 1999).

4.4.3.3. ***Sex ratio induction of sex change and sex reversal***

Through experiments with high density populations of worms, Kegel & Pfannenstiel (1983) concluded that individuals change sex in response to unbalanced sex ratios, with the adult sex ratio oscillating around 1:1. Indeed, Charnov & Bull (1989) predicted that in species with early sex changers, i.e., individuals that essentially mature directly as the second sex, as might be the case in *O. puerilis* (see Discussion), the sex ratio is expected to be less biased towards the first sex. However, in their study, Kegel & Pfannenstiel (1983) excluded sexually mature primary males capable of fertilization from their sex ratio calculation, including them would result in the male-biased sex ratio typical of protandry (Avise and Mank, 2009). Indeed, male-biased sex ratios are indeed the norm in both laboratory populations as well as in nature (Berglund, 1991; Premoli & Sella, 1995; Sella & Ramella, 1999; personal observation).

Nevertheless, the finding that individuals change sex in response to unbalanced sex ratios remains valid, as sex ratio induction of sex change (Ross, 1990) has been observed in some sequential hermaphrodites (Shapiro & Lubbock, 1980). For instance, in the sea goldie *Pseudanthias squamipinnis*, which typically lives in a strict single-male harem in small groups, multiple males can coexist in larger groups. In these large groups, the experimental removal of males results in a one-to-one sex change in the females (Shapiro, 1981). Just like *O. puerilis*, *P. squamipinnis* is also capable of bidirectional sex change in response to sex-ratio fluctuations.

4.4.4. **Size-Advantage Hypothesis and Bidirectional Sex Change in *O. puerilis***

Charnov (1982) highlighted *Ophryotrocha* as a unique genus for investigating sex allocation due to its diversity of sexual systems, even among closely related, often sympatric species. Berglund (1991) conducted comparative studies on *O. puerilis* and its gonochoristic congener *O. labronica* to investigate the adaptive value of these different sexual systems. The size-advantage hypothesis proposes that sequential hermaphroditism occurs when the reproductive value of the two sexes changes with body size (Ghiselin, 1969).

Berglund (1991) found that female fecundity was equally size-dependent in both species; however, in *O. labronica*, larger males dominated access to females over smaller males, resulting in a male size advantage that likely reinforces separate sexes (Berglund, 1991). In contrast, in *O. puerilis*, males had higher mating success when smaller, as females rejected mates larger than themselves (Berglund, 1990, 1991). At a certain body size, it thus becomes advantageous for a male to change sex to female, maintaining the size-specific fecundity differences characteristic of protandry.

Similarly, bidirectional hermaphroditism is thought to evolve when the reproductive value of the two sexes changes more than once (Manabe et al., 2007; see Fig. 4.4). In *O. puerilis*, small individuals benefit from being male due to female preference and may change sex when they grow larger, anticipating a higher reproductive value as females.

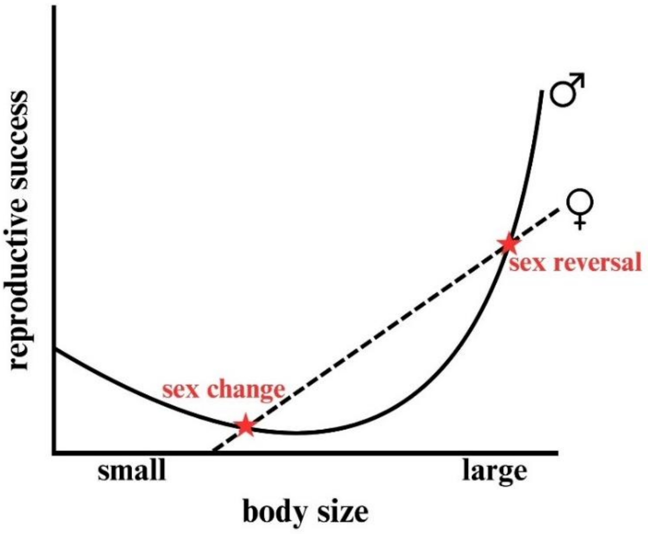

Figure 4.4. Supposed adaptive value of bidirectional sex change in *O. puerilis*. Adapted from Nakashima (1987).

However, at a certain larger body size, if other females are present and male-male competition is high, becoming a large male may be even more advantageous, as large males may dominate reproductive opportunities, exponentially increasing their reproductive value (Fig. 4.4). Indeed, at high densities, hormonal control of sex change by females is thought to intensify male-male competition (Sella & Ramella, 1999). Berglund (1990) also observed that large males sometimes interrupted mating pair formation between females and smaller males.

However, in his experiment, small males were, on average, only 4 segments smaller than females (~ 18.5 segments vs. ~ 22.6 segments). Since typical sex change occurs at ~ 16 – 18 segments, these small males may themselves be considered relatively large, as the protandric male phase begins at 9 segments. It would be interesting to see how these sexually mature primary males fare in these contests and whether they are preferred by females. Young males may also enhance their reproductive success by engaging in sneak matings, as reproduction occurs through pseudocopulation.

Nevertheless, bidirectional sex change is also adaptive in monogamous conditions, which can be explained by the traditional size-advantage hypothesis. For instance, in the pair-culture, initially size-matched females form a breeding pair through sex reversal by the relatively smaller female, as the larger individual is better suited for the female role due to size-dependent fecundity. After spawning, the high reproductive costs for females slow their growth, and the male eventually outgrows the female (growth-rate advantage; Berglund, 1986). This relative size difference then triggers simultaneous sex changes in both individuals. Additionally, as mentioned previously, population densities can fall to single digits in some seasons; therefore, such bidirectional sex change would allow for pair formation between any two conspecifics (low density hypothesis; Kuwamura et al., 2011).

4.4.5. **Conflict over sexual roles**

Berglund (1990) observed that conspecifics fought over sexual roles, resulting in severe injuries and even death. These conflicts were not sex-specific, males injured each other in the presence of a female, presumably for access to the female (Berglund, 1990), and the injured male was often found isolated from the pair (Berglund, 1986). Similarly, females also injured each other in the presence of a male, presumably fighting over the female role, leading to reproductive suppression in the losing female (Berglund, 1990).

Interestingly, when two females were kept together, there was increased variance in growth (Berglund, 1986), possibly due to competitive growth to reinforce sexual roles (see Discussion). He also observed that females tended to prefer males smaller than themselves, presumably due to conflict over the female role (Berglund, 1990). Since relative size determines sex roles, pairing with a male larger than herself would trigger sex changes in both individuals, leading her to lose female status.

4.5. **Synthesis and Rationale for Empirical Chapters**

The substantial historical body of work on *O. puerilis* reveals a species with extreme phenotypic plasticity in its life-history strategy. Sex allocation in this annelid is governed by a highly dynamic interplay between polygenic predispositions, environmental factors (e.g., temperature, nutrition), and social cues (e.g., pheromones and relative size).

However, although social influence on sex allocation in adults is well established, the process by which juveniles initially determine their sex role and developmental trajectory in complex social environments remains largely unexplored. In particular, since relative size determines phenotypic sex, it is unknown whether juveniles actively modulate growth in response to social competition to establish size hierarchies that reinforce future sex roles.

The empirical chapters of this thesis aim to address these questions. Chapter 6 provides the first evidence of strategic growth modulation in an invertebrate, demonstrating how growth plasticity contributes to the resolution of reproductive conflict. Chapter 5 investigates how early-life social environments shape developmental trajectories and resulting sex allocation in juveniles.

Worms used in this study were collected by Dr. Sergi Taboada in 2015 from the harbour of Blanes in Spain (NW Mediterranean, 41°40.4680N, 2°47.9430E) using pig and cow bones experimentally deployed for ca. 3 months inside wire cages at 5 m of depth (Taboada et al., 2016). Upon collection, bones were place into containers with seawater without supplementary oxygenation, which induced the worms to vacate the bones, thus easing their collection. Worms were sorted by eye and later barcoded at the Museo Nacional de Ciencias Naturales de Madrid. Several specimens of *O. puerilis* were shipped alive to the Laboratoire d'Éthologie Expérimentale et Comparée, Université Sorbonne Paris Nord, where a culture of this worm was established in 2018.

References

Åkesson, B. (1967). On the biology and larval morphology of *Ophryotrocha puerilis* Claparède & Metschnikov (Polychaeta). *Ophelia*, *4*(1), 111-119.

Åkesson, B. (1974). Morphology and life history of *Ophryotrocha maculata* sp. n.(Polychaeta, Dorvilleidae). *Zoologica Scripta*, *2*(4), 141-144.

Åkesson, B. (1994). Evolution of viviparity in the genus *Ophryotrocha* (Polychaeta, Dorvilleidae). In *Proceedings of the 4th International Conference on Polychaeta. Memoirs of the National Museum of Natural History* (Vol. 162, pp. 29-35).

Avise, J. C., & Mank, J. E. (2009). Evolutionary perspectives on hermaphroditism in fishes. *Sexual Development*, *3*(2-3), 152-163.

Bacci, G. (1951). Existence of true males and females in a hermaphrodite population of *Ophryotrocha puerilis*. *Experientia*, *7*(6), 222-224.

Bacci, G., & La Greca, M. (1953). Genetic and morphological evidence for subspecific differences between Naples and Plymouth populations of *Ophryotrocha puerilis*. *Nature*, *171*(4364), 1115-1115.

Bacci, G. (1965). Sex determination and genic balance of *Ophryotrocha puerilis*, a hermaphrodite polychaete worm. *Nature*, *207*(5002), 1208-1209.

Bacci, G., & Bortesi, O. (1961a). Pure males and females from hermaphroditic strains of *Ophryotrocha puerilis*. *Experientia*, *17*(5), 229-230.

Bacci, G., & Bortesi, O. (1961b). Existence of multiple sex genotypes in the hermaphrodite polychaete worm *Ophryotrocha puerilis siberti*. *Nature*, *190*(4778), 838-838.

Bacci, G., & Bortesi, O. (1967). The reestablishment of sex balance in *Ophryotrocha puerilis* through interactions between individuals from arrhenogenous and thelygenous lines. *Experientia*, *23*(6), 448-449.

Bacci, G., & Voria, P. (1970). The influence of temperature upon the change from the male to the female phase in *Ophryotrocha puerilis puerilis*, a polychaete worm. *Experientia*, *26*, 1273-1274.

Banse, K. (1963). Polychaetous annelids from Puget Sound and the San Juan Archipelago, Washington. *Proceedings of the Biological Society of Washington*, *76*, 197-208.

Berglund, A. (1986). Sex change by a polychaete: effects of social and reproductive costs. *Ecology*, *67*(4), 837-845.

Berglund, A. (1990). Sequential hermaphroditism and the size-advantage hypothesis: an experimental test. *Animal Behaviour*, *39*(3), 426-433.

Berglund, A. (1991). To change or not to change sex: a comparison between two *Ophryotrocha* species (Polychaeta). *Evolutionary Ecology*, *5*, 128-135.

Berruti, G. (1980). The effect of starvation on the appearance of the definitive upper jaw in *Ophryotrocha puerilis* (Annelida, Polychaeta). *Italian Journal of Zoology*, *47*(1-2), 71-74.

Buston, P., & Clutton-Brock, T. (2022). Strategic growth in social vertebrates. *Trends in Ecology & Evolution*, *37*(8), 694-705.

Charnov, E. L., & Bull, J. (1977). When is sex environmentally determined?. *Nature*, *266*(5605), 828-830.

Charnov, E. L. (1982). *The Theory of Sex Allocation* (Vol. 18). Princeton University Press.
Claparède, E., & Metschnikow, E. (1869). Beiträge zur Kenntnis der Entwickelungsgeschichte der Chaetopoden. *Zeitschrift für Wissenschaftliche Zoologie*, *19*, 163–205.

Dahlgren, T. G., Åkesson, B., Schander, C., Halanych, K. M., & Sundberg, P. (2001). Molecular phylogeny of the model annelid *Ophryotrocha*. *The Biological Bulletin*, *201*(2), 193-203.

Ghiselin, M. T. (1969). The evolution of hermaphroditism among animals. *The Quarterly Review of Biology*, *44*(2), 189-208.

Hartmann, M., & Huth, W. (1936). Untersuchungen über die Geschlechtsbestimmung und Geschlechtsumwandlung von *Ophryotrocha puerilis*. *Zoologische Jahrbücher (Physik)*, 56, 389-439.

Hartmann, M., & Lewinski, G. V. (1938). Untersuchungen über die Geschlechtsbestimmung und Geschlechtsumwandlung von *Ophryotrocha puerilis*. II. Versuche über die Wirkung von Kalium, Magnesium und Kupfer. *Zoologische Jahrbücher (Physiologie)*, 58, 551-574.

Hartmann, M., & von Lewinski, G. (1940). Untersuchungen über die Geschlechtsbestimmung und Geschlechtsumwandlung von *Ophryotrocha puerilis*. III. Die stoffliche Natur der vermännlichenden Wirkung “starker” Weibchen (“Eistoffe”). *Zool. Jahrb. Abt. Allg. Zool. Physiol. Tiere*, *60*, 1-12.

Jaccarini, V., AGius, L., Schembri, P. J., & Rizzo, M. (1983). Sex determination and larval sexual interaction in *Bonellia viridis* Rolando (Echiura: Bonelliidae). *Journal of Experimental Marine Biology and Ecology*, *66*(1), 25-40.

Jacobsohn, S. (1999). Characterization of novel F-actin envelopes surrounding nuclei during cleavage of a polychaete worm. *The International Journal of Developmental Biology*, 43(1), 19–26.

Kegel, B., & Pfannenstiel, H. D. (1983). Evaluation of the pair-culture effect in *Ophryotrocha puerilis* (Polychaeta: Dorvilleidae). I. Pair-culture effect and sex ratio. *Helgoländer Meeresuntersuchungen*, *36*, 205-213.

Kuwamura, T., Suzuki, S., & Kadota, T. (2011). Reversed sex change by widowed males in polygynous and protogynous fishes: female removal experiments in the field. *Naturwissenschaften*, *98*, 1041-1048.

Marchionni, V., & Rolando, A. (1979). The effect of bonellin on the timing of sex inversion and on fertility in *Ophryotrocha puerilis*. *Atti della Società Toscana di Scienze Naturali, Memorie*, *Serie B, 86*, 355–362.

Marchionni, V., & Rolando, A. (1981). Sex reversal in *Ophryotrocha puerilis puerilis* (Polychaeta, Dorvilleidae) induced by ethereal extracts of female phase individuals. *Italian Journal of Zoology*, *48*(2), 91-96.

Martin, D., Abelló, P., & Cartes, J. (1991). A new species of *Ophryotrocha* (Polychaeta: Dorvilleidae) commensal in Geryon longipes (Crustacea: Brachyura) from the western Mediterranean Sea. *Journal of Natural History*, *25*(2), 279-292.

Müller, H. (1962). Über die Sexualität des Polychaeten *Ophryotrocha puerilis*, ihre Determination und ihren Einfluss auf Drüsentätigkeit und auf Apparatenentwicklung. *Zeitschrift für Morphologie und Ökologie der Tiere, 52*(1), 1-32.

McIntosh, W. C. (1885). Notes from the St. Andrews Marine Laboratory (under the Fishery Board for Scotland). No. III. 2. On a new British Staurocephalus. *The Annals and Magazine of Natural History, including Zoology, Botany and Geology. Series 5, 16*(96), 480–487.

Ockelmann, K. W., & Åkesson, B. (1990). *Ophryotrocha socialis* n. sp., a link between two groups of simultaneous hermaphrodites within the genus (Polychaeta, Dorvilleidae). *Ophelia*, *31*(3), 145-162.

Paavo, B., Bailey-Brock, J. H., Åkesson, B., & Nylund, A. (2000). Morphology and life history of *Ophryotrocha adherens* sp. nov.(Polychaeta, Dorvilleidae). *Sarsia*, *85*(3), 251-264.

Parenti, U. (1964). Inversione sessuale limitata a due metameri in *Ophryotrocha gracilis*. *Italian Journal of Zoology*, *31*(1), 25-30.

Paxton, H., & Åkesson, B. (2007). Redescription of *Ophryotrocha puerilis* and *O. labronica* (Annelida, Dorvilleidae). *Marine Biology Research*, *3*(1), 3-19.

Paxton, H., & Åkesson, B. (2010). The *Ophryotrocha labronica* group (Annelida: Dorvilleidae)—with the description of seven new species. *Zootaxa*, *2713*(1), 1-24.

Paxton, H., & Åkesson, B. (2011). The *Ophryotrocha diadema* group (Annelida: Dorvilleidae), with the description of two new species. *Zootaxa*, *3092*(1), 43-59.

Pfannenstiel, H. D. (1975). Mutual influence on the sexual differentiation in the protandric polychaete *Ophryotrocha puerilis*. In *Intersexuality in the animal kingdom* (pp. 48-56). Berlin, Heidelberg: Springer Berlin Heidelberg.

Pfannenstiel, H. D. (1977). Experimental analysis of the 'Paarkultureffekt'in the protandric polychaete, *Ophryotrocha puerilis* Clap. Mecz. *Journal of Experimental Marine Biology and Ecology*, *28*(1), 31-40.

Pfannenstiel, H. D. (1978). Endocrinology of polychaete reproduction and sexual development. *Italian Journal of Zoology*, *45*(2), 171-188.

Pfannenstiel, H. D., & Grünig, C. (1990). Spermatogenesis and sperm ultrastructure in the polychaete genus *Ophryotrocha* (Dorvilleidae). *Helgoländer Meeresuntersuchungen*, *44*, 159-171.

Pfannenstiel, H. D., & Grünig, C. H. (1982). Yolk formation in an annelid (*Ophryotrocha puerilis*, Polychaeta). *Tissue and Cell*, *14*(4), 669-680.

Pfannenstiel, H. D., Grothe, C., & Kegel, B. (1982). Studies on *Ophryotrocha geryonicola* (Polychaeta: Dorvilleidae). *Helgoländer Meeresuntersuchungen*, *35*(1), 119-125.

Premoli, M. C., & Sella, G. (1995). Sex economy in benthic polychaetes. *Ethology Ecology & Evolution*, *7*(1), 27-48.

Prevedelli, D., Massamba N'Siala, G., & Simonini, R. (2005). The seasonal dynamics of six species of Dorvilleidae (Polychaeta) in the harbour of La Spezia (Italy). *Marine Ecology*, *26*(3-4), 286-293.

Prevedelli, D., N'siala, G. M., & Simonini, R. (2006). Gonochorism vs. hermaphroditism: relationship between life history and fitness in three species of *Ophryotrocha* (Polychaeta: Dorvilleidae) with different forms of sexuality. *Journal of Animal Ecology*, *75*(1), 203-212.

Ross, R. M. (1990). The evolution of sex-change mechanisms in fishes. *Environmental Biology of Fishes*, *29*, 81-93.

Sella, G. (1980). The genetic system controlling the body length at which sex change occurs in *Ophryotrocha puerilis* (Annelida, Polychaeta). *Atti della Società Toscana di Scienze Naturali, Processi Verbali e Memorie***,** Serie B, 87, 197–203.

Sella, G., & Ramella, L. (1999). Sexual conflict and mating systems in the dorvilleid genus *Ophryotrocha* and the dinophilid genus *Dinophilus*. *Hydrobiologia*, *402*, 203-213.

Shapiro, D. Y. (1981). Size, maturation and the social control of sex reversal in the coral reef fish *Anthais squamipinnis*. *Journal of Zoology*, *193*(1), 105-128.

Simonini, R. (2002). Distribution and ecology of the genus *Ophryotrocha* (Polychaeta: Dorvilleidae) in Italian harbors and lagoons. *Vie et Milieu/Life & Environment*, 59-65.

Simonini, R., Grandi, V., Massamba-N'Siala, G., Martino, M. P., Castelli, A., & Prevedelli, D. (2010). Diversity, habitat affinities and diet of *Ophryotrocha* species (Polychaeta, Dorvilleidae) living in Mediterranean harbour habitats. *Vie et Milieu/Life & Environment*, 27-38.

Svensson, S. G. B., Strohmeier, T., Rastrick, H., Garcia, A. A., Lock, E. J., Sveier, H., & Jansen, H. M. (2023). Life history traits for *Ophryotrocha craigsmithi* (Wiklund, Glover & Dahlgren, 2009), a candidate species in integrated multitrophic aquaculture. *Frontiers in Marine Science*, *10*, 1116765.

Szaniawski, H. (1974). Some Mesozoic scolecodonts congeneric with recent forms. *Acta Palaeontologica Polonica*, *19*(2).

Taboada, S., Bas, M., Leiva, C., Garriga, M., Sardá, R., & Avila, C. (2016). Life after death: shallow-water Mediterranean invertebrate communities associated with mammal bones. *Marine Ecology*, *37*(1), 164-178.

Taboada, S., Leiva, C., Bas, M., Schult, N., & McHugh, D. (2017). Cryptic species and colonization processes in *Ophryotrocha* (Annelida, Dorvilleidae) inhabiting vertebrate remains in the shallow-water Mediterranean. *Zoologica Scripta*, *46*(5), 611-624.

Tempestini, A., Massamba-N'Siala, G., Vermandele, F., Beaudreau, N., Mortz, M., Dufresne, F., & Calosi, P. (2020). Extensive gene rearrangements in the mitogenomes of congeneric annelid species and insights on the evolutionary history of the genus *Ophryotrocha*. *BMC genomics*, *21*(1), 815.

Thornhill, D. J., Dahlgren, T. G., & Halanych, K. M. (2009). Evolution and ecology of *Ophryotrocha* (Dorvilleidae, eunicida). *Annelids in Modern Biology*, 242-256.

Tilic, E., & Rouse, G. W. (2024). Hardly Venus's servant—morphological adaptations of Veneriserva to an endoparasitic lifestyle and its phylogenetic position within Dorvilleidae (Annelida). *Organisms Diversity & Evolution*, *24*(1), 67-83.

Westheide, W. (1984). The concept of reproduction in polychaetes with small body size: adaptations in interstitial species. In: Fischer, A., & Pfannenstiel, H. D. (eds) *Polychaete reproduction*. Gustav Fischer Verlag, Stuttgart, p. 265-287.

Wiklund, H., Glover, A. G., & Dahlgren, T. G. (2009). Three new species of *Ophryotrocha* (Annelida: Dorvilleidae) from a whale-fall in the North-East Atlantic. *Zootaxa*, *2228*(1), 43-56.

Zhang, D. S., Zhou, Y. D., Wang, C. S., & Rouse, G. W. (2017). A new species of *Ophryotrocha* (Annelida, Eunicida, Dorvilleidae) from hydrothermal vents on the Southwest Indian Ridge. *ZooKeys*, (687), 1.

CHAPTER 5

Game Over: Conflict Resolution Through Strategic Growth in an Invertebrate

Pooja Radhakrishnan[1], Noé Saboul[1], Raquel Monclús[1], Sergi Taboada[2], Maria Cristina Lorenzi[1]

[1] LEEC, Laboratoire d'Éthologie Expérimentale et Comparée, Université Sorbonne Paris Nord, Villetaneuse (France)

[2] Dept. de Biodiversidad y Biología Evolutiva, Museo Nacional de Ciencias Naturales, Madrid (Spain)

Accepted in *Functional Ecology*
27th Aug 2025

Abstract

1. In some multimember groups with unequal partitioning of reproduction and poor breeding opportunities outside the group, natural selection has favoured the evolution of adaptive mechanisms such as strategic growth modulation. Strategic growth is a form of body growth plasticity where individuals make growth adjustments calibrated to their closest competitor, establishing a size hierarchy that defines who has priority in reproduction.
2. In this study we explored the occurrence of strategic growth in an invertebrate, the marine annelid worm *Ophryotrocha puerilis*, and investigated its underlying mechanisms via growth curve analysis.
3. Size-matched juvenile worms exposed to different social environments established size hierarchies by following distinct developmental trajectories, and the intensity and duration of growth spurts correlated to the level of competition within their social environment. In monogamous environments, the onset of reproduction led to the weakening of the size hierarchy.
4. Conversely, in reproductively competitive environments, the onset of reproduction led to the stabilisation of size differences, supporting the idea that individuals actively regulate their growth relative to rivals to mitigate reproductive conflict and size hierarchies emerge as a result of these strategic adjustments.
5. This study provides the first evidence for strategic body growth in an invertebrate and explores both the establishment of the size hierarchy as well as its eventual dissolution upon conflict resolution.

Keywords: reproductive skew, *Ophryotrocha*, social regulation, reproduction, size hierarchy

5.1. Introduction

In animals, individuals typically undergo a clearly identifiable juvenile developmental period during which they experience rapid body growth (Case, 1978; West et al., 2001). While in most species juvenile growth follows a typical trajectory, significant individual variation is widespread, as body growth is highly sensitive to environmental factors. For instance, as the study of socially-mediated phenotypic plasticity reveals (Croll et al., 2023; Metcalf, 2024), tadpoles of the wood frog, *Rana sylvatica,* exhibit slower growth in response to perceived high intraspecific competition, even when conspecific density is visually simulated using mirrors (Rot-Nikcevic et al., 2006). Juvenile growth trajectories can also be influenced by sexual signals – which serve as predictive cues of reproductive opportunities (West-Eberhard, 2003; Kasumovic & Brooks, 2011). An example of this is observed in the Australian redback spiders, *Latrodectus hasselti*, where males mature earlier and at a smaller body size in the presence of mature virgin females (Stoltz et al., 2012).

Subtle conspecific cues may also trigger a sophisticated form of adaptive, plastic growth called strategic body growth, which has been observed in multimember groups characterised by size-based dominance hierarchies (Heg et al., 2004; Hamilton & Benincasa, 2022; Buston & Clutton-Brock, 2022). In such groups, variation in body size is not primarily due to preferential access to resources, nor is it contingent solely on cues from the overall social environment, such as population density, sex ratio, or mate availability (Hamilton & Heg, 2008; Buston & Clutton-Brock, 2022). Instead, group members regulate their growth by making nuanced adjustments proportional to fine-grained changes in the size of their closest competitors, resulting first in the establishment, and subsequently in the maintenance, of a size hierarchy (Tibbetts et al., 2022; Buston & Clutton-Brock, 2022). Here, following Buston & Clutton-Brock's review (2022), we use the broad and inclusive definition of strategic growth as "adaptive plasticity of body size in response to fine-grain changes in social conditions".

This form of socially responsive growth, previously referred to as social control or social suppression of size and growth (Borowsky, 1973; Volpato & Fernandes, 1994), has been well documented in social vertebrates, where the largest individuals typically monopolise reproduction. This leads to high reproductive skew and the formation of a subordinate breeding queue as dispersal is limited by environmental constraints and a lack of outside options (Kokko

& Johnstone, 1999; Buston, 2003, 2004; Branconi et al., 2020). One form of strategic growth in such groups occurs following the disappearance or death of the dominant individual: the largest subordinate breeder ascends in rank and displays a strategic growth spurt. In the cylindrical sandperch *Parapercis cylindrica*, the intensity of this growth spurt is proportional to the number of females in the group, allowing the male to successfully defend the harem (Walker & McCormick, 2009). Similarly, in the cooperatively breeding Damaraland mole-rat *Fukomys damarensis*, female non-breeders that assume the breeding role undergo marked growth spurts upon rank ascension (Thorley et al., 2018).

Moreover, since body size is one of the intrinsic attributes associated with position in the hierarchy due to its significance in influencing fecundity or dominance (Holekamp & Strauss, 2016; Dehnen et al., 2022), competition is expected to be the highest when competitors are closer in body size (close competitor strategy; Enquist et al., 1990; Hamilton & Benincasa, 2022). This interindividual conflict triggers another form of strategic growth where individuals make strategic adjustments in body size relative to conspecifics, particularly their closest competitors, triggering a ‘domino effect’ where the body size of each individual is limited by the size of the individual immediately above it in rank (Heg et al., 2004; Russell et al., 2004; Wong et al., 2016).

Two mechanisms have been described to reduce or even resolve conflict when individuals are size-matched. Conflict could be resolved through competitors self-regulating growth (Hamilton & Benincasa, 2020; Buston & Clutton-Brock, 2022), i.e., ‘losers’ strategically downregulating their growth (subordinate self-restraint) (Wong et al., 2016), or, on the other hand, winners strategically upregulating their growth (competitive growth) (Reed et al., 2019; Huchard et al., 2016). For instance, in the emerald coral goby *Paragobiodon xanthosomus*, subordinates suppress their growth by effectively ‘dieting’ as they approach the size of dominants, thereby maintaining size differences that minimize conflict and reduce the risk of eviction (Wong et al., 2008). Interestingly, in the clown anemonefish, *Amphiprion percula*, even ‘losers’ in pair confrontations grow faster than isolated individuals, suggesting that, in a pair, the presence of a competitor triggers growth modulation in both individuals but a more pronounced accelerated growth by the ‘winner’ until a ‘safe’ conflict-resolving size ratio is reached (Reed et al., 2019).

Strategic body growth has rarely been explored in invertebrates, despite the presence of well-known examples of social hierarchies among them (Tibbetts et al., 2022). For example, in the paper wasp *Polistes dominulus*, larger females in hibernation aggregations are more likely to become dominant at colony establishment in spring (Dapporto et al., 2006). Similarly, in the eusocial snapping shrimp *Synalpheus regalis*, the largest female is the sole breeder, and her body size strongly predicts both fecundity and colony size (Duffy, 1996). If relative size among conspecifics is an important determinant of social status, and if social status determines breeding status, which in turn influences fitness, then natural selection should favour mechanisms of body growth modulation that enhance competitive abilities, such as strategic growth. Therefore, we conducted this study on *Ophryotrocha puerilis*, a common, interstitial, shallow-water Mediterranean annelid worm (Paxton & Åkesson, 2007; Thornhill, 2009). Preliminary studies on small groups of same-aged and similar-sized worms revealed that individuals displayed differential growth rates despite being fed *ad libitum*; these data, together with existing evidence of apparent reproductive conflicts between same-sex worms, where larger males win contests over access to females and larger females retain the female role (Berglund 1986, 1990), suggest the potential for strategic growth.

In the wild, *O. puerilis* experiences significant fluctuations in population densities (Premoli & Sella, 1995; Prevedelli et al., 2005; Taboada et al., 2016, 2017), which might create conditions promoting phenotypic plasticity in reproduction-associated traits (Charnov, 1982; Schärer, 2009). Additionally, Taboada et al. (2016, 2017) documented that *O. puerilis* are opportunistic colonizers by experimentally placing bones at different depths in the Mediterranean seafloor, where they were frequently the first and most abundant invertebrates to arrive. These colonies contained individuals at all life stages, including larvae, juveniles, adults, and egg masses, and haplotype analyses indicated temporal stability, with many haplotypes persisting over time (Taboada et al., 2017). Similar natural aggregations have also been reported in mussel beds and anthropogenic substrates such as shipping ropes (Premoli & Sella, 1995; Sella & Ramella, 1999). While long-term tracking of individual group membership is limited by technical constraints (e.g., small size, marine environment, interstitial lifestyle), the co-occurrence of several life stages and evidence for temporal haplotype stability suggest that individuals are likely to engage in repeated interactions within stable colonies. This predictability might enable individuals to continually evaluate their position within the group and modulate their body growth in response to both coarse- and fine-grained changes in their closest competitors, conditions predictive of strategic growth (Walker & McCormick, 2009; Buston & Clutton-Brock, 2022).

Here, we test whether juvenile *O. puerilis* worms establish size hierarchies and display strategic growth. We expect that if worms establish size hierarchies, size-matched juveniles would consistently develop size differences over time. Second, while we expect the establishment of a size hierarchy to resolve the reproductive conflict, we also expect that entering the reproductive phase would alter interindividual dynamics, i.e., that conflict-related size differences might peak before the onset of reproduction, and consequently diminish thereafter, in the absence of mate competition. Finally, by analysing the worms' body growth curves, we investigate how juveniles sharing the same environment regulate their body growth in relation to others. Specifically, we expect that the intensity and duration of growth spurts depend on both the number of competitors and an individual's relative rank, with higher-ranking individuals exhibiting more intense and prolonged growth spurts, whereas lower-ranking individuals may exhibit shorter growth spurts as they concede the competition.

5.2. Methods

5.2.1 Study Model

Ophryotrocha puerilis are marine annelid worms, with adult body size ranging from 4-7 mm (18-26 segments) up to 13 mm (35 segments) (Paxton & Åkesson, 2007; Prevedelli et al., 2005, 2006). This species is so far the only known protandrous (male-first) sequential hermaphrodite in the genus *Ophryotrocha* (Paxton & Åkesson, 2007) and are relatively long-lived (4-5 months on average; Prevedelli et al., 2006). Although described as protandrous, female-to-male sex reversal can occur, typically triggered by subtle social cues such as relative size among conspecifics (Hartmann & Huth, 1936), sex ratio (Kegel & Pfannenstiel, 1983), or interindividual conflict (Berglund, 1986, 1990), as well as by stressors such as injury (Hartmann & Huth, 1936) or starvation (Berruti, 1980). However, it is almost never observed in isolation among well-fed and healthy females.

Reproduction occurs through pseudocopulation, a form of external fertilization in which both female and male release their gametes almost simultaneously into a jelly-like mucous casing secreted by the female (Fig. 1A, B, C) (Paxton & Åkesson, 2007). They are continuous spawners without a defined reproductive season (Pfannenstiel & Grünig, 1982; Jacobson, 1999), and spawning occurs every 5 days in the pair-culture (Berglund, 1986, 1990). Female fecundity is highly size-dependent (Berglund, 1986, 1990): small females may produce about 30 eggs in their first cocoon, while larger females can lay up to 350-400 eggs per clutch

(Åkesson; 1967). Both parents protect the cocoon. Eggs develop into embryos and mature into larvae within 8 days (Fig. 1D). They have direct development (lacking a planktonic larval phase); juveniles (Fig. 1E) mature as males at a body size of 9 segments before switching sex to females (Paxton & Åkesson, 2007).

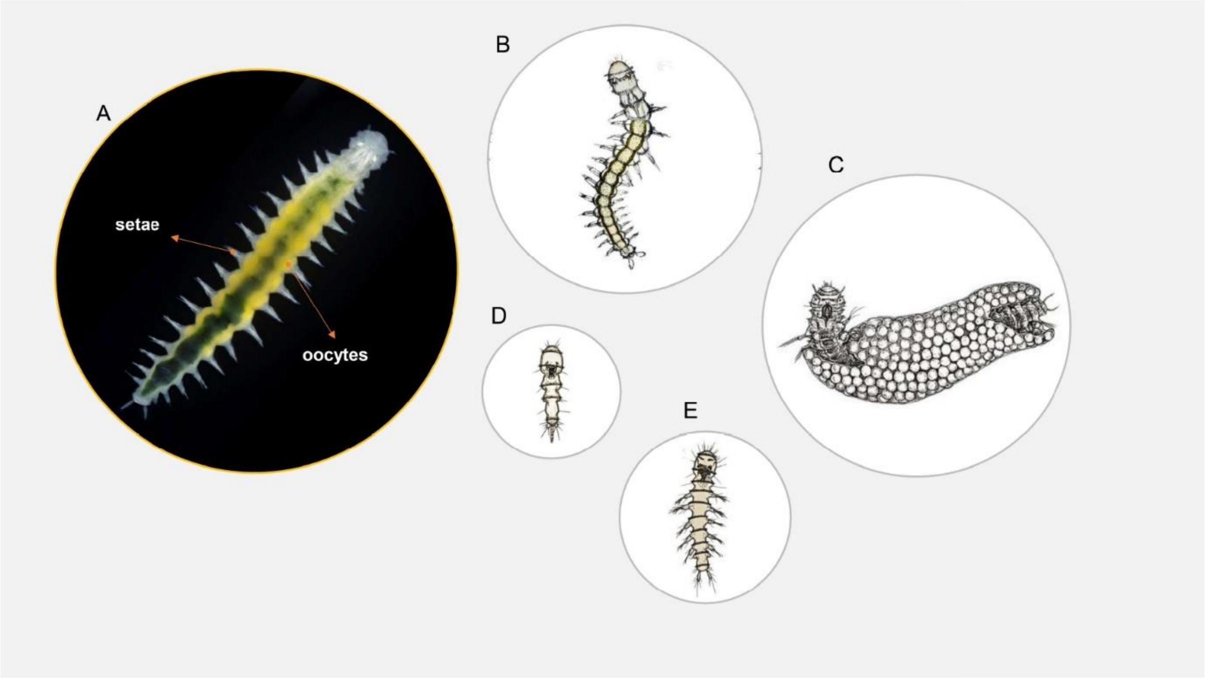

Figure 1. Life cycle stages of *Ophryotrocha puerilis*. A) Adult female with visible oocytes in yellow; B) Adult male; C) Adult female with a newly laid egg cocoon; D) Newly emerged larva; E) five-segment juvenile, the stage at which individuals were introduced into experimental treatments.

(A) Modified from SLU Artdatabanken (2025); (B) Modified from original figures by Claparède & Mecznikow (1869) available at: *http://www.biodiversitylibrary.org/item/159258;* (C) Modified from Åkesson (1967, Fig.1). (D) Modified from Åkesson (1967, Fig. 2G); (E) Modified from Åkesson (1967, Fig. 3C)

Sex change to female is influenced by environmental factors (e.g., temperature and seasonality; Prevedelli et al., 2006; Radhakrishnan et al., in prep.), as well as by the number and size of conspecifics (Pfannenstiel, 1977; Berglund, 1986, 1990, 1991; Radhakrishnan et al., in prep.). For example, while sex change typically occurs around 23 segments in lab cultures (Paxton & Åkesson, 2007), isolated worms may undergo sex change earlier, around 16-22 segments (Paxton & Åkesson, 2007; P.R. personal observation). Therefore, sex change to female is plastic and context-dependent, and is determined by the appearance of oocytes visible through their transparent bodies (see Fig. 1A) rather than age or body size alone.

These annelids are commonly found in harbours, wharfs, or eutrophic coastal areas (Prevedelli et al., 2005; Simonini et al., 2010; Taboada et al., 2016, 2017). Worms used in this study are descending from worms collected in 2015 from the harbour of Blanes Spain, NW Mediterranean, 41°40.4680N, 2°47.9430E), and barcoded at the the Natural History Museum of London.

5.2.2. Experimental Procedure

To maximize genetic diversity and minimize potential influence of relatedness on growth outcomes, parental families (n=128) were created by separately pairing males (10 segments) and females (>18 segments) obtained from two distinct laboratory mass cultures. Parental pairs were left undisturbed for up to two weeks in separate bowls, and parents were removed 3-4 days after the females laid cocoons. One-segment larvae (see Fig. 1D) emerged from these cocoons after 5-7 days. These larvae were left to develop for a week until they became juveniles (see Fig. 1E) and then used to form the experimental groups, ensuring that no individuals within a group were siblings. While individual identity could not be tracked throughout the experiment, this design reduces the likelihood that genotype alone explains the consistent growth differences observed.

Juvenile worms (4-7 segments, 12-14 days old, n=170) were randomly allocated into four social environments (Fig. 2): Pairs (two juveniles in the same bowl, n= 55) or Triplets (three juveniles in the same bowl, n= 30). As a control, we also set up juveniles in Isolation (n=55) and in Groups of adults (n=30) (where individuals were kept in groups of one 21-segments female and three 14-segments males). These social environments are ecologically relevant for the species as these worms often colonise new environments and live in relatively stable populations in small interstices (Thornhill, 2009; Taboada et al., 2017).

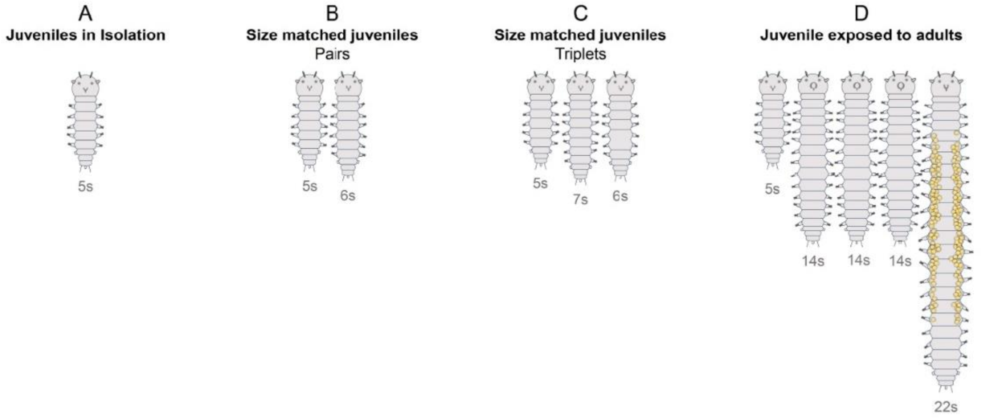

Figure 2. Juvenile *Ophryotrocha puerilis* worms (5–8 segments; see Fig. 1E) were assigned to one of four social environments: A) Isolation, where individuals were housed alone; B) Pairs of size-matched juveniles; C) Triplets of size-matched juveniles; and D) Exposure to adults in a male-biased environment.

s = segment count; oocytes of adult females in yellow. Body size was measured as the number of segments bearing setae (see Fig. 1A). Sex change was noted as the day of appearance of oocytes visible through the coelom (see Fig. 1A).

Worms were kept in glass bowls with 10 ml filtered artificial marine water (35 ‰ salinity) for 60 days. Water was changed weekly, and worms were fed pureed spinach *ad libitum* at each change. The experimental bowls were maintained in an incubator at an ambient temperature of 21°C, with a 24-hour dark photoperiod.

5.2.3. Measurements

Body size was expressed as the number of body segments with setae (setigerous segments, annelid body size varies in length and diameter because of their hydrostatic skeleton, Kier, 2012) and was measured in juveniles every 2-5 days throughout the experiment. The minute body size of the worms, combined with the mucous layer covering their bodies (Murray et al., 2012) and their marine lifestyle, makes individual marking particularly challenging. Therefore, in Pairs and Triplets, worms were differentiated from their partners using a ranking system with the largest worm designated as Rank 1 (the next as Rank 2, and the next - in Triplets - as Rank 3). Before sex change, either there was no possibility of misidentifying the worms, because the size of the smaller worm in Pairs or the smallest or intermediate worm in Triplets reached on a given day was smaller than the size of the partner(s) at the previous measurement or, when this was not true, we assigned sizes assuming that size variation between subsequent measurements was the smallest for each worm (e.g., if at time t a worm was 7 segments and the other 9 segments and at time t+1 they were 9 and 11 segments, we assigned a growth of 2 segments at each worm rather than a growth of 4 segments to one and 0 to the other). This was a conservative approach leading to minimize growth rate differences, i.e., differences in growth rate curve parameters might be even larger than calculated (see below). As for the calculation for the establishment of the size hierarchy (see below), this was based on the size difference at any given day and therefore did not require individual recognition. Typically, the size difference became apparent within the first 3-4 days, after which it remained constant or, more commonly, increased over time (see Results), therefore reducing the likelihood of mistaking identifying individuals from day 4 onwards. After sex change, females can be distinguished from males by the presence of mature oocytes that are distributed along the length of the coelomic cavity and are visible under a stereomicroscope due to the worms' transparent body walls (see Fig. 1A) (Paxton & Åkesson, 2007).

We noted the age at sex change when oocytes were observed. In all bowls (except Isolation), egg laying occurred after one worm changed sex to female (data not recorded); egg cocoons were promptly removed to avoid changes in group composition.

Any occurrence of mortality led to the exclusion of that replicate. However, if an adult male or female died in the Groups of adults treatment, the replicate was included, as this treatment represented an environment where mortality may naturally be higher due to interindividual aggression (Berglund, 1986).

5.2.4. Replication statement

Scale of inference	Scale at which the factor of interest is applied	Number of replicates at the appropriate scale	
Individual (n = 285 individuals)	Bowl (n = 170 bowls)	One individual in Isolation	n = 55 bowls
		Pairs	n = 55 bowls
		Triplets	n = 30 bowls
		One individual in a Groups of adults	n = 30 bowls

5.2.5. Statistical analyses

Statistical analyses were performed in R (version 4.3.1) http:// www.r-project.org (R Core Team, 2023) using the packages: “lme4” (Bates et al., 2015), “drc” (Ritz et al., 2015) and “zoo” (Zeileis et al., 2020).

5.2.5.1. Size Hierarchy in Pairs and Triplets

Size difference was calculated as the difference in the number of setigerous segments between the worms sharing a bowl. In the analyses of the variation of size differences across time, we noted the day of the first sex change in each bowl, set this day as 0 and calculated the days to that day and from that day until the next individual in the bowl changed sex (in any case no longer than day 60).

We used Generalized Linear Mixed-effects Models (GLMMs) to assess how time influenced the variation in size differences as a function of the social environment. We ran two separate models: the first included the data before sex change of Rank 1 individuals (i.e., from the start of the experiment to day -1) and the second included the data after the change (i.e., from the day 0 to the sex change by any other worm in the bowl). As size differences were count data, we used a Poisson distribution with a log link function. We incorporated a random effect Bowl Identity to account for repeated measures over time and interdependency of body size between individuals sharing the same bowl. Data were checked for overdispersion.

5.2.5.2. Deriving Growth Curve Parameters

We used the Gompertz and Logistic models as candidate models to fit individual growth data, as these curves captured the characteristic sigmoidal (S-shaped) growth pattern exhibited by juvenile worms. Initial parameter estimates were approximated based on the observed data for both Gompertz and Logistic curves. For the Logistic model, ‘lower’ and ‘upper’ values were set as the minimum and maximum observed body sizes, respectively. The ‘slope’ parameter, which corresponds to the maximum growth rate (hereafter referred to as *growth rate*) was set to 0.2. Size at the asymptote was fixed to 30, and the ‘age at inflection’ (the age when rapid growth rate begins to decline) was set to 6. For the Gompertz model, ‘lower’ and ‘upper’ values were set as the minimum and maximum observed body sizes, respectively, and slope was set to 0.1. Unlike the Logistic model, the Gompertz model does not have a fixed asymptote; instead, the asymptotic size is estimated from the data during model fitting.

We used the ‘zoo’ package for interpolation to fill in missing values, if any. These initial values served as estimates, acting as reference points for the optimisation of parameter values for each model. During model fitting, the optimisation process (using the ‘drm’ function from the ‘drc’ package) fine-tunes these estimates to obtain parameter values that best align with the observed data. We tested these final parameter values for both Logistic and Gompertz for goodness of fit using Akaike Information Criterion (AIC) values to determine which model best described variation in the data. The Logistic model was a better fit; therefore, we used the parameter estimates for logistic curves for all further analysis.

5.2.5.3. Analysis of Growth Curve Parameters

We compared differences in body growth parameters (growth rate, age at inflection and asymptote) within and between Pairs and Triplets using LMMs with Rank as the predictor variable. We incorporated Bowl Identity as a random factor to account for the non-independence of body growth trajectories between worms within any environment. We checked for multicollinearity using variance inflation factors (VIF).

Finally, we ran two LMs (and their relative post-hoc pairwise comparisons), one considering the highest and another for the lowest ranks in Pairs and Triplets. In the first model we compared growth curve parameters of Rank 1 individuals in Pairs and Triplets with worms in Isolation because, if worms exhibited competitive growth (*sensu* Huchard et al., 2016; Reed et al., 2019), we expected Rank 1 worms in Pairs or Triplets to display faster growing strategies than worms in Isolation. In the second model, we compared the lowest ranks in Pairs and

Triplets (Rank 2 and Rank 3, respectively) with worms in Groups of adults; we expected worms in Groups of adults to also display slower-growing strategies because the presence of large adult worms should relegate juveniles to the lowest rank (as also reported by Buston, 2003, in the clownfish *Amphiprion percula*).

5.3. Results

5.3.1. Size Hierarchy in Pairs and Triplets

While all the size-matched juveniles established a size difference after we set up Pairs and Triplets, the social environment (Pairs, Triplets) significantly affected the pattern of variation of such size differences over time, both before and after sex change (Fig. 3, Table 1).

5.3.1.1. Pairs

The size-matched juvenile worms established a size difference shortly after pairing (Fig. 3A). This size difference progressively grew until the average size ratio between them had grown up to 77% (a nearly four-segment difference) (Fig. 4A). However, while a size hierarchy was established, it was not maintained. Following the sex change by Rank 1 worms (i.e., from male to female, identified by the appearance of oocytes) around day 16, the size difference gradually and significantly diminished, weakening the size hierarchy (Fig. 3A, Table 2). The two worms were size matched by the end of the experiment (see below, asymptote), and in some cases, Rank 2 had outgrown Rank 1.

3.1.2. Triplets

Size-matched juveniles established a size difference shortly after setting up the Triplets (Fig. 3B). This size difference progressively and significantly increased until the average size ratio between the Rank 1 and Rank 2 worms reached 86%, and the size ratio between the Rank 2 and Rank 3 worms reached 84% (approx. a two-segment difference in both) (Fig. 4B, Table 2). The size hierarchy established between all three worms was maintained thereafter (Fig. 3B). Following the sex change by Rank 1 individuals, the size difference between the Rank 1 worms (now a female) and the Rank 2 worms kept gradually and significantly increasing, albeit at a more modest pace. However, the size-hierarchy was maintained without significant variation between the Rank 2 and Rank 3 worms (which were still males) until one of the two changed sex to female or until the end of the experiment (Fig. 3B, Table 2).

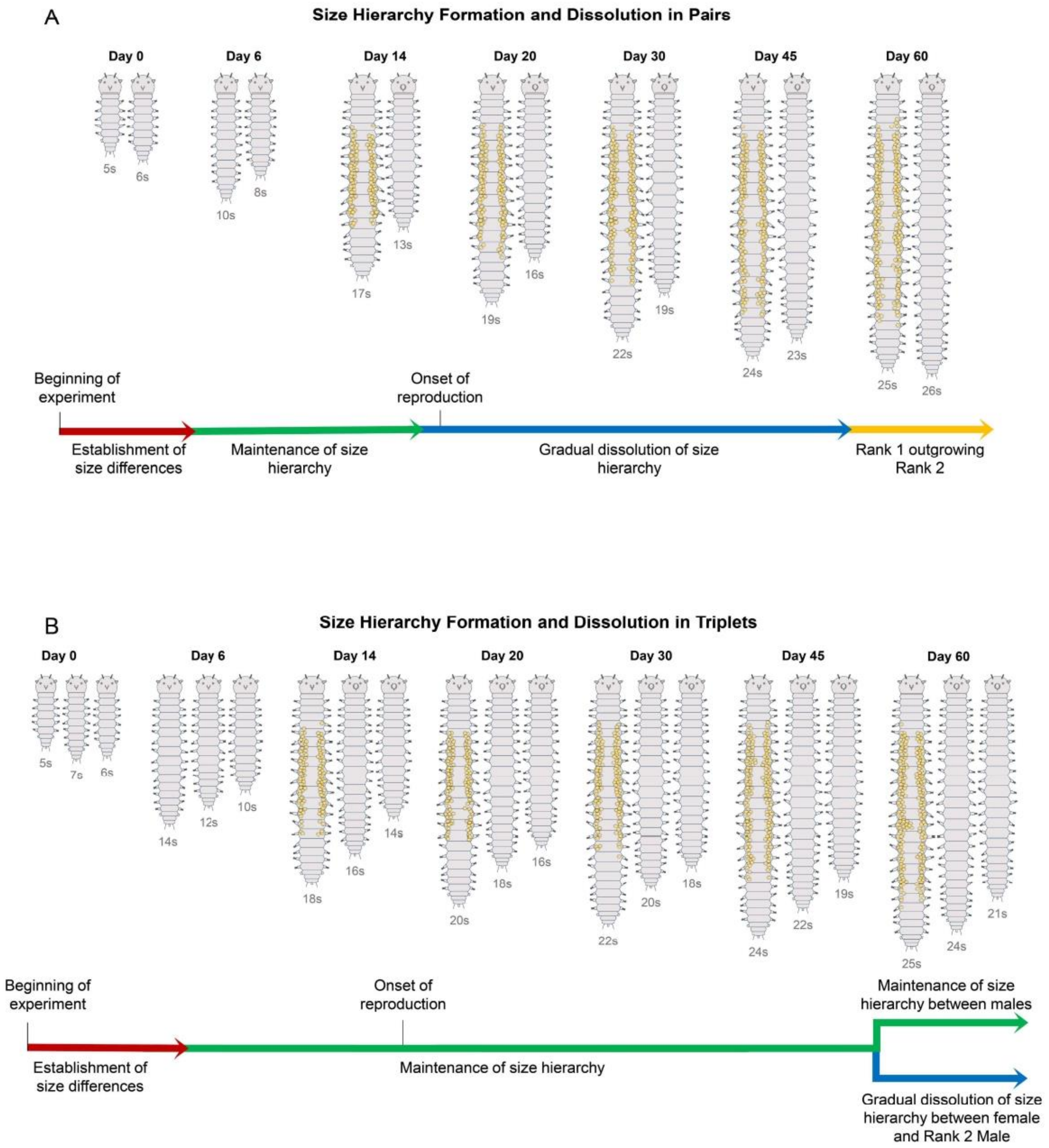

Figure 3. Schematic representation of size hierarchy formation and dissolution in Pairs and Triplets based on average body sizes calculated for each day. s = segment count; oocytes of adult females in yellow. Sex change was noted as the day of appearance of oocytes visible through the coelom (see Fig. 1A). Onset of reproduction was noted as the day when a cocoon was laid. Both worms in Pairs and in Triplets entered the experiment as size matched (day 0) and established size differences early (day 6, on average). A) Pairs: the size hierarchy was maintained until the onset of reproduction, after which size differences gradually diminished. By the end of the experiment, Rank1 individuals started to outgrow Rank 2. B) Triplets: the size hierarchy was maintained even after the onset of reproduction. Eventually, size differences between the female and Rank 2 gradually diminish, while size differences between Rank 2 and Rank 3 males are maintained.

Table 1. The results of the overall GLMM analyses on the variation in body size difference with social environment and time before and after sex change from male to female – typically, by the largest worm – in Pairs and Triplets. Significant values are in bold.

Time period	**Source of variation**	**Reference level**	**Level**	**Estimate ± SE**	**χ^2**	**P**
Before Sex Change	**Social environment**	Pair	Triplets	-0.758± 0.187	16.376	< **0.0001**
	Time			0.089± 0.010	77.057	< **0.0001**
	Social environment*Time	Pairs*Time	Triplets*Time	-0.039± 0.017	4.960	**0.026**
Number of observations: 434 Number of Pairs and Triplets: 66						
After Sex Change	Social environment	Pair	Triplets	-0.304± 0.204	2.211	0.137
	Time			-0.020± 0.002	99.803	< **0.0001**
	Social environment *Time	Pairs*Time	Triplets*Time	0.027± 0.004	50.142	< **0.0001**
Number of observations: 924 Number of Pairs and Triplets: 61						

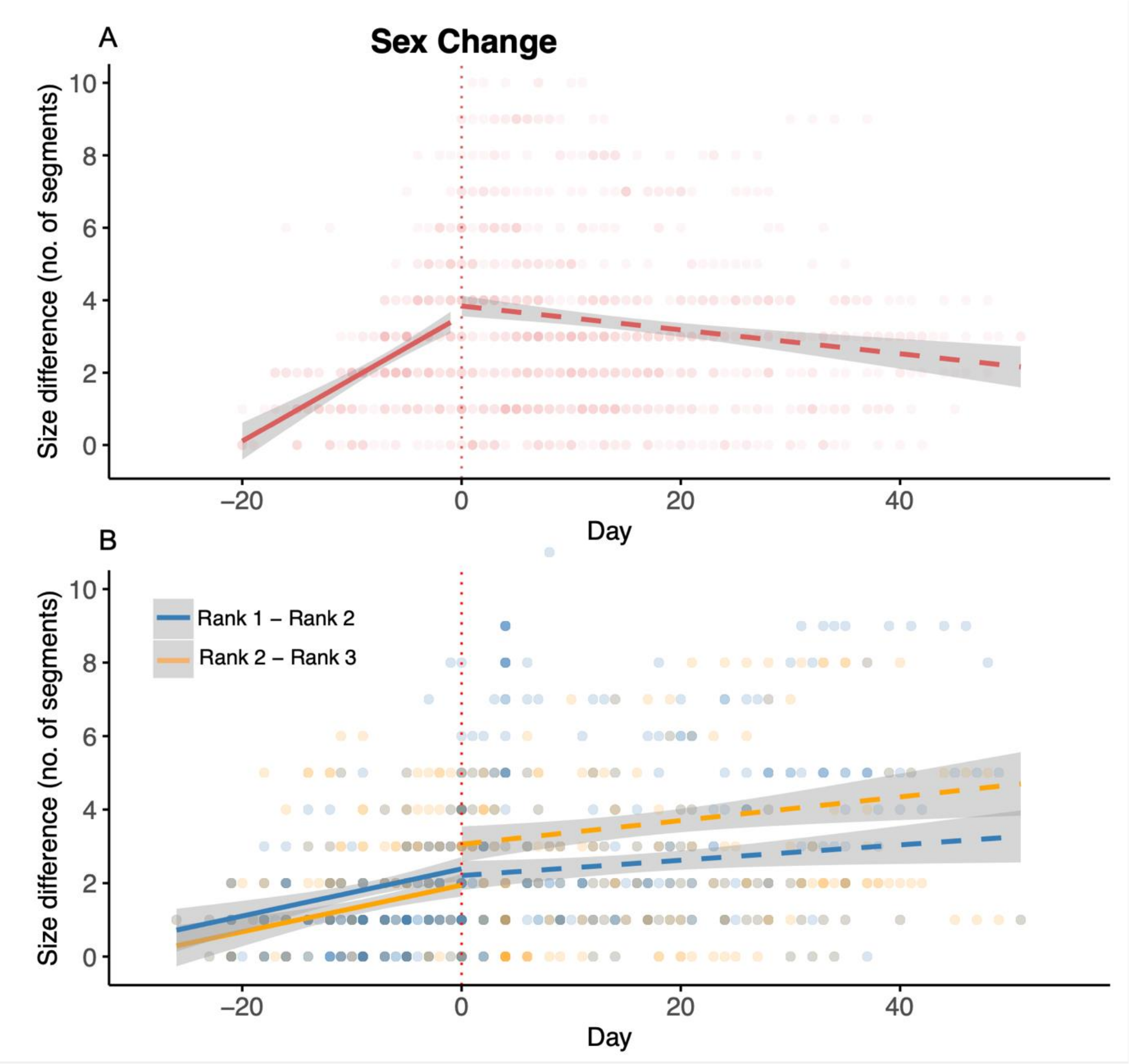

Figure 4. The establishment of a size hierarchy and its subsequent dynamics in Pairs and Triplets. In both Pairs A) and Triplets B), the size differences between the worms increased until Rank 1 worms changed sex (from male to female) at day 0. Following sex change, the size difference between the Rank 1 and Rank 2 worms diminished significantly in Pairs A). In Triplets, it kept increasing significantly with time, whereas that between Rank 2 and Rank 3 was maintained B). Raw data are shown in colour; the lines represent the predicted values from the GLMMs and the shading the 95% confidence intervals.

Table 2. The results of the post hoc GLMM analyses on the variation in body size difference with time before and after sex change from male to female – typically, by the largest worm – in Pairs and Triplets. Significant values are in bold.

Social environment	**Source of variation**	**Term of interest**	**Estimate ± SE**	**χ^2**	**P**
Pairs	**Time before Sex Change**	Rank 1-Rank 2	0.090 ± 0.010	77.085	**< 0.0001**
	Time after Sex Change	Rank 1-Rank 2	- 0.020 ± 0.002	100.15	**< 0.0001**
Triplets	**Time before Sex Change**	Rank 1-Rank 2	0.052 ± 0.014	13.376	**< 0.001**
		Rank 2-Rank 3	0.050 ± 0.013	15.966	**< 0.0001**
	Time after Sex Change	Rank 1-Rank 2	0.007 ± 0.003	5.774	**0.016**
		Rank 2-Rank 3	0.003 ± 0.003	0.587	0.443

5.3.2. Adjusting growth rates

5.3.2.1. Pairs

Paired worms exhibited rank-specific growth trajectories. The growth trajectory of Rank 1 individuals was characterised by a highly significant, pronounced, and prolonged growth spurt, as indicated by the steeper slope and later age at inflection compared to Rank 2 individuals (Fig. 5A, B; Table 3). However, even if their juvenile growth spurt was weaker and shorter, by the end of the experiment, Rank 2 individuals reached a larger body size than Rank 1 individuals (Fig. 5C, Table 3).

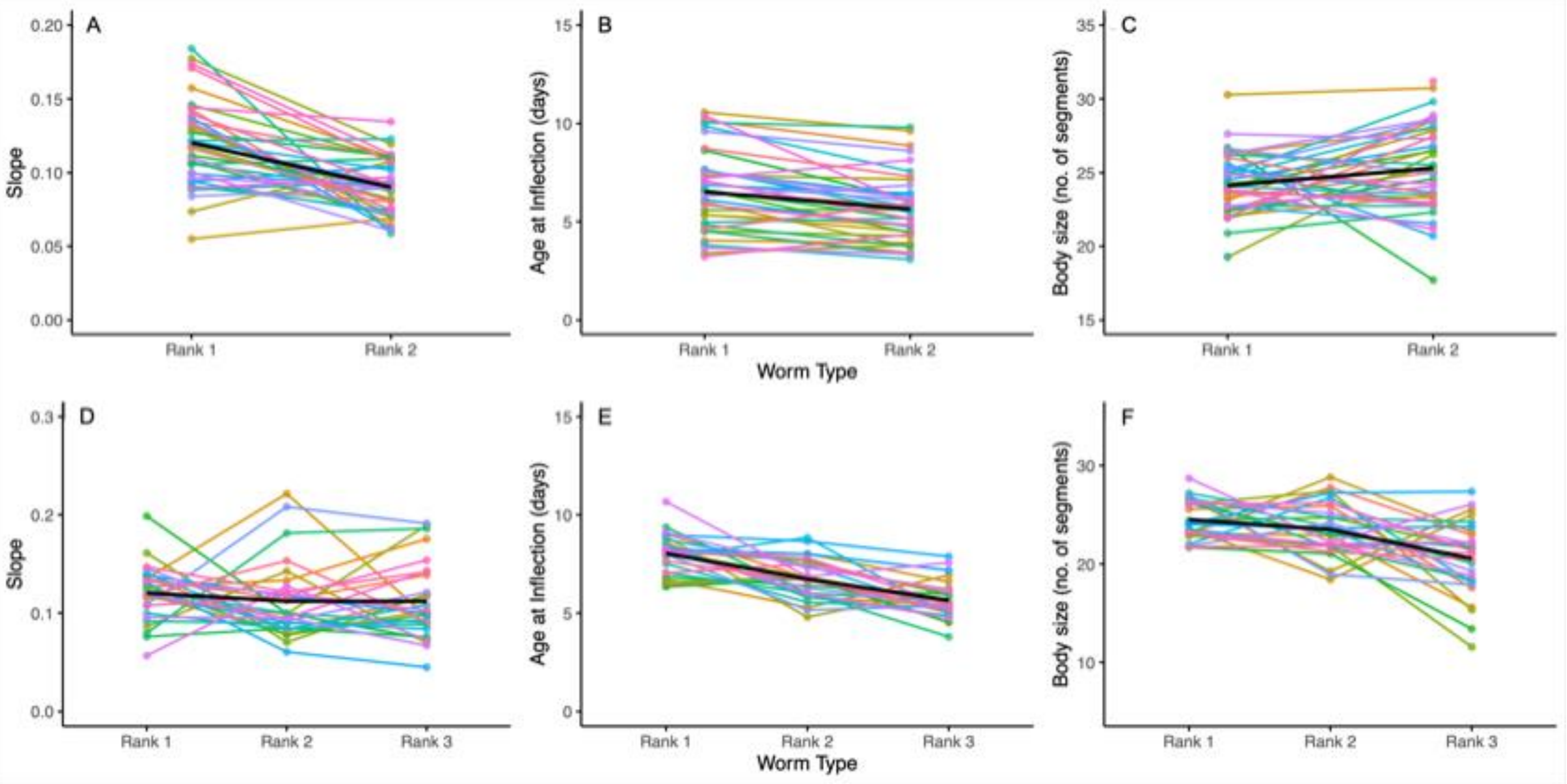

Figure 5. The interindividual relationships between worms in Pairs and Triplets for each growth parameter. Each line connects individuals sharing the same bowl. Lines are coded by colour for each Triplet and are consistent across the three parameters. Bold black lines highlight average values. A) Maximum growth rate (slope) of Rank 1 and Rank 2 worms in Pairs; B) Age at inflection of Rank 1 and Rank 2 worms in Pairs; C) Asymptotic body size of Rank 1 and Rank 2 worms in Pairs; D) Maximum growth rate (slope) of Rank 1, 2 and 3 worms in Triplets; E) Age at inflection of Rank 1, 2 and 3 worms in Triplets; F) Asymptotic body size of Rank 1, 2 and 3 worms in Triplets.

Table 3. The results of the GLMM analyses on growth curve parameters in Pairs and Triplets (all comparisons: df = 1). Significant values are in bold.

Social environment	**Term of interest**	**Source of variation**	**Estimate ± SE**	$\chi 2$	**P**
Pairs	Growth rate (slope)	Intercept	0.15 ± 0.008		**< 0.0001**
		Rank	-0.03 ± 0.005	35.729	**< 0.0001**
	Age at inflection	Intercept	7.405 ± 0.402		**< 0.0001**
		Rank	- 0.911 ± 0.182	24.93	**< 0.0001**
	Asymptotic body size	Intercept	23.023 ± 0.868		**< 0.0001**
		Rank	1.135 ± 0.539	4.432	**0.035**
Number of observations: 78 Number of Pairs: 40					
Triplets	Growth rate (slope)	Intercept	0.123 ± 0.01		**< 0.0001**
		Rank	- 0.004 ± 0.005	0.832	0.362
	Age at inflection	Intercept	9.203 ± 0.267		**< 0.0001**
		Rank	-1.199 ± 0.117	105.16	**< 0.0001**
	Asymptotic body size	Intercept	26.607 ± 0.825		**< 0.0001**
		Rank	-1.981 ± 0.373	28.182	**< 0.0001**
Number of observations: 84 Number of Pairs: 28					

5.3.2.2.| Triplets

Worms in Triplets exhibited rank-specific growth trajectories. Unlike Pairs, where Rank 1 and Rank 2 worms exhibited significantly different growth patterns, the three worms in Triplets grew at similar rates, as evidenced by comparable slopes (Fig. 5D; Table 3). However, the duration of the growth spurt was significantly associated with rank, as indicated by significantly different ages at inflection (Fig. 5E; Table 3). Rank 1 worms had the longest growth spurt, growing at the maximum rate for the longest duration, followed by Rank 2, and finally Rank 3 worms, which had the shortest growth spurt (Fig. 5E). By the end of the experiment, the body size of individuals in Triplets was significantly negatively associated with rank, with Rank 1 individuals exhibiting the largest body size (Fig. 5F; Table 3).

5.3.2.3. Is there a single fast-growth trajectory?

The highest ranks in Pairs and Triplets and the Isolated worms had significantly different body growth trajectories (slope: $\chi^2 = 25.396$, df = 2, $P < 0.0001$; age at inflection: $\chi^2 = 16.873$, df = 2, $P < 0.001$; asymptotic body size: $\chi^2 = 65.881$, df = 2, $P < 0.0001$).

Isolated worms exhibited significantly lower maximum growth rates in comparison to the highest ranks in both Pairs and Triplets, though their age at inflection was comparable to that of Rank 1 worms in Pairs but occurred significantly earlier than Rank 1 worms in Triplets (Table 4). Despite their slower growth rates, Isolated worms ultimately achieved the largest asymptotic body size (Table 4), and were the largest among all the experimental worms by the end of the experiment, likely because, lacking a mate, they did not reproduce during the experiment. Additionally, Rank 1 worms in Pairs grew similarly to Rank 1 worms in Triplets and reached a similar asymptotic size. However, the duration of the growth spurt was significantly longer for Rank 1 worms in Triplets (Table 4).

5.3.2.4. Is there a single slow-growth trajectory?

Overall, the lowest ranks in Pairs and Triplets and the worms in Groups of adults exhibited similar growth rates but differed in the duration of their spurt and reached highly significantly different asymptotic size (slope: $\chi^2 = 3.0769$, df = 2, $P = 0.215$; age at inflection: $\chi^2 = 7.575$, df = 2, $P = 0.023$; asymptotic body size: $\chi^2 = 36.979$, df = 2, $P < 0.0001$).

Worms in Groups of adults reached their age at inflection significantly later than Rank 2 in Pairs and Rank 3 worms in Triplets (Table 4). By the end of the experiment, worms in Groups of adults had an asymptotic body size significantly smaller than Rank 2 worms in Pairs but similar to that of Rank 3 worms in Triplets (Table 4).Additionally, Rank 2 worms in Pairs and Rank 3 worms in Triplets had similar duration of the growth spurt but by the end of the experiment Rank 2 worms in Pairs reached a larger body than Rank 3 worms in Triplets (Table 4).

Table 4: The results of the GLM Post-Hoc comparisons of growth curve parameters across highest (Isolated worms, Pairs Rank 1 and Triplets Rank 1) and lowest rank worms (worms in Groups of adults, Pairs Rank 2 and Triplets Rank 3). For the lowest ranks, the overall model on slope was non-significant; as a consequence, we did not perform post-Hoc tests. Significant values are in bold.

	Post hoc comparison	**Reference level**	**Level**	**Growth curve parameter**	**Estimate ± SE**	**χ2**	**P**
Highest ranks	Isolated worm *vs* Pairs Rank 1	Isolated worm	Pairs Rank 1	Slope	0.025 ± 0.006	20.593	**<0.0001**
				Age at inflection	0.177 ± 0.434	0.166	0.684
				Asymptotic body size	- 3.849 ± 0.548	49.302	**<0.0001**
	Isolated worm *vs* Triplets Rank 1	Isolated worm	Pairs Rank 1	Slope	0.025 ± 0.006	18.256	**<0.0001**
				Age at inflection	1.695 ± 0.394	18.494	**<0.0001**
				Asymptotic body size	- 3.479 ± 0.599	33.723	**<0.0001**
	Pairs Rank 1 *vs* Triplets Rank 1	Isolated worm	Pairs Rank 1	Slope	~ 0± 0.001	~ 0	0.999
				Age at inflection	1.518 ± 0.429	12.526	**0.0004**
				Asymptotic body size	0.370 ± 0.520	0.508	0.476
Lowest ranks	Pairs Rank 2 *vs* worms in Group	Worms in Group	Pairs Rank 2	Age at inflection	- 0.910 ± 0.405	5.037	**0.025**
				Asymptotic body size	5.266 ± 1.000	27.721	**<0.0001**
	Triplets Rank 3 *vs* worms in Group	Worms in Group	Triplets Rank 3	Age at inflection	- 0.908 ± 0.326	7.757	**0.005**
				Asymptotic body size	0.494 ± 1.217	0.165	0.685
	Pairs Rank 2 *vs* Triplets Rank 3	Pairs Rank 2	Triplets Rank 3	Age at inflection	0.002 ± 0.366	~ 0	0.996
				Asymptotic body size	- 4.771 ± 0.823	33.587	**<0.0001**

5.4. Discussion

Our results provide evidence of strategic growth in the annelid worm *Ophryotrocha puerilis.* As predicted, worms did not adhere to a single predetermined growth trajectory but modulated their body growth based on interindividual dynamics, such as their body size relative to conspecifics in their social environment, establishing their relative positions in a size hierarchy. To our knowledge, this is the first investigation providing empirical support for strategic growth in an invertebrate.

Size-matched juveniles in both Pairs and Triplets followed significantly different growth trajectories shortly after the start of the experiment, establishing an early size difference that progressively grew into a size hierarchy. Within these social environments, growth trajectories were specific to individual rank, i.e., position in the size hierarchy. In Pairs, Rank 1 worms consistently showed a pronounced and extended growth spurt, and this rapid growth led to Rank 1 worms achieving a larger body size earlier than their respective Rank 2 partners, as shown by increasing size differences. In Triplets, while worms exhibited similar accelerated growth, the duration varied among ranks, with Rank 1 worms showing the longest growth spurt, followed by Rank 2 and then Rank 3 worms.

Comparing high-rank worms across social environments (among which, 93% of the worms in Pairs and 60% of the worms in Triplets changed sex early, i.e., before day 20), we found that Rank 1 worms in Pairs and Triplets grew at similar rates, but both grew faster than worms in Isolation (suggesting competitive growth). Furthermore, the end of the growth spurt was significantly different among Rank 1 worms in Pairs and Triplets and Isolated worms, and occurred sequentially depending on the social context, with those in Isolation exhibiting the shortest growth spurt, followed by those in Pairs, and finally those in Triplets.

These differences cannot be attributed to status-dependent growth (*sensu* Heg et al., 2004), as all juveniles, at least in Pairs, would acquire the breeding status, yet their growth trajectories differed. Moreover, these differences cannot solely be explained by sex-change-induced differential growth rates (e.g., proportional to polygyny potential; Walker & McCormick 2009; Munday et al., 2009), nor sex-specific variations in growth (Munday et al., 2006), because they differed depending on the social environment. Indeed, the intensity of the growth spurt was associated with the number of individuals in the social environment (Pairs vs. Triplets) as well as an individual's rank among conspecifics within that environment (Rank-1 vs. lower-ranks), further reinforcing the notion that growth acceleration is a response to competition, as also observed in the Kalahari meerkat *Suricata suricatta* (Huchard et al., 2016), and the clown anemonefish *Amphiprion percula* (Reed et al., 2019; Iwata et al., 2020). Consequently, the end of the growth spurt likely signifies conflict resolution, similar to the reduction in aggressive interactions observed once size hierarchies is established in *A. percula* (Wong et al., 2016), and the cichlid *Neolamprologus pulcher* (Heg et al., 2004).

Our results are consistent with the suggestion that *O. puerilis* exhibit strategic growth in response to fine-grained changes in partners' size. Other possible explanations for differential growth rates among group members appear unlikely. Differential growth rates within multimember groups are well-documented and have been attributed primarily to factors as diverse as intrinsic attributes, maternal rank, winner-loser state, resource monopolisation, etc. (Dehnen et al., 2022; Tibbetts et al., 2022; Hamilton & Benincasa, 2022). For instance, unequal priority of access to food among individuals contributes to status-dependent growth rates in bonobos *Pan paniscus* (Vervaecke et al., 1999), and three-spined sticklebacks *Gasterosteus aculeatus* (Sneddon et al., 2006). However, in our experiment, it is unlikely that unequal access to food or early-life conditions influenced the growth trajectories, as all experimental worms were reared and exposed to identical and stable early-life conditions and received *ad libitum* food.

In contrast, the strategic growth hypothesis posits that variations in growth should exceed differences expected by chance, access to food, and initial size, proposing that the establishment of size hierarchies serves primarily to resolve interindividual conflicts over dominant positions (Buston, 2003; Heg et al., 2004; Hamilton & Heg, 2008; Walker & McCormick, 2009). Within established size-based hierarchies, group members not only arrange themselves in a linear 'pecking order' pattern (Strauss et al., 2022), but they also exhibit rank-specific growth patterns, thereby maintaining size differences that are critical for minimising rank-related conflict (Fitzgerald et al., 2022; Tibbetts et al., 2022). Therefore, a key distinction between status-dependent growth differences and strategic growth is that while the size differences typical of dominance hierarchies arise from status-dependent feedback loops (Dehnen et al., 2022), in species exhibiting strategic growth, these differences arise and are maintained through dominants interfering with subordinates' growth or through self-regulating mechanisms (e.g., Buston, 2003; Buston & Cant, 2006; Huchard et al., 2016; Russell et al., 2004; Fitzgerald et al., 2022; Benvenuto & Lorenzi, 2023). For instance, in the naked mole-rat *Heterocephalus glaber*, subordinate females previously housed with a dominant female showed an impressive 82% weight gain when newly paired with a male (Dengler-Crish & Catania, 2007), and growth rates tripled in subordinate males of the cichlid *N. pulcher* when an opportunity to assume the dominant position arose (Culbert et al., 2019). In protandrous sequential hermaphrodites such as *O. puerilis*, rank-related conflict may arise from competition for the female role, as the second sex is typically associated with greater reproductive success (Benvenuto et al., 2017; Roberts et al., 2021).

In the beginning of our experiment, where a clear size hierarchy had not yet been established, size-matched individuals in Pairs and Triplets had an equal opportunity to assume the Rank 1 position and become female, resulting in interindividual conflict (as discussed by Chase & Seitz, 2011; Dehnen et al., 2022). Previous studies on size-matched individuals in species displaying strategic growth have demonstrated that size ratios close to 1 lead to accelerated growth and a 'tug of war' growth pattern among rivals, where the acceleration of body growth in one individual triggers a pronounced bout of accelerated growth in a rival (competitive growth; Huchard et al., 2016; Reed et al., 2019; Iwata et al., 2020). For example, Huchard et al. (2016) induced weight gain in subordinate *S. suricatta* meerkats by supplementing their diet and found that this weight gain triggered a bout of accelerated growth in the closest, same sex subordinate above them without any food supplementation, possibly to reinforce the dominant status.

Typically, once a size hierarchy is established, agonistic behaviours diminish, and the hierarchy stabilises as the subordinate acknowledges its position, conceding the competition by downregulating growth (Wong et al., 2016; Benvenuto & Lorenzi, 2023). In our study, divergence in growth trajectories occurred relatively early in Pairs (around day 5), as seen by the early age at inflection, which signifies the cessation of accelerated growth, and thus can be interpreted as the moment when an individual concedes the competition. This is consistent with observations of early conflict resolution in size-matched dyads that occurred after just 1 day in the clown anemonefish *A. percula* (Wong et al., 2016). Early conflict resolution in pairs is likely due to the condition of enforced monogamy, where the interests of the two worms aligned quickly, leading to the prospective formation of a male-female breeding pair. Direct breeding-pair formation has also been observed in pairs of sexually immature anemonefish that differentiated directly into males and females (Iwata et al., 2020).

In Triplets, however, the size hierarchy was maintained, typically until the Rank 2 individuals changed sex. Unlike Pairs, where growth rate differed between the two, worms in triplets had comparable and high maximum growth rates, suggesting similar efforts by all individuals for Rank 1 position. However, the duration of the growth spurt varied significantly among the three, with each worm reaching its age at inflection in a sequential manner (Rank 3 conceded the competition first, followed by Rank 2 and then Rank 1). Unlike the condition of enforced monogamy in Pairs, the Triplets environment introduced mate competition between rivals. First, although sex change by Rank 1 worms partially resolved the conflict over which competitor reproduced as a female first, the size difference between the Rank 1 and Rank 2 worms kept increasing – likely evidence for a persistent competition over hierarchical position (for example, female-female competition for limited resources, including sperm; Stockley & Bro-Jørgensen, 2011; Pla et al., 2020). In contrast, the maintenance of the size difference between Rank 2 and Rank 3 worms (as well as between juveniles and adult males in Groups of adults) could be attributed to male-male competition as the males engage in continued conflict over fertilisation after the onset of reproduction. Indeed, Berglund (1986, 1991) reported heightened conflict between same-sex individuals in *O. puerilis*, often resulting in severe injuries from biting and even death. Males competed for access to females, with larger males frequently winning contests (Berglund, 1991), while females competed for the female mating role, often resulting in the 'loser' reverting to the male role (Berglund, 1986, 1990).

Our study demonstrates that *O. puerilis* exhibits finely tuned reaction norms for body growth that are highly responsive to social dynamics. Such plasticity is highly adaptive in environments where competition is variable and population densities fluctuate significantly, as is typical in the wild (Sella & Ramella, 1999). In such fluctuating social conditions—where individuals may face periods of high competition for mates or limited opportunities for reproduction—it may be more advantageous for individuals to modulate their growth rate and developmental trajectories in response to social cues. Individuals may grow rapidly and mature early in isolation in novel habitats, as predicted by Ghiselin (1969), or adopt slower growth trajectories when joining established groups where they may be relegated to the lowest rank to avoid conflict with group members. Such adaptability also facilitates opportunistic strategies critical to the success of colonizing species such as *O. puerilis* (Clark, 1978; Prevedelli et al., 2006; Taboada et al., 2017) and reflect the general adaptive advantage of plasticity in organisms facing variable ecological and social environments. Such inter- and intra-individual variation in phenotypic responses is also observed in the brown shrimp *Crangon crangon*, where individuals rapidly and reversibly adjust their coloration to enhance camouflage in response to their dynamic estuarine habitat (Siegenthaler et al., 2018).

There is now clear evidence of convergent evolution of the ability to modulate body growth in diverse vertebrates (Buston & Clutton-Brock, 2022), and our study extends this understanding to invertebrates. Indeed, our findings support the hypothesis that competition among individuals, be it for reproduction as females (e.g., Young et al., 2006; Dubuc & Clutton-Brock, 2019) or as males (e.g., Hamilton & Heg 2008; Heg, 2010), repeatedly and independently drives the evolution of the ability to modulate body growth depending on the size of close competitors in both vertebrates and invertebrates.

Direct measures of reduced social conflict as a consequence of hierarchy formation and rank-specific fitness outcomes would offer even more robust support for the strategic growth hypothesis in *O. puerilis*. Future studies should incorporate behavioural observations, individual identification, and parentage analysis, particularly in group settings where male–male competition is intense.

5.5. Conclusions

The present study highlights the highly context-dependent plasticity in growth trajectories in the marine worm *O. puerilis*, observed not only between worms exposed to different social environments but also between ranks. Our findings suggest that individual growth strategies are strongly influenced by social dynamics and are likely shaped by different social factors, particularly the intensity of sexual conflict, the individual's competitive ability relative to the others, and by its subsequent rank in the size hierarchy. How individuals assess reciprocal qualities, through behavioural interactions, chemical exchanges, or both, and whether it is a matter of high-rank individuals manipulating decisions of lower ranks or rather reciprocal quality assessments and individual decisions is a matter of debate (e.g., Benvenuto & Lorenzi, 2023), and a challenge for future investigations.

References

Bates, D., Maechler, M., Bolker, B., Walker, S., Christensen, R. H. B., Singmann, H., Dai, B., Grothendieck, G., Green, P. and Bolker, M. B. (2015). Package 'lme4'. *Convergence*, *12*(1), 2.

Benvenuto, C., Coscia, I., Chopelet, J., Sala-Bozano, M., & Mariani, S. (2017). Ecological and evolutionary consequences of alternative sex-change pathways in fish. Scientific Reports, 7(1), 1-12.

Benvenuto, C., & Lorenzi, M. C. (2023). Social regulation of reproduction: control or signal? *Trends in Ecology & Evolution*, *38*, 1028-1040.

Berglund, A. (1986). Sex change by a polychaete: effects of social and reproductive costs. *Ecology,* 67(4), 837-845.

Berglund, A. (1990). Sequential hermaphroditism and the size-advantage hypothesis: an experimental test. *Animal Behaviour*, *39(*3), 426-433.

Berglund, A. (1991). To change or not to change sex: a comparison between two *Ophryotrocha* species (Polychaeta). *Evolutionary Ecology*, *5*, 128-135.

Berruti, G. (1980). The effect of starvation on the appearance of the definitive upper jaw in *Ophryotrocha puerilis* (Annelida, Polychaeta). *Italian Journal of Zoology*, 47(1-2), 71-74.

Borowsky, R. L. (1973). Social control of adult size in males of *Xiphophorus variatus. Nature*, *245*(5424), 332-335.

Branconi, R., Barbasch, T. A., Francis, R. K., Srinivasan, M., Jones, G. P., & Buston, P. M. (2020). Ecological and social constraints combine to promote evolution of non-breeding strategies in clownfish. *Communications Biology*, *3*(1), 649.

Buston, P. (2003). Size and growth modification in clownfish. *Nature*, *424(6945),* 145-146.

Buston, P. M. (2004). Territory inheritance in clownfish. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *271*, S252-S254.

Buston, P. M., & Cant, M. A. (2006). A new perspective on size hierarchies in nature: patterns, causes, and consequences. *Oecologia*, *149*, 362-372.

Buston, P., & Clutton-Brock, T. (2022). Strategic growth in social vertebrates. *Trends in Ecology & Evolution, 37*(8), 694-705.

Case, T. J. (1978). On the evolution and adaptive significance of postnatal growth rates in the terrestrial vertebrates. *The Quarterly Review of Biology*, *53*(3), 243-282.

Chase, I. D., & Seitz, K. (2011). Self-structuring properties of dominance hierarchies: a new perspective. *Advances in genetics*, *75,* 51-81.

Charnov, E.L. (1982) The Theory of Sex Allocation. Princeton University Press, Princeton, New Jersey, USA.

Croll, J. C., van Kooten, T., & de Roos, A. M. (2024). An energetic approach to the evolution of growth curve plasticity. *Theoretical Ecology*, *17*(1), 13-33.

Culbert, B. M., Balshine, S., & Gilmour, K. M. (2019). Physiological regulation of growth during social ascension in a group-living fish. *Physiological and Biochemical Zoology*, *92*(2), 211-222.

Dapporto, L., Palagi, E., Cini, A., & Turillazzi, S. (2006). Prehibernating aggregations of *Polistes dominulus*: an occasion to study early dominance assessment in social insects. *Naturwissenschaften*, *93*, 321-324.

Dehnen, T., Arbon, J. J., Farine, D. R., & Boogert, N. J. (2022). How feedback and feed-forward mechanisms link determinants of social dominance. *Biological Reviews*, *97*(3), 1210-1230.

Dengler-Crish, C. M., & Catania, K. C. (2007). Phenotypic plasticity in female naked mole-rats after removal from reproductive suppression. *Journal of Experimental Biology*, *210*(24), 4351-4358.

Dubuc, C., & Clutton-Brock, T. H. (2019). Male immigration triggers increased growth in subordinate female meerkats. *Ecology and Evolution*, *9*(3), 1127-1134.

Duffy, J. E. (1996). Eusociality in a coral-reef shrimp. *Nature*, *381*(6582), 512-514.

Enquist, M., Leimar, O., Ljungberg, T., Mallner, Y. & Segerdahl, N. 1990. A test of the sequential assessment game: fighting in the cichlid fish *Nannacara anomala*. *Animal Behaviour, 40,* 1–14.

Fitzgerald, L.M., Harrison, H.B., Coker, D.J., Saenz-Agudelo, P., Srinivasan, M., Majoris, J.E., Boström Einarsson, L., Pujol, B., Bennett-Smith, M., Thorrold, S.R., Planes, S., Jones G. P. & Berumen L. (2022). Rank change and growth within social hierarchies of the orange clownfish, *Amphiprion percula*. *Marine Biology*, *169*(10), 128.

Ghiselin, M. T. (1969). The evolution of hermaphroditism among animals. *The Quarterly review of biology*, *44*(2), 189-208.

Hamilton, I. M., & Benincasa, M. D. (2022). Emergence of size-structured dominance hierarchies through size-dependent feedback. *Philosophical Transactions of the Royal Society B*, *377*(1845), 20200449.

Hamilton, I. M., & Heg, D. (2008). Sex differences in the effect of social status on the growth of subordinates in a co-operatively breeding cichlid. *Journal of Fish Biology*, *72*(4), 1079-1088.

Hartmann, M., & Huth, W. (1936). Untersuchungen über die Geschlechtsbestimmung und Geschlechtsumwandlung von *Ophryotrocha puerilis*. *Zoologische Jahrbücher (Physik)*, 56, 389-439.

Heg, D. (2010). Status-dependent and strategic growth adjustments in female cooperative cichlids. *Behavioral Ecology and Sociobiology*, *64*, 1309-1316.

Heg, D., Bender, N., & Hamilton, I. (2004). Strategic growth decisions in helper cichlids. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *271*(6), S505-S508.

Holekamp, K. E., & Strauss, E. D. (2016). Aggression and dominance: an interdisciplinary overview. *Current Opinion in Behavioral Sciences*, *12*, 44-51.

Huchard, E., English, S., Bell, M. B., Thavarajah, N., & Clutton-Brock, T. (2016). Competitive growth in a cooperative mammal. *Nature*, *533*(7604), 532-534.

Iwata, E., Yoshida, T., Abe, T., Takahara, K., & Masuda, K. (2020). Behavioural differences between breeding and nonbreeding pairs of protandry monogamous false clown anemonefish *Amphiprion ocellaris*. *Journal of Fisheries*, *8*(2), 808-816.

Kasumovic, M. M., & Brooks, R. C. (2011). It's all who you know: the evolution of socially cued anticipatory plasticity as a mating strategy. *The Quarterly Review of Biology*, *86*(3), 181-197.

Kegel, B., & Pfannenstiel, H. D. (1983). Evaluation of the pair-culture effect in *Ophryotrocha puerilis* (Polychaeta: Dorvilleidae). I. Pair-culture effect and sex ratio. *Helgoländer Meeresuntersuchungen*, 36, 205-213.

Kier, W. M. (2012). The diversity of hydrostatic skeletons. *Journal of Experimental Biology*, *215*(8), 1247-1257.

Kokko, H., & Johnstone, R. A. (1999). Social queuing in animal societies: a dynamic model of reproductive skew. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *266*(1419), 571-578.

Metcalfe, N. B. (2024). How important is hidden phenotypic plasticity arising from alternative but converging developmental trajectories, and what limits it? *Journal of Experimental Biology*, *227*(Suppl 1), jeb246010.

Munday, P. L., Buston, P. M., & Warner, R. R. (2006). Diversity and flexibility of sex-change strategies in animals. *Trends in Ecology & Evolution*, *21*(2), 89-95.

Munday, P. L., Ryen, C. A., McCormick, M. I., & Walker, S. P. W. (2009). Growth acceleration, behaviour and otolith check marks associated with sex change in the wrasse *Halichoeres miniatus*. *Coral Reefs*, *28*, 623-634.

Murray, H. M., Gallardi, D., Gidge, Y. S., & Sheppard, G. L. (2012). Histology and mucous histochemistry of the integument and body wall of a marine polychaete worm, *Ophryotrocha* n. sp. (Annelida: Dorvilleidae) associated with Steelhead Trout Cage Sites on the South Coast of Newfoundland. *Journal of Marine Sciences*, *2012*(1), 202515.

Paxton, H., & Åkesson, B. (2007). Redescription of *Ophryotrocha puerilis* and *O. labronica* (Annelida, Dorvilleidae). *Marine Biology Research*, *3*(1), 3-19.

Pfannenstiel, H. D. (1977). Experimental analysis of the 'Paarkultureffekt'in the protandric polychaete, *Ophryotrocha puerilis* Clap. Mecz. *Journal of Experimental Marine Biology and Ecology*, *28*(1), 31-40.

Pla, S., Benvenuto, C., Capellini, I. & Piferrer, F. (2020). A phylogenetic comparative analysis on the evolution of sequential hermaphroditism in seabreams (Teleostei: Sparidae). *Scientific Reports*, *10*(1), 3606.

Premoli, M. C., & Sella, G. (1995). Sex economy in benthic polychaetes. *Ethology, Ecology & Evolution*, *7*(1), 27-48.

Prevedelli, D., Massamba N'Siala, G., & Simonini, R. (2005). The seasonal dynamics of six species of Dorvilleidae (Polychaeta) in the harbour of La Spezia (Italy). *Marine Ecology*, *26*(3-4), 286-293.

Prevedelli, D., N'SIALA, G. M., & Simonini, R. (2006). Gonochorism vs. hermaphroditism: relationship between life history and fitness in three species of *Ophryotrocha* (Polychaeta: Dorvilleidae) with different forms of sexuality. *Journal of Animal Ecology*, *75*(1), 203-212.

Radhakrishnan, P., Saboul, N., Monclús, R., Taboada, S. & Lorenzi, M.C. (2025). Data from: Game over: Conflict resolution through strategic growth in an invertebrate. Dryad Digital Repository. https://doi.org/10.5061/dryad.mkkwh71bg

R Core Team (2024). R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.

Reed, C., Branconi, R., Majoris, J., Johnson, C., & Buston, P. (2019). Competitive growth in a social fish. *Biology Letters*, *15*(2), 20180737.

Ritz, C., Baty, F., Streibig, J.C., and Gerhard, D. (2015). Dose-Response Analysis Using R. PLoS ONE, 10(12), e0146021.

Roberts, B. H., Morrongiello, J. R., Morgan, D. L., King, A. J., Saunders, T. M., & Crook, D. A. (2021). Faster juvenile growth promotes earlier sex change in a protandrous hermaphrodite (barramundi Lates calcarifer). *Scientific reports*, *11*(1), 2276.

Rot-Nikcevic, I., Taylor, C. N., & Wassersug, R. J. (2006). The role of images of conspecifics as visual cues in the development and behavior of larval anurans. *Behavioral Ecology and Sociobiology*, *60*, 19-25.

Russell, A. F., Carlson, A. A., McIlrath, G. M., Jordan, N. R., & Clutton-Brock, T. (2004). Adaptive size modification by dominant female meerkats. *Evolution*, *58*(7), 1600-1607.

Schärer, L. (2009). Tests of sex allocation theory in simultaneously hermaphroditic animals. *Evolution*, *63*(6), 1377-1405.

Sella, G., & Ramella, L. (1999). Sexual conflict and mating systems in the dorvilleid genus *Ophryotrocha* and the dinophilid genus *Dinophilus*. *Hydrobiologia*, *402*, 203-213.

Siegenthaler, A., Mastin, A., Dufaut, C., Mondal, D., & Benvenuto, C. (2018). Background matching in the brown shrimp *Crangon crangon*: adaptive camouflage and behavioural-plasticity. *Scientific Reports*, *8*(1), 3292.

Simonini, R., Grandi, V., Massamba-N'Siala, G., Martino, M. P., Castelli, A., & Prevedelli, D. (2010). Diversity, habitat affinities and diet of *Ophryotrocha* species (Polychaeta, Dorvilleidae) living in Mediterranean harbour habitats. *Vie et Milieu/Life & Environment*, 27-38.

Sneddon, L. U., Hawkesworth, S., Braithwaite, V. A., & Yerbury, J. (2006). Impact of environmental disturbance on the stability and benefits of individual status within dominance hierarchies. *Ethology*, *112*(5), 437-447.

Stockley, P., & Bro-Jørgensen, J. (2011). Female competition and its evolutionary consequences in mammals. *Biological Reviews*, *86*(2), 341-366.

Stoltz, J. A., Andrade, M. C., & Kasumovic, M. M. (2012). Developmental plasticity in metabolic rates reinforces morphological plasticity in response to social cues of sexual selection. *Journal of Insect Physiology*, *58*(7), 985-990.

Strauss, E. D., Curley, J. P., Shizuka, D., & Hobson, E. A. (2022). The centennial of the pecking order: current state and future prospects for the study of dominance hierarchies. *Philosophical Transactions of the Royal Society B*, *377*(1845), 20200432.

Taboada, S., Bas, M., Leiva, C., Garriga, M., Sardá, R., & Avila, C. (2016). Life after death: shallow-water Mediterranean invertebrate communities associated with mammal bones. *Marine Ecology*, *37*(1), 164-178.

Taboada, S., Leiva, C., Bas, M., Schult, N., & McHugh, D. (2017). Cryptic species and colonization processes in *Ophryotrocha* (Annelida, Dorvilleidae) inhabiting vertebrate remains in the shallow-water Mediterranean. *Zoologica Scripta*, *46*(5), 611-624.

Thorley, J., Katlein, N., Goddard, K., Zöttl, M., & Clutton-Brock, T. (2018). Reproduction triggers adaptive increases in body size in female mole-rats. *Proceedings of the Royal Society B*, *285*(1880), 20180897.

Thornhill, D.J., Dahlgren, T.G., Halanych, K.M. (2009). Evolution and ecology of *Ophryotrocha* (Dorvilleidae, Eunicida). In: Shain DH, editor. *Annelids in modern biology*. Hoboken (NJ): Wiley-Blackwell. pp. 242–256.

Tibbetts, E. A., Pardo-Sanchez, J., & Weise, C. (2022). The establishment and maintenance of dominance hierarchies. *Philosophical Transactions of the Royal Society B*, *377*(1845), 20200450.

Vervaecke, H., De Vries, H., & Van Elsacker, L. (1999). An experimental evaluation of the consistency of competitive ability and agonistic dominance in different social contexts in captive bonobos. *Behaviour*, *136*(4), 423-442.

Volpato, G. L., & Fernandes, M. O. (1994). Social control of growth in fish. *Brazilian Journal of Medical and Biological Research*, *27*, 797-797.

Walker, S. P., & McCormick, M. I. (2009). Sexual selection explains sex-specific growth plasticity and positive allometry for sexual size dimorphism in a reef fish. *Proceedings of the Royal Society B: Biological Sciences*, *276*(1671), 3335-3343.

West, G. B., Brown, J. H., & Enquist, B. J. (2001). A general model for ontogenetic growth. *Nature*, *413*(6856), 628-631.

West-Eberhard, M. J. (2003). *Developmental plasticity and evolution*. Oxford University Press.

Wong, M. Y., Munday, P. L., Buston, P. M., & Jones, G. P. (2008). Fasting or feasting in a fish social hierarchy. *Current Biology*, *18*(9), R372-R373.

Wong, M. Y., Uppaluri, C., Medina, A., Seymour, J., & Buston, P. M. (2016). The four elements of within-group conflict in animal societies: an experimental test using the clown anemonefish, *Amphiprion percula*. *Behavioral Ecology and Sociobiology*, *70*, 1467-1475.

Young, A. J., Carlson, A. A., Monfort, S. L., Russell, A. F., Bennett, N. C., & Clutton-Brock, T. (2006). Stress and the suppression of subordinate reproduction in cooperatively breeding meerkats. *Proceedings of the National Academy of Sciences*, *103*(32), 12005-12010.

Zeileis A, Grothendieck G (2005). zoo: S3 Infrastructure for Regular and Irregular Time Series. *Journal of Statistical Software*, 14(6), 1–27.

CHAPTER 6

Early-life social environment shapes sex allocation in a sex-changing worm

Pooja Radhakrishnan[1]*, Noé Saboul*[1]*, Maria Cristina Lorenzi*[1]

[1]LEEC, Laboratoire d'Éthologie Expérimentale et Comparée, Université Sorbonne Paris Nord, Villetaneuse (France)

Accepted in *Ethology Ecology & Evolution*
16 October 2025

Abstract

Sexual plasticity allows individuals to maximize fitness by adjusting sex allocation in response to environmental cues. Sequential hermaphroditism, or sex change, is expected to evolve when the reproductive value (i.e., expected future reproductive success) of one sex increases disproportionately with body size. While socially mediated sex change is well documented in adults, little is known about how juveniles respond to social cues during development. Here, we investigated sex allocation plasticity in size- and age-matched juveniles of the marine annelid *Ophryotrocha puerilis* exposed to four social environments: isolation, exposure to adults, and size-matched pairs and triplets. Isolated juveniles changed sex early, whereas nearly all juveniles exposed to adults deferred sex change, suggesting that the presence of adults modulates the timing of sex change. Juveniles in pairs and triplets consistently formed mating pairs, with one individual changing sex to female early, supporting what we term the *matchmaking hypothesis*, which proposes that juveniles use conspecific cues to adjust their developmental trajectories and resulting sex allocation. We discuss the adaptive value of early-life social sensitivity and the potential for sex role preferences in juveniles. This study highlights how social environments shape developmental trajectories in sequential hermaphrodites, beginning earlier in life than previously recognized.

Keywords: sex allocation, plasticity, sequential hermaphroditism, social mediation of sex change, juvenile, *Ophryotrocha*

6.1. Introduction

In most animals, sex is fixed and individuals are gonochoristic, developing either as male or female. In hermaphroditic species, by contrast, individuals are cosexual, capable of expressing both sexual functions either simultaneously (simultaneous hermaphrodites) or sequentially (sequential hermaphrodites) (Leonard, 2018). The adaptive value of sequential hermaphroditism, or sex change, has long been explained by the size-advantage hypothesis, originally proposed by Ghiselin (1969), and developed further by Warner (1975, 1988) and Charnov (1982). According to this model, sex change is favoured when the reproductive value of one sex increases more steeply with body size than that of the other, allowing individuals to maximize their lifetime fitness by functioning first as the sex with size-independent fitness, and changing to the size-dependent sex once a critical size threshold is reached.

Since female fecundity generally scales positively with size (particularly in fishes and invertebrates, where sequential hermaphroditism is most common; Pla et al., 2022), the direction of sex change (male to female, or protandry; versus female to male, or protogyny) is largely determined by the shape of the male fitness curve, which in turn depends on the mating system, as male reproductive success is often mediated by sexual selection (Guallart et al., 2013; Beukeboom & Perrin, 2014; Vega-Frutis et al., 2014; Benvenuto et al., 2017; Kuwamura et al., 2020).

Protandry generally occurs in species with random mating or non-size-assortative monogamy, where female preference for male size is weak or absent, and sperm competition is low (Kuwamura et al., 2020). In these systems, male reproductive success is relatively independent of size (Casas & Suborido-Rey, 2021), favouring early male function and subsequent change to female. Protogyny, by contrast, is typically associated with mating systems under strong sexual selection, where large males achieve disproportionate reproductive success by monopolising mates or territories (Munday et al., 2006; Kadota, 2022), making it adaptive to begin life as a female and change sex when large enough to compete successfully for mates.

While Ghiselin’s model emphasizes intrinsic factors such as size and age in determining the optimal size at and direction of sex change, Charnov’s (1982) sex allocation theory expands this by incorporating extrinsic social factors as critical determinants of an individual’s optimal sex allocation. This framework posits that sex allocation, defined as the relative investment in male versus female function, is shaped both by an individual's own condition (e.g., body size, age, or physiological state) and by its condition relative to conspecifics, including factors like position in the size hierarchy, social status, and group composition (Charnov, 1982; West, 2009). Individuals are expected to change sex when the reproductive payoff from the second sex exceeds that of remaining in their current sex, i.e., when it maximizes their reproductive value (Munday et al., 2006).

Accordingly, the social environment becomes a key determinant of an individual's reproductive value, since the relative benefits of being male or female are often context-dependent and vary with the composition of the social group (Munday et al., 2006; Beukeboom & Perrin, 2014). For instance, in the protogynous goby *Coryphopterus glaucofraenum* Gill, 1863, isolated females rarely change sex, but the presence of a competitor triggers sex change (Cole & Shapiro, 1995). In haremic and protogynous species like the bluehead wrasse *Thalassoma bifasciatum* Bloch, 1791 and the cleaner wrasse *Labroides dimidiatus* Valenciennes, 1839, size determines social status, which in turn regulates sex change (Warner & Swearer, 1991; Munday et al., 2010). Other factors such as sex ratio (Shapiro & Lubbock, 1980), size-ratio among conspecifics (Ross et al., 1983), presence of smaller conspecifics (Cole & Shapiro, 1995), as well as potential for sperm competition and reproductive skew (Munoz & Warner, 2003, 2004) can also modulate the timing and likelihood of sex change.

Among marine invertebrates, theoretical models predict that socially mediated sex change should be strongly favoured in species with frequent or unpredictable changes in local social structure, such as those with planktonic larvae that settle in novel habitats (Hoagland, 1978). Yet, empirical evidence from direct-developing species such as *Crepidula coquimbensis* Brown and Olivares, 1996, *C.* cf. *onyx*, and *C. incurva* Broderip, 1834 suggests that environmental unpredictability, rather than dispersal mode alone, may be the key selective pressure favouring social mediation of sex change (Collin et al., 2005; Brante et al., 2012). If social mediation of sex change is adaptive under such variable conditions, responsiveness to conspecific cues should not be limited to adults but also extend to juveniles, which may experience similar ecological pressures. However, despite considerable research on social influences shaping sex allocation in adults, little is known about how juveniles respond to social cues during development.

Here, we experimentally test how early-life social environments shape sex allocation in the marine annelid *Ophryotrocha puerilis* Claparède & Mecznikow, 1869, a protandrous sequential hermaphrodite (Paxton & Åkesson, 2007). This species is an ideal model for studying socially mediated sex change due to its remarkable plasticity and responsiveness to social cues (. However, direct observations of *O. puerilis* in their natural habitat remain particularly challenging because of their small size (adults: 4–7 mm, Thornhill et al., 2009; juveniles: 0.7–1 mm, Åkesson, 1967; P.R., personal observation) and their marine environment. As such, many aspects of their natural ecology, such as their prevalent social structure and behaviour in the wild remain poorly understood, though laboratory studies have revealed various environmental and social influences on adult sex allocation.

Early experimental work using artificial selection for delayed or early sex change showed that *O. puerilis* exhibits polygenic sex determination, i.e., multiple genes influence whether an individual initially functions as male or female (Bacci & Bortesi, 1961a, b; Bacci, 1965). Müller (1962) proposed that while individuals may have a genetic predisposition toward maleness or femaleness, environmental and physiological factors can modulate these tendencies. This view has been supported by other studies conducted both before and after Müller's work, showing that sex change can be influenced by environmental conditions such as temperature (Bacci & Voria, 1970) and water mineral ion concentration (Hartmann & von Lewinski, 1938), as well as physiological factors like injury (Hartmann & Huth, 1936) and starvation (Berruti, 1980).

Moreover, *O. puerilis* also shows an impressive ability to modulate sex allocation in response to social cues. Studies have shown that subtle conspecific cues such as relative body size among conspecifics (Hartmann & Huth, 1936), pheromonal and chemical signalling (Hartmann & von Lewinski, 1940; Marchionni & Rolando, 1979, 1981; Pfannenstiel, 1975, 1977, 1978), direct physical contact (Müller, 1962), sex ratio among conspecifics (Kegel & Pfannenstiel, 1983) and interindividual conflict (Berglund, 1986, 1990) can all modulate patterns of sex allocation. However, these effects have primarily been observed in adults, in the context of sex reversal (i.e., when individuals that have already changed sex to female subsequently change back to male).

In this study, we exposed juvenile worms to a range of early-life social environments reflecting natural variation in population density and social group composition: complete isolation, pairs and triplets of size-matched juveniles, and high-density, male-biased adult groups. We considered three possible outcomes based on previous research in other species. First, under the *induction hypothesis* (Ross, 1990), social interactions may be necessary to trigger sex change, so isolated juveniles should delay sex change. Second, under the *inhibition* *hypothesis* (Ross, 1990), the presence of larger or adult conspecifics may suppress sex change, so juveniles raised with adults should delayed sex change. Third, among pairs and triplets of size-matched juveniles, we propose the *matchmaking hypothesis*, positing that juveniles may independently respond to social cues by adjusting their developmental trajectories and eventual sex allocation to facilitate mating pair formation. We therefore expect that one individual will either develop directly as a female (i.e., skipping the initial male phase) or go through a transient male phase and change sex to female early.

6.2. Methods

Study Model

Ophryotrocha puerilis is a small marine polychaete worm first described by Claparède and Mecznikow (1869) from specimens collected at the Stazione Zoologica in Naples. The authors observed that even adult individuals exhibited juvenile or rudimentary features compared to other polychaetes, which led them to designate it the type species of a new genus and name it *puerilis*. It is the only known sequential hermaphrodite in its genus (Paxton & Åkesson, 2007; Thornhill et al., 2009) and is an opportunistic colonizer often found in interstitial spaces, such as mussel colonies (Taboada et al., 2016, 2017). The laboratory population used in this study originated from samples collected in Spain (Blanes, NW Mediterranean, 41°40.4680N, 2°47.9430E) in 2015 (Sergi Taboada, pers. comm.).

Body size is measured as segment count, a reliable method due to the hydrostatic skeletons of annelids (Kier, 2012). *Individuals* typically mature first as males at a body size of approximately 9 segments and may change sex to female at 14-16 segments (Paxton & Åkesson, 2007). Mating occurs via pseudocopulation, during which gametes are simultaneously released into a gelatinous mucous cocoon (Paxton & Åkesson, 2007). Juveniles emerge from cocoons after 7-8 days at 1-2 segments and continue growing by adding chaetigerous segments (Åkesson, 1967). Typically, generation time is approximately 35 days at 20°C (Åkesson, 1967), and lifespan in laboratory conditions is about 5 months (Prevedelli et al., 2006).

Experimental Procedure

We took newly emerged juveniles from their cocoons at one week of age and two body segments of size and isolated them for one week, ensuring the same age and body size while minimizing potential early-life social effects. All experimental juveniles were sourced from 76 parental pairs to ensure they were not related, and measured 4-7 segments long and 12-14 days old at the start of the experiment. If an individual died during the experiment, the replicate was excluded.

Using the same dataset as Radhakrishnan et al. (in press), we explored whether *O. puerilis* juveniles adjust the timing of sex change in response to different social conditions. Juveniles were exposed to four ecologically relevant conditions over 60 days: isolation (n = 55), pairs (n = 55), triplets (n = 30), and a group of adults (n = 30). Juveniles in the adult treatment were housed with three 14-segment males and one 21-segment female, reflecting a typical male-biased sex ratio observed in high-density wild populations (Premoli & Sella, 1995).

Worms were housed in glass bowls with 10ml filtered artificial marine water (35% salinity) maintained in thermostatic cabinets set at 21°C under a 24-hour dark photoperiod cycle. Water changes were conducted weekly to minimize accumulation of metabolites, and spinach was offered *ad libitum* as food.

Measurements

Every 2-5 days, worms were screened for sex change, identified by the first appearance of oocytes visible through the transparent body wall under a stereomicroscope (Kegel & Pfannenstiel, 1983; Paxton & Åkesson, 2007). The day of first oocyte appearance was recorded as the *timing of sex change,* and segment number on that day was recorded as *body size at sex change*. The first individual to change sex in a replicate was typically larger than its partner(s). Therefore, in pairs and triplets, individuals were categorized by relative size at the time of first sex change: "Large" (early sex changer in pairs and triplets), "Intermediate" (triplets only), and "Small" (later sex changers in pairs and triplets) (see Results).

Statistical Analysis

The timing of sex change was analysed across social environments using a log-rank test for survival analyses. Statistical analyses were performed in R (version 4.3.1) http:// www.r-project.org (R Core Team, 2023) using the “survival” (Therneau et al., 2000) and “lme4” (Bates et al., 2015) packages.

To explore variation in timing and body size at sex change, we ran Generalized Linear Mixed Models (GLMMs) with either timing or body size as the response variable, and juvenile size category as a seven-level fixed predictor: 2 levels for pairs (Large, Small); 3 levels for triplets (Large, Intermediate, Small); and 1 level each for juveniles in isolation or groups of adults. In these models, social environment and bowl ID were included as random factor to account for correlated responses within social environments and bowls.

As these first analyses indicated significant differences, we further tested for variation in timing of and body size at sex change *within* social environment using GLMMs (bowl ID as random factor), and for variation *between* social environments among juveniles belonging to the same size-categories using GLMs. As both response variables were count data, models for Poisson-distributed data were used; whenever the data were overdispersed, we added a case-level random factor.

6.3. Results

6.3.1. *Rate of Sex Change Across Social Environments and Size Categories*

Overall, during the 60-day experiment, of the 238 surviving worms across social environments, 177 (74.4%) changed sex to female, while 60 (25.2%) deferred sex change (Fig. 1). Among the total 170 replicates, 37 were excluded either due to mortality or other incidental factors.

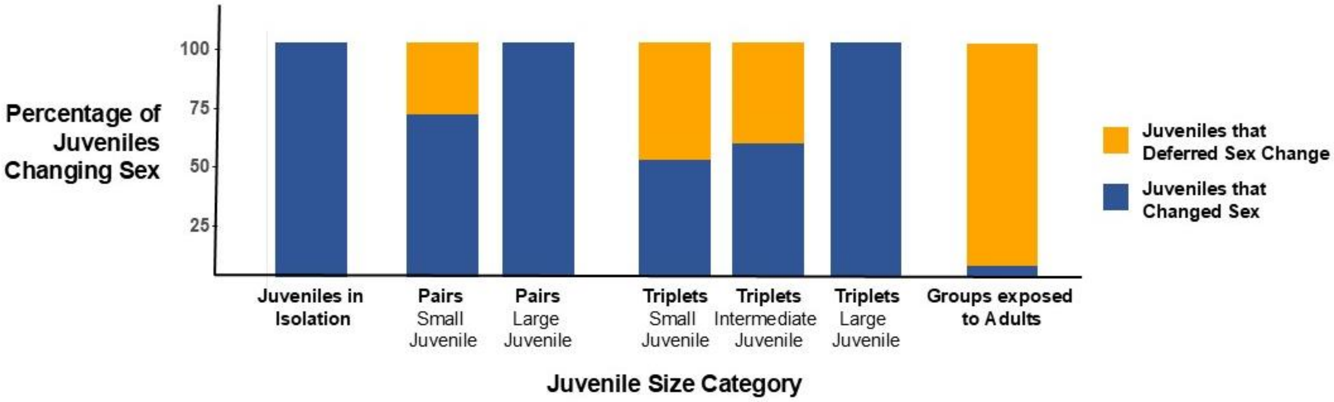

Figure 1: **Percentage of juvenile sex change across social environments and juvenile categories.** Juveniles either changed sex (blue) or deferred sex change (orange). Each bar represents a specific juvenile type by size category: isolated juveniles; small and large juveniles in pairs; small, intermediate, and large juveniles in triplets; and juveniles exposed to groups of adults.

6.3.2. *Do worms follow a fixed sex change schedule?*

The timing of sex change was highly plastic and strongly influenced by social environment (Table 1). The analysis of the rate of sex change revealed that worms exhibited different sex change strategies across social environments, with individuals of the same size class showing consistent trends (Log-rank test, $P < 0.0001$) (Fig. 2).

Table 1. Variability in the Timing and Body Size at Sex Change Across Different Social Environments

Social Environment	Juvenile Size Category	Timing of Sex Change (Mean ± SD)	Body Size at Sex Change (Mean ± SD)	No. of observations
Isolated		15.688 ± 4.887	16.25 ± 1.578	48
Pairs	Small juvenile	41.821 ± 12.362	22.286 ± 2.891	28
	Large juvenile	14.539 ± 4.129	16.846 ± 1.548	39
Triplets	Small juvenile	37.867 ± 14.432	20.467 ± 2.722	15
	Intermediate juvenile	32.790 ± 15.27	20.632 ± 3.76	19
	Large juvenile	20.125 ± 10.381	19.313 ± 2.619	48
Groups		26	18	1

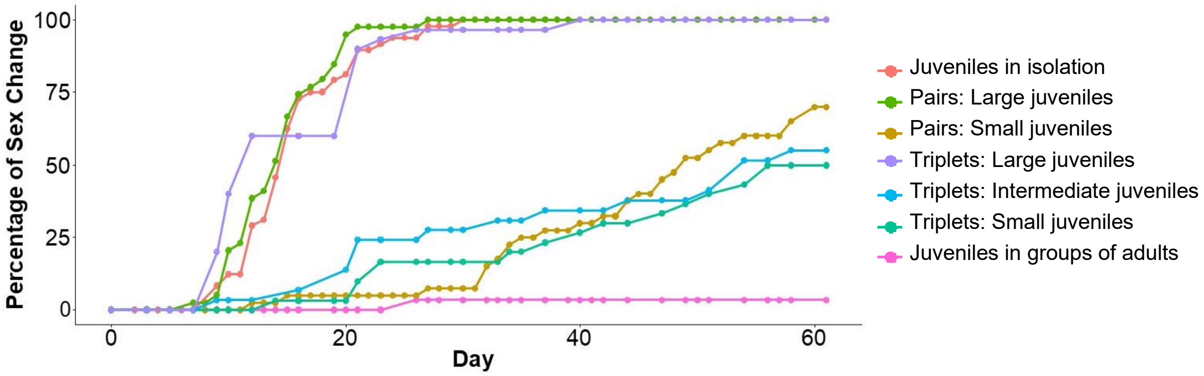

Figure 2. Rate of male to female sex change in each social environment for all juvenile size categories shown as cumulative percentages

Among early sex changers (i.e., the largest worms in pairs and triplets and worms in Isolation), there was a significant difference in the timing of sex change (GLMM: $\chi^2 = 14.54$, df = 2, P = 0.001). Conversely, the late sex changers (i.e., the small juveniles in pairs and triplets, and intermediate juveniles in triplets and the juveniles in groups of adults), changed sex at only marginally significant different times (GLMM: $\chi^2 = 5.615$ df = 2, P = 0.060).

6.3.3. *Does sex change become inevitable after reaching a threshold body size?*

The timing of sex change was associated with body size in significantly different ways depending on the juvenile size category across social environments, suggesting that sex change was not inevitable after reaching a certain body size (GLMM, interaction size*juvenile size-category: $\chi^2 = 32.535$, df = 5, $P < 0.0001$; juvenile category: $\chi^2 = 111.462$, df = 6, $P < 0.0001$; body size: $\chi^2 = 120.042$, df = 1, $P < 0.0001$) (Fig. 3).

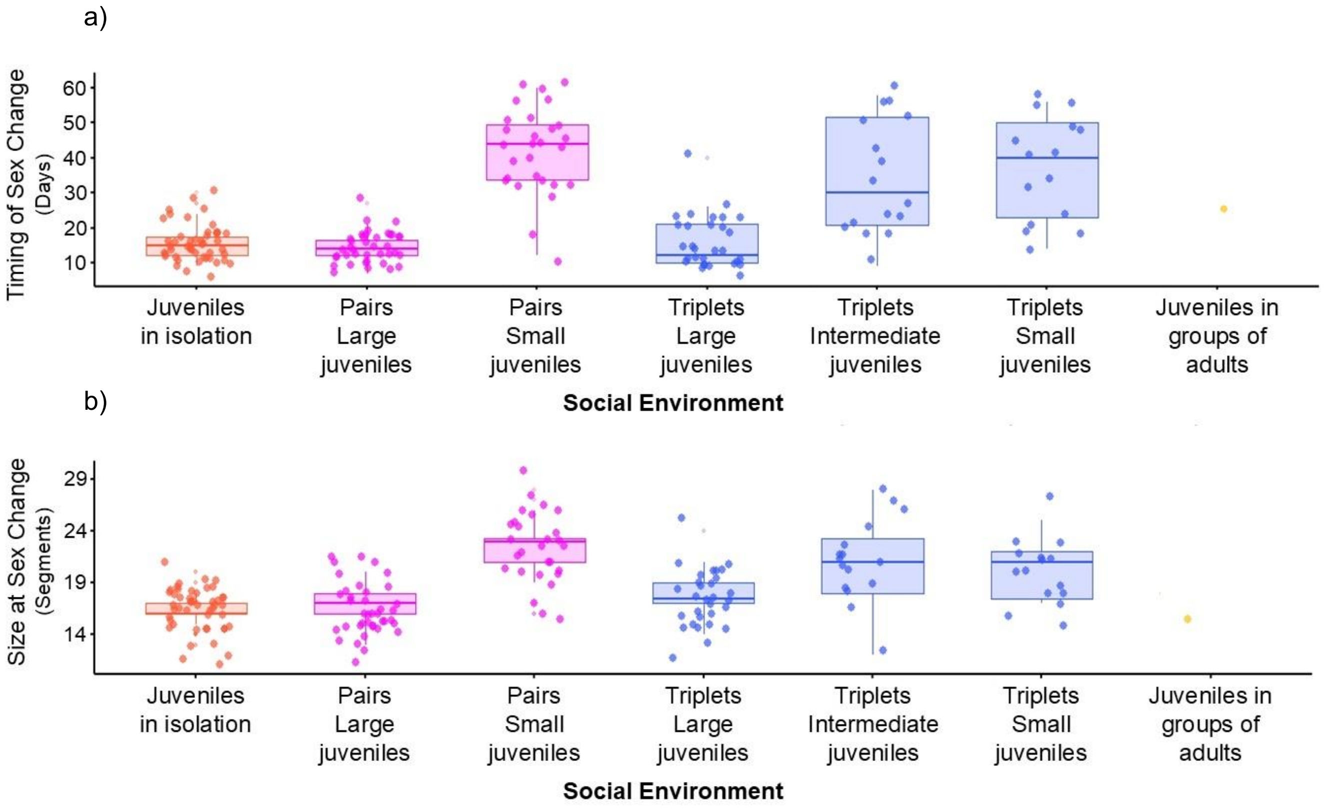

Figure 3. Timing and body size at sex change to female across social environments and juvenile body size categories. Timing was measured as number of days to sex change from the beginning of the experiment, body size as number of segments. In the box plots, the horizontal line represents the median, the box edges represent the 25th and 75th percentiles (IQR) and the whiskers extend to 1.5 × IQR. Raw data are jittered both vertically and horizontally to avoid overlap.

6.3.4. *Do worms vary the timing of sex change depending on the social environment?*

The timing of sex change varied significantly between the juveniles belonging to the different size-categories across social environments (GLMM: $\chi^2 = 220.43$, df = 6, $P < 0.0001$). Similarly, the body size at sex change also varied significantly among them (GLMM: $\chi^2 = 48.831$, df = 6, $P < 0.0001$). Therefore, we examined the variation in the timing and body at sex change within treatments.

6.3.4.1. *Pairs*

Despite sharing identical environments, the larger juvenile worms within each replicate consistently changed sex significantly earlier (almost 3 weeks before) than their partners (Table 1, Fig. 3a), but at a significantly smaller body size (approx. 75% of the partner's eventual size at sex change) (Table 1, Fig. 3b).

6.3.4.2. *Triplets*

Similarly, the largest juveniles in triplets changed sex significantly earlier (2-2.5 weeks before) compared to their partners (GLMM: $\chi^2 = 30.336$, df = 2, $P < 0.0001$) (Table 2, Fig. 3a). However, despite the significant differences in the timing of sex change among the three worms in triplets, their body sizes at sex change were not significantly different (GLMM: $\chi^2 = 1.5649$, df = 2, $P = 0.457$) (Table 2, Fig. 3b).

Table 2. Outcomes of GLMM testing for the variation in the timing and body size at sex change within Pairs and Triplets.

Social Environment	**Term of Interest**	**Source of Variation**	**Reference Level**	**Level**	**Estimate ± SE**	χ^2	**P**
Pairs	Timing of Sex Change	Intercept			3.733 ± 0.029		**<0.0001**
		Juvenile size-category	Small juvenile	Large juvenile	-1.062 ± 0.053	192.44	**<0.0001**
	Body Size at Sex Change	Intercept			3.104 ± 0.040		**<0.0001**
		Juvenile size-category	Small juvenile	Large juvenile	-0.280 ± 0.056	25.059	**<0.0001**
	Number of observations: 67 (in n = 39 Pairs)						
Triplets	Timing of Sex Change	Intercept			3.565 ± 0.1164		**<0.0001**
		Juvenile size-category	Small juvenile	Intermediate juvenile	-0.164 ± 0157		0.294
				Large juvenile	-0.654 ± 0.135		**<0.0001**
	Body Size at Sex Change	Intercept			3.019 ± 0.057		**<0.0001**
		Juvenile size-category	Small juvenile	Intermediate juvenile	0.008 ± 0.076		0.916
				Large juvenile	-0.058 ± 0.066		0.378
	Number of observations: 82 (in n = 30 Triplets)						

6.4. Discussion

We found that juvenile *Ophryotrocha puerilis* worms allocate reproductive resources strategically by adopting distinct developmental pathways in response to conspecific cues. Despite being age- and size-matched, worms in pairs and triplets adopted complementary sexes and directly formed mating pairs, supporting the *matchmaking hypothesis*. One individual consistently changed to female early and at a smaller body size (as small as 12 segments) and remained female, while its partner(s) prolonged the male phase, with many deferring sex change beyond the 60-day experimental period. Early sex changers were consistently larger than their partners at sex change. Isolated juveniles similarly changed sex early and remained female, whereas nearly all juveniles exposed to groups of adults (19 out of 20) deferred sex change despite growing to sizes of up to 25 segments. Taken together, these results demonstrate that: i) neither absolute age nor body size predicts sex change in *O. puerilis*, ii) the timing and body size at sex change to female are highly variable, with substantial overlap in male and female sizes, and iii) juveniles consistently develop into complementary sexes in the absence of adults.

Direct mating pair formation among size-matched juveniles in the absence of adults aligns with early observations by Müller (1962) in *O. puerilis*, and has been similarly observed in the clown anemonefish *Amphiprion ocellaris* Cuvier, 1830 (Iwata et al., 2020). In the absence of adults, there are no immediate mating opportunities, making early sex change to female functionally equivalent to shortening the non-reproductive phase, provided another juvenile is present. The emergence of complementary sexes among pairs of worms was first observed in adults of *O. puerilis* by Hartmann & Huth (1936), who reported that when two females were paired, one reverted to male. This "pair culture effect" is thought to be mediated by a contact pheromone inhibiting ootropic hormone production (Müller, 1962; Pfannenstiel, 1975, 1977, 1978). Since this pheromone is female-specific, it is unlikely to influence the timing of sex change among juveniles in same-age groups, though it remains a plausible explanation for the prolongation of the male phase among juveniles exposed to adults. For instance, in the simultaneously hermaphroditic congener *O. diadema* Åkesson, 1976, sex allocation is influenced by conspecific waterborne chemical cues (Schleicherovà et al., 2006; Santi et al., 2018).

Our findings among isolated worms align with Ghiselin's (1969) predictions that juveniles of protandrous invertebrates arriving at a new patch should develop directly as females and later arrivals as males as: in male-skewed populations (Premoli & Sella, 1995), females have greater mate-finding advantages. Early sex change in the absence of adults or in low density conditions is also observed in the slipper snail *C. fornicata* Linnaeus 1758, sex change occurs at smaller sizes in newly established colonies (Hoagland, 1978; Richter & Luque, 2004). Moreover, the capacity for bidirectional sex change in *O. puerilis* (Paxton & Åkesson, 2007), and the relatively short duration of reversal (~5 days; Berglund, 1986, 1990) suggests that sex change is flexible rather than fixed, and is achieved quickly, therefore enabling individuals to revert to the male phase if social conditions change.

The prolongation of the male phase among the 'small' partners in pairs and triplets, as well as among juveniles exposed to adults, can be explained by the exponential size-fecundity relationship in females: small females may lay about 30 eggs in their first clutch, while larger females can lay up to 350 eggs per clutch (Prevedelli et al., 2006). Delaying sex change in the presence of larger individuals would therefore be adaptive, enhancing the overall reproductive success of the pair (size-advantage hypothesis; Ghiselin, 1969). Deferment of sex change among juveniles exposed to adults is also consistent with the inhibition hypothesis (Ross, 1990), which proposes that the presence of larger or dominant individuals suppresses sex change in smaller or subordinate conspecifics that has been widely documented across both vertebrates (see reviews by Munday et al., 2006; Godwin, 2009; Lamm et al., 2015) and invertebrates (Table 3).

Indeed, social mediation of sex change is likely highly adaptive for an opportunistic colonizer such as *O. puerilis*. Colonization studies using experimentally placed bones at various depths have documented successful recruitment, indicating that *O. puerilis* can disperse despite lacking a planktonic larval stage (Taboada et al., 2016, 2017). Additionally, haplotype and demographic data indicate that once established, populations remain relatively stable over time, maintained through a dynamic combination of persistent individuals and transient newcomers (Taboada et al., 2016, 2017). Population densities in the wild fluctuate widely, ranging from isolated individuals to dense, male-skewed aggregations (Premoli & Sella, 1995; Sella & Ramella, 1999; Prevedelli et al., 2005; Taboada et al., 2016, 2017). Together, these ecological conditions should promote a high degree of phenotypic plasticity, allowing both adults and juveniles to maximize fitness in variable social environments by dynamically modulating their sex allocation (or age of sexual maturation) to current social context.

Similar ecological and social dynamics appear to underlie socially mediated sex change in a variety of marine invertebrates. For example, in protandrous limpets such as *Patella ferruginea* Gmelin, 1791, female scarcity triggers early sex change (Rivera-Ingraham et al., 2011; Guallart et al., 2013),

Table 3: Social Cues Mediating Sex Change in Invertebrates[1]

Species	**Direction of Sex Change**	**Social Cue Mediating Sex Change**	**References**
Slipper limpets			
Crepidula convexa	Protandrous	Presence of, or association with, larger males or females triggers sex change	Ambrogio & Pechenik, 2008; Le Cam et al., 2014
Crepidula cf. *onyx*	Protandrous	Presence of female delays sex change	Collin et al., 2005
Crepidula cf. *marginalis*	Protandrous	Presence of smaller individuals triggers earlier sex change	Collin, 2013; Carrillo-Baltodano & Collin (2015)
Crepidula coquimbensis	Protandrous	Proportion of males in the aggregation	Brante et al., 2012
Crepidula incurva	Protandrous	Presence of female delays sex change	Collin et al., 2005
Crepidula fornicata	Protandrous	Presence of female inhibits or delays sex change; population sex ratio; density of females; in newly established colonies, sex change occurs at smaller sizes	Hoagland, 1978; Richter & Luque, 2004
Crepidula lingulata	Protandrous	Presence of females delays or inhibits sex change; isolation triggers sex change	Collin, 2000
Crepidula neritoidea	Protandrous	Presence of female delays or inhibits sex change	Soong & Chen, 1991; Chen & Soong, 2002; Richter & Luque, 2004
Crepidula norrisiarum	Protandrous	Presence of female delays sex change; in the absence of females, the largest male changes sex	Warner et al., 1996; Richter & Luque, 2004

Continued on next page

---

[1] **Footnote:** This table is limited to instances of and factors influencing social mediation of sex change in invertebrates, as this phenomenon has already been extensively documented in vertebrates through several review papers (see Munday et al. 2006; Godwin, 2009; Lamm et al., 2015).

Table 3. *Social Cues Mediating Sex Change in Invertebrates (Continued)*

Species	Direction of SC	Social Cue Mediating SC	References
Coral-lovers			
Coralliophila abbreviata	Protandrous	Presence of females may inhibit or delay sex change; skewed male-biased sex ratio triggers earlier sex change	Johnston & Miller, 2007
Coralliophila meyendorffii	Protandrous	Presence of female inhibits or delays sex change	Richter & Luque, 2004; Collin, 2013
Coralliophila neritoidea	Protandrous	Presence of female inhibits or delays sex change	Chen et al., 1998; Richter & Luque, 2004
Coralliophila violacea	Protandrous	Presence of female inhibits or delays sex change	Soong & Chen, 1991; Chen et al., 1998; Collin, 2013
Limpets			
Patella aspera	Protandrous	Earlier sex change due to lower density of larger individuals caused by exploitation targeting females	Martins et al., 2017
Patella ferruginea	Protandrous bidirectional	Low density of larger individuals triggers sex change	Rivera-Ingraham et al. 2011; Guallart et al., 2013.
Patella vulgata	Protandrous bidirectional	Earlier sex change due to lower density of larger individuals caused by exploitation targeting larger individuals	Le Quesne & Hawkins, 2006; Borges et al., 2016
Owl limpet Lottia gigantea	Protandrous	Low density triggers sex change; presence of many larger conspecifics delays sex change due to territoriality	Wright, 1989; Fenberg and Roy 2012; Collin 2013

Continued on next page

Table 3. *Social Cues Mediating Sex Change in Invertebrates (Continued)*

Species	Direction of SC	Social Cue Mediating Sex Change	References
Oysters			
Crassostrea gigas	Protandry with Bi-directional	Isolation triggers sex change; gregariousness delays sex change	Yasuoka & Yusa, 2016
Crustaceans			
Athanas kominatoensis	Protandrous	Relative size, males delay sex change due to male-male competition	Nakashima, 1987
Pandalus borealis	Protandrous	Low density of females promotes earlier sex change	Koeller et al., 2000
Pandalus latirostis	Protandrous	Males delay sex change due to male-male competition	Chiba et al., 2003, Chiba, 2007
Pandalus jordani	Protandrous	Earlier sex change due to lower density of larger individuals caused by exploitation targeting larger individuals	Fenberg & Roy, 2008
Heterotanais oerstedii	Protogynous	Females delay sex change in the presence of males	Kakui & Hiruta, 2022
Chondrochelia dubia	Protogynous	Females delay sex change in the presence of male	Kakui & Hiruta, 2022

while fishing pressure causes earlier sex change in *P. aspera* Röding, 1798 and *P. vulgata* Linnaeus, 1758 (Le Quesne & Hawkins, 2006; Borges et al., 2016; Martins et al., 2017). Similarly, males of the protandrous sea snails *Coralliophila neritoidea* Lamarck, 1816 (Chen et al., 1998; Richter & Luque, 2004), *C. violacea* Kiener, 1836(Soong & Chen, 1991), *C. lingulata* Gould, 1846 (Collin, 2000), and *C. fornicata* (Hoch & Cahill, 2012) delay sex change when females are present. In *Pandalus jordani* Rathbun, 1902, selective harvesting of large individuals prompts earlier sex change (Fenberg & Roy, 2008), while in *P. borealis* Krøyer, 1838, sex change occurs at smaller sizes under high female density due to reduced growth (Koeller et al., 2000). In the protandrous oyster *Crassostrea gigas* Thunberg, 1793, isolation triggers early sex change, while group living delays it (Yasuoka & Yusa, 2016). Similarly, in protogynous species like the shrimps *Heterotanais oerstedii* Krøyer, 1842, *Chondrochelia dubia* Krøyer, 1842, and *Nesotanais* sp. aff. *ryukyuensis* Kakui et al., 2010, females delay sex change in the presence of males, who defend them in tubular shelters (Kakui & Hiruta, 2022).

An important feature of sequential hermaphroditism is that the second sex typically experiences greater reproductive success (Warner et al., 1975; Benvenuto et al., 2017; Roberts et al., 2021). Additionally, small males in protandrous species may incur high energetic costs in sperm production to fertilize the eggs of highly fecund females (Pla et al., 2020). This disparity in reproductive success and energetic costs between the sexes may drive competition for the second sex, leading to conflict over sexual roles. In *O. puerilis*, Berglund (1990) observed that individuals fought over sexual roles, often resulting in severe injuries and even death. These conflicts were not sex-specific; males injured each other in the presence of a female, presumably for access to the female (Berglund, 1990), and the injured male was often found isolated from the pair (Berglund, 1986). Similarly, females injured each other in the presence of a male, presumably over the opportunity to assume the female role, resulting in reproductive suppression in the losing female (Berglund, 1990). However, in the current study, we did not observe any overt aggression or injuries among juveniles in pairs or triplets, suggesting that physical aggression is unlikely to be the primary mechanism in those contexts.

Among juveniles exposed to adults, however, we observed a higher incidence of interindividual conflict (30% mortality), consistent with earlier studies (Kegel & Pfannenstiel, 1983; Berglund, 1986). Juveniles in groups of large adults were often observed crawling upside down along the underside of the water surface, an avoidance behaviour presumably to evade aggression from adults, and which was not observed in any other social environment. A strikingly similar avoidance behaviour is observed in the cooperatively breeding cichlid *Neolamprologus pulcher* Trewavas and Poll, 1952, where evicted subordinates hover near the surface to avoid aggression from dominant breeders occupying shelters below (Zöttl et al., 2013). Size-based aggression and territoriality have also been reported in the gonochoristic polychaete *Ophthalmonoe pettiboneae* Petersen & Britayev, 1997, where individuals fight to monopolize hosts and may evict or kill smaller conspecifics, who often avoid conflict altogether (Britayev & Martin, 2021). In *O. puerilis*, the observed avoidance behaviours and mucous trail construction (Sella & Ramella, 1999) may serve similar functions in reducing conflict and modulating social interactions.

Indeed, social status, dominance interactions, and territoriality are known to influence phenotypic sex in several sequential hermaphrodites. In the bidirectional and haremic bluebanded goby *Lythrypnus dalli* Gilbert, 1890, size-matched juveniles resolve conflict over sex roles by first establishing a dominance hierarchy and the dominant individual becomes male, while subordinates change sex to female (Solomon-Lane et al., 2016). Similarly, in the territorial limpet *Lottia gigantea* G.B. Sowerby I, 1834, sex change is regulated by social status, population density, aggression, and sex-ratio pheromones (Wright, 1989). Recent work shows that *O. puerilis* also forms size-based hierarchies within social groups (Radhakrishnan et al., in press), suggesting that individuals may use relative size as a cue to make independent decisions about sex allocation based on their condition relative to others (Benvenuto & Lorenzi, 2023). For instance, in the protogynous Midas cichlid *Cichlasoma citrinellum* Günther, 1864, relative size among juveniles can predict their phenotypic sex even before sexual maturity (Francis & Barlow, 1993). Similarly, in the protogynous goby *Trimma caudomaculatum* Yoshino & Araga, 1975, a mere difference of 2.5 mm (~6%) can determine sex roles between same-sex individuals (Tomatsu et al., 2018). In our study, an initial difference of just two body segments may have been sufficient to trigger sex-specific developmental trajectories.

In the protandrous barramundi *Lates calcarifer* Bloch 1970, juveniles that grow faster tend to change sex earlier, although not at a smaller body size (Roberts et al., 2021). In contrast, the early sex changers in our study changed sex at significantly smaller body sizes (~5 segments difference). One possible explanation is that once sex roles are established, individuals poised to become female may accelerate growth to reach the minimum threshold required for sex change (sex change-induced growth spurt; McCormick et al., 2010). Although female fecundity is highly size-dependent, becoming a small female is still advantageous if it shortens the non-reproductive male phase (Ross, 1987).

The timing and body size at sex change in *O. puerilis* is therefore likely mediated by multiple interacting social factors, including size asymmetries, the threat of aggression, and composition of social group. Our findings contribute a novel perspective to the adult-centred paradigm by demonstrating that juveniles adjust developmental trajectories and sex allocation in response to conspecific cues, suggesting that sensitivity to social cues may begin earlier in development than previously recognized. Future research should explore the precise mechanisms underlying these processes to provide a more nuanced understanding of sex allocation strategies in *O. puerilis*. For example, paternity analyses could help determine whether the female and largest male in the environment exert reproductive monopoly, strengthening the idea that juveniles actively compete for the female role to maximize fitness. Additionally, developing individual marking techniques would enhance investigations by enabling longitudinal studies of social behaviour, growth, conflict, and sex allocation within larger social groups.

6.5. Conclusion

Our study reveals a high degree of reaction norm plasticity in the timing of sex change in *Ophryotrocha puerilis*, driven by subtle conspecific cues in the social environment during early-life. In the wild, population densities fluctuate from isolated individuals to dense aggregations (Premoli & Sella, 1995; Sella & Ramella, 1999; Prevedelli et al., 2005). Such unpredictability in mating opportunities and competition explains the evolution of dynamic adjustment of phenotypic sex in *O. puerilis*. Additionally, our findings reveal that juveniles modulate their developmental trajectories and resulting sex allocation in a condition-dependent manner, challenging the traditional view of juveniles as passive responders to social cues (see Benvenuto & Lorenzi, 2023).

Supplemental Materials

Table 1S: *Post-hoc comparisons among all experimental juveniles across social environments*

Comparison	Reference	Source of Variation	Estimate ± SE	χ^2	P
Isolated vs. L1 Pairs	Isolated	L1 Pair	0.981 ± 0.047	454.92	**<0.0001**
Isolated vs. L2 Pairs	Isolated	L2 Pair	-0.076 ± 0.056	1.878	0.171
Isolated vs. L1 Triplets	Isolated	L1 Triplets	0.881 ± 0.056	234.49	**<0.0001**
Isolated vs. L2 Triplets	Isolated	L2 Triplets	0.737 ± 0.054	177.27	**<0.0001**
Isolated vs. L3 Triplets	Isolated	L3 Triplets	0.249 ± 0.049	26.461	**<0.0001**
L1 Pairs vs. L1 Triplets	L1 Pair	L1 Triplets	-0.099 ± 0.051	3.8166	0.052
L1 Pairs vs. L2 Triplets	L1 Pair	L2 Triplets	-0.243 ± 0.050	24.623	**<0.0001**
L1 Pairs vs. L3 Triplets	L1 Pair	L3 Triplets	-0.732 ± 0.044	283.56	**<0.0001**
L2 Pairs vs. L1 Triplets	L1 Triplets	L2 Pair	-0.957 ± 0.059	250.73	**<0.0001**
L2 Pairs vs. L2 Triplets	L2 Pair	L2 Triplets	0.813 ± 0.058	193.55	**<0.0001**
L2 Pairs vs. L3 Triplets	L2 Pair	L3 Triplets	0.325 ± 0.053	38.701	**<0.0001**

References

Åkesson, B. (1967). On the biology and larval morphology of *Ophryotrocha puerilis* Claparède & Metschnikov (Polychaeta). *Ophelia*, *4*(1), 111-119.

Allsop, D. J., & West, S. A. (2003). Constant relative age and size at sex change for sequentially hermaphroditic fish. *Journal of Evolutionary Biology*, *16*(5), 921-929.

Ambrogio, O. V., & Pechenik, J. A. (2008). When is a male not a male? Sex recognition and choice in two sex-changing species. *Behavioral Ecology and Sociobiology*, *62*, 1779-1786.

Avise, J. C., & Mank, J. E. (2009). Evolutionary perspectives on hermaphroditism in fishes. *Sexual Development*, *3*(2-3), 152-163.

Bacci, G., & La Greca, M. (1953). Genetic and morphological evidence for subspecific differences between Naples and Plymouth populations of *Ophryotrocha puerilis*. *Nature*, *171*(4364), 1115-1115.

Bacci, G., & Bortesi, O. (1961a). Pure males and females from hermaphroditic strains of *Ophryotrocha puerilis*. *Experientia*, *17*(5), 229-230.

Bacci, G., & Bortesi, O. (1961b). Existence of multiple sex genotypes in the hermaphrodite polychaete worm *Ophryotrocha puerilis siberti*. *Nature*, *190*(4778), 838-838.

Bacci, G. (1965). Sex determination and genic balance of *Ophryotrocha puerilis*, a hermaphrodite polychaete worm. *Nature*, *207*(5002), 1208-1209.

Bacci, G., & Bortesi, O. (1967). The reestablishment of sex balance in *Ophryotrocha puerilis* through interactions between individuals from arrhenogenous and thelygenous lines. *Experientia*, *23*(6), 448-449.

Bacci, G., & Voria, P. (1970). The influence of temperature upon the change from the male to the female phase in *Ophryotrocha puerilis puerilis*, a polychaete worm. *Experientia*, *26*, 1273-1274.

Bates, D., Mächler, M., Bolker, B. & Walker, S. (2015) Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*, 67, 1–48.

Benvenuto, C., Coscia, I., Chopelet, J., Sala-Bozano, M., & Mariani, S. (2017). Ecological and evolutionary consequences of alternative sex-change pathways in fish. *Scientific Reports*, *7*(1), 9084.

Benvenuto C, Lorenzi MC. (2023). Social regulation of reproduction: control or signal? *Trends Ecol. Evol.* 38, 1028–1040.

Berglund, A. (1986). Sex change by a polychaete: effects of social and reproductive costs. *Ecology*, *67*(4), 837-845.

Berglund, A. (1990). Sequential hermaphroditism and the size-advantage hypothesis: an experimental test. *Animal Behaviour*, *39*(3), 426-433.

Berruti, G. (1980). The effect of starvation on the appearance of the definitive upper jaw in *Ophryotrocha puerilis* (Annelida, Polychaeta). *Italian Journal of Zoology*, *47*(1-2), 71-74.

Beukeboom LW, Perrin N. (2014). The diversity of sexual cycles. In *The evolution of sex determination*, pp. 18–36. Oxford, UK: Oxford University Press.

Borges, C. D., Hawkins, S. J., Crowe, T. P., & Doncaster, C. P. (2016). The influence of simulated exploitation on *Patella vulgata* populations: protandric sex change is size-dependent. *Ecology and Evolution*, *6*(2), 514-531.

Brante, A., Vilches, C., & Quiñones, A. (2012). Role of population traits and local social interactions in sex-change plasticity of the protandric marine gastropod *Crepidula coquimbensis*. *Marine Ecology Progress Series*, *458*, 123-132.

Britayev, T. A., & Martin, D. (2021). Behavioral traits and territoriality in the symbiotic scaleworm *Ophthalmonoe pettiboneae*. *Scientific Reports*, *11*(1), 12408.

Carrillo-Baltodano, A., & Collin, R. (2015). *Crepidula* slipper limpets alter sex change in response to physical contact with conspecifics. *The Biological Bulletin*, *229*(3), 232-242.

Casas, L., & Saborido-Rey, F. (2021). Environmental cues and mechanisms underpinning sex change in fish. *Sexual Development*, *15*(1-3), 108-121.

Charnov, E. L., & Bull, J. (1977). When is sex environmentally determined?. *Nature*, *266*(5605), 828-830.

Charnov, E. L. (1982). *The Theory of Sex Allocation* (Vol. 18). Princeton University Press.

Chen, M. H., Yang, Y. W., & Soong, K. (1998). Preliminary observations on change of sex by the coral-inhabiting snails *Coralliophila violacea* (Lamarck) (Gastropoda: Coralliophilidae). *Journal of Experimental Marine Biology and Ecology*, *230*(2), 207-212.

Chen, M. H., & Soong, K. (2002). Estimation of age in the sex-changing, coral-inhabiting snail *Coralliophila violacea* from the growth striae on opercula and a mark–recapture experiment. *Marine Biology*, *140*, 337-342.

Chiba, S., Goshima, S., & Shinomiya, Y. (2003). Male-male competition selects for delayed sex change in the protandrous shrimp *Pandalus latirostris*. *Marine Biology*, *142*, 1153-1157.

Chiba, S. (2007). A review of ecological and evolutionary studies on hermaphroditic decapod crustaceans. *Plankton and Benthos Research*, *2*(3), 107-119.

Cole, K. S., & Shapiro, D. Y. (1995). Social faciliation and sensory mediation of adult sex change in a cryptic, benthic marine goby. *Journal of Experimental Marine Biology and Ecology*, *186*(1), 65-75.

Collin, R. (2000). Sex change, reproduction and development of *Crepidula adunca* and *C. lingulata* (Gastropoda: Calyptraeidae) *Veliger* 43: 24–33.

Collin, R., McLellan, M., Gruber, K., & Bailey-Jourdain, C. (2005). Effects of conspecific associations on size at sex change in three species of calyptraeid gastropods. *Marine Ecology Progress Series*, *293*, 89-97.

Collin, R. (2013). Phylogenetic patterns and phenotypic plasticity of molluscan sexual systems. *Integrative and Comparative Biology*, *53*(4), 723-735.

Espinosa, F., Guerra-Garcia, J. M., Fa, D., & Garcia-Gomez, J. C. (2006). Aspects of reproduction and their implications for the conservation of the endangered limpet, *Patella ferruginea*. *Invertebrate Reproduction & Development*, *49*(1-2), 85-92.

Fenberg, P. B., & Roy, K. (2008). Ecological and evolutionary consequences of size-selective harvesting: how much do we know? *Molecular Ecology*, *17*(1), 209-220.

Fenberg, P. B., & Roy, K. (2012). Anthropogenic harvesting pressure and changes in life history: insights from a rocky intertidal limpet. *The American Naturalist*, *180*(2), 200-210.

Francis, R. C., & Barlow, G. W. (1993). Social control of primary sex differentiation in the Midas cichlid. *Proceedings of the National Academy of Sciences*, *90*(22), 10673-10675.

Ghiselin, M. T. (1969). The evolution of hermaphroditism among animals. *The Quarterly review of biology*, *44*(2), 189-208.

Godwin, J. (2009, May). Social determination of sex in reef fishes. In *Seminars in Cell & Developmental Biology* (Vol. 20, No. 3, pp. 264-270). Academic Press.

Guallart, J., Calvo, M., Acevedo, I., & Templado, J. (2013). Two-way sex change in the endangered limpet *Patella ferruginea* (Mollusca, Gastropoda). *Invertebrate Reproduction & Development*, *57*(3), 247-253.

Hartmann, M., & Huth, W. (1936). Untersuchungen über die Geschlechtsbestimmung und Geschlechtsumwandlung von *Ophryotrocha puerilis*. *Zoologische Jahrbücher (Physik)*, 56, 389-439.

Hartmann, M., & von Lewinski, G. (1938). Untersuchungen über die Geschlechtsbestimmung und Geschlechtsumwandlung von *Ophryotrocha puerilis*. II. Versuche über die Wirkung von Kalium, Magnesium und Kupfer. *Zoologische Jahrbücher (Physiologie)*, 58, 551-574.

Hoagland KE. 1978. Protandry and the evolution of environmentally mediated sex change: a study of the Mollusca. *Malacologia* 17: 365–391.

Iwata, E., Yoshida, T., Abe, T., Takahata, K., & Masuda, K. (2020). Behavioural differences between breeding and nonbreeding pairs of protandry monogamous false clown anemonefish *Amphiprion ocellaris*. *Journal of Fisheries*, 8, 808– 816.

Johnston, L., & Miller, M. W. (2007). Variation in life-history traits of the corallivorous gastropod *Coralliophila abbreviata* on three coral hosts. *Marine Biology*, *150*(6), 1215-1225.

Kakui, K., & Hiruta, C. (2022). Protogynous hermaphroditism in Crustacea: a new example from Tanaidacea. *Canadian Journal of Zoology*, *100*(8), 481-487.

Kegel, B., & Pfannenstiel, H. D. (1983). Evaluation of the pair-culture effect in *Ophryotrocha puerilis* (Polychaeta: Dorvilleidae). I. Pair-culture effect and sex ratio. *Helgoländer Meeresuntersuchungen*, *36*, 205-213.

Kier, W. M. (2012). The diversity of hydrostatic skeletons. *Journal of Experimental Biology*, *215*(8), 1247-1257.

Koeller, P., Mohn, R., & Etter, M. (2000). Density dependant sex change in northern shrimp, *Pandalus borealis*, on the Scotian Shelf. *Journal of Northwest Atlantic Fishery Science*, *27*.

Kuwamura, T., Sunobe, T., Sakai, Y., Kadota, T., & Sawada, K. (2020). Hermaphroditism in fishes: an annotated list of species, phylogeny, and mating system. *Ichthyological Research*, *67*, 341-360.

Lamm, M. S., Liu, H., Gemmell, N. J., & Godwin, J. R. (2015). The need for speed: neuroendocrine regulation of socially-controlled sex change. *Integrative and Comparative Biology*, *55*(2), 307-322.

Le Cam, S., Riquet, F., Pechenik, J. A., & Viard, F. (2014). Paternity and gregariousness in the sex-changing sessile marine gastropod *Crepidula convexa*: comparison with other protandrous *Crepidula* species. *Journal of Heredity*, *105*(3), 397-406.

Le Quesne, W. J. F., & Hawkins, S. J. (2006). Direct observations of protandrous sex change in the patellid limpet *Patella vulgata*. *Journal of the Marine Biological Association of the United Kingdom*, *86*(1), 161-162.

Leonard, J. L. (2018). The evolution of sexual systems in animals. *Transitions between sexual systems: understanding the mechanisms of, and pathways between, dioecy, hermaphroditism and other sexual systems*, 1-58.

Marchionni, V., & Rolando, A. (1979). The effect of bonellin on the timing of sex inversion and on fertility in *Ophryotrocha puerilis*. *Atti della Società Toscana di Scienze Naturali, Memorie*, *Serie B, 86*, 355–362.

Marchionni, V., & Rolando, A. (1981). Sex reversal in *Ophryotrocha puerilis puerilis* (Polychaeta, Dorvilleidae) induced by ethereal extracts of female phase individuals. *Italian Journal of Zoology*, *48*(2), 91-96.

Mariani, S., Sala-Bozano, M., Chopelet, J., & Benvenuto, C. (2013). Spatial and temporal patterns of size-at-sex-change in two exploited coastal fish. *Environmental biology of fishes*, *96*, 535-541.

Martins, G. M., Borges, C. D., Vale, M., Ribeiro, P. A., Ferraz, R. R., Martins, H. R., ... & Hawkins, S. J. (2017). Exploitation promotes earlier sex change in a protandrous patellid limpet, *Patella aspera* Röding, 1798. *Ecology and Evolution*, *7*(10), 3616-3622.

Müller, H. (1962). Über die Sexualität des Polychaeten *Ophryotrocha puerilis*, ihre Determination und ihren Einfluss auf Drüsentätigkeit und auf Apparatenentwicklung. *Zeitschrift für Morphologie und Ökologie der Tiere, 52*(1), 1-32.

Munday, P. L., Buston, P. M., & Warner, R. R. (2006). Diversity and flexibility of sex-change strategies in animals. *Trends in Ecology & Evolution*, *21*(2), 89-95.

Munday, P. L., Kuwamura, T., & Kroon, F. J. (2010). Bidirectional sex change in marine fishes. In: Cole KS (ed) Reproduction and sexuality in marine fishes: patterns and processes. University of California Press, Berkeley, California, pp 241–271.

Munoz, R. C., & Warner, R. R. (2003). A new version of the size-advantage hypothesis for sex change: incorporating sperm competition and size-fecundity skew. *The American Naturalist*, *161*(5), 749-761.

Munoz, R. C., & Warner, R. R. (2004). Testing a new version of the size-advantage hypothesis for sex change: sperm competition and size-skew effects in the bucktooth parrotfish, *Sparisoma radians*. *Behavioral Ecology*, *15*(1), 129-136.

Nakashima, Y. (1987). Reproductive strategies in a partially protandrous shrimp, *Athanas kominatoensis* (Decapoda: Alpheidae): sex change as the best of a bad situation for subordinates. *Journal of Ethology*, *5*(2), 145-159.

Paxton, H., & Åkesson, B. (2007). Redescription of *Ophryotrocha puerilis* and *O. labronica* (Annelida, Dorvilleidae). *Marine Biology Research*, 3(1), 3-19.

Pfannenstiel, H. D. (1975). Mutual influence on the sexual differentiation in the protandric polychaete *Ophryotrocha puerilis*. In *Intersexuality in the animal kingdom* (pp. 48-56). Berlin, Heidelberg: Springer Berlin Heidelberg.

Pfannenstiel, H. D. (1977). Experimental analysis of the 'Paarkultureffekt' in the protandric polychaete, *Ophryotrocha puerilis* Clap. Mecz. *Journal of Experimental Marine Biology and Ecology*, *28*(1), 31-40.

Pfannenstiel, H. D. (1978). Endocrinology of polychaete reproduction and sexual development. *Italian Journal of Zoology*, *45*(2), 171-188.

Pla, S., Benvenuto, C., Capellini, I., & Piferrer, F. (2020). A phylogenetic comparative analysis on the evolution of sequential hermaphroditism in seabreams (Teleostei: Sparidae). *Scientific Reports*, *10*(1), 3606.

Pla, S., Benvenuto, C., Capellini, I., & Piferrer, F. (2022). Switches, stability and reversals in the evolutionary history of sexual systems in fish. *Nature Communications*, *13*(1), 3029.

Premoli, M. C., & Sella, G. (1995). Sex economy in benthic polychaetes. *Ethology Ecology & Evolution*, *7*(1), 27-48.

Prevedelli, D., Massamba N'Siala, G., & Simonini, R. (2005). The seasonal dynamics of six species of Dorvilleidae (Polychaeta) in the harbour of La Spezia (Italy). *Marine Ecology*, *26*(3-4), 286-293.

Prevedelli, D., N'siala, G. M., & Simonini, R. (2006). Gonochorism vs. hermaphroditism: relationship between life history and fitness in three species of *Ophryotrocha* (Polychaeta: Dorvilleidae) with different forms of sexuality. *Journal of Animal Ecology*, *75*(1), 203-212.

Radhakrishnan, P., Saboul, N., Monclús, R., Taboada, S. & Lorenzi, M.C. (in press). Game over: Conflict resolution through strategic growth in an invertebrate. *Functional Ecology*.

Richter, A., & Luque, Á. A. (2004). Sex change in two mediterranean species of Coralliophilidae (Mollusca: Gastropoda: Neogastropoda). *Journal of the Marine Biological Association of the United Kingdom*, *84*(2), 383-392.

Rivera-Ingraham, G. A., Espinosa, F., & García-Gómez, J. C. (2011). Environmentally mediated sex change in the endangered limpet *Patella ferruginea* (Gastropoda: Patellidae). *Journal of Molluscan Studies*, *77*(3), 226-231.

Roberts, B. H., Morrongiello, J. R., Morgan, D. L., King, A. J., Saunders, T. M., & Crook, D. A. (2021). Faster juvenile growth promotes earlier sex change in a protandrous hermaphrodite (barramundi *Lates calcarifer*). *Scientific Reports*, *11*(1), 2276.

Ross, R. M., Losey, G. S., & Diamond, M. (1983). Sex change in a coral-reef fish: dependence of stimulation and inhibition on relative size. *Science*, *221*(4610), 574-575.

Ross, R. M. (1987). Sex-change linked growth acceleration in a coral-reef fish, *Thalassoma duperrey*. *Journal of Experimental Zoology*, *244*(3), 455-461.

Ross, R. M. (1990). The evolution of sex-change mechanisms in fishes. *Environmental Biology of Fishes*, *29*, 81-93.

Santi, M., Picchi, L., & Lorenzi, M. C. (2018). Dynamic modulation of reproductive strategies in a simultaneous hermaphrodite and preference for the male role. *Animal Behaviour*, *146*, 87-96.

Schleicherová D, Lorenzi MC, Sella G. 2006. How outcrossing hermaphrodites sense the presence of conspecifics and suppress female allocation. *Behavioural Ecology*, 17:1–5.

Sella, G., & Ramella, L. (1999). Sexual conflict and mating systems in the dorvilleid genus *Ophryotrocha* and the dinophilid genus *Dinophilus*. *Hydrobiologia*, *402*, 203-213.

Shapiro, D. Y., & Lubbock, R. (1980). Group sex ratio and sex reversal. *Journal of Theoretical Biology*, *83*(3), 411-426.

Solomon-Lane, T. K., Shvidkaya, P., Thomas, A., Williams, M. M., Rhyne, A., Rogers, L., & Grober, M. S. (2016). Juvenile social status predicts primary sex allocation in a sex changing fish. *Evolution & Development*, *18*(4), 245-253.

Soong, K., & Chen, J. L. (1991). Population structure and sex-change in the coral-inhabiting snail *Coralliophila violacea* at Hsiao-Liuchiu, Taiwan. *Marine Biology*, *111*, 81-86.

Taboada, S., Bas, M., Leiva, C., Garriga, M., Sardá, R., & Avila, C. (2016). Life after death: shallow-water Mediterranean invertebrate communities associated with mammal bones. *Marine Ecology*, *37*(1), 164-178.

Taboada, S., Leiva, C., Bas, M., Schult, N., & McHugh, D. (2017). Cryptic species and colonization processes in *Ophryotrocha* (Annelida, Dorvilleidae) inhabiting vertebrate remains in the shallow-water Mediterranean. *Zoologica Scripta*, *46*(5), 611-624.

Therneau, T. M., & Grambsch, P. M. (2000). *Modeling survival data: Extending the Cox model.* Springer.

Thornhill DJ, Dahlgren TG, Halanych KM. 2009. Evolution and ecology of *Ophryotrocha* (Dorvilleidae, Eunicida). In: Shain DH, editor. *Annelids in modern biology*. Hoboken (NJ): Wiley-Blackwell. pp. 242–256.

Tomatsu, S., Ogiso, K., Fukuda, K., Deki, M., Dewa, S. I., Manabe, H., Sakurai, M., Shinomiya, A., & Sunobe, T. (2018). Multi-male group and bidirectional sex change in the gobiid fish, *Trimma caudomaculatum*. *Ichthyological Research, 65*(4), 502-506.

Vega-Frutis, R., Macías-Ordóñez, R., Guevara, R., & Fromhage, L. (2014). Sex change in plants and animals: A unified perspective. *Journal of Evolutionary Biology, 27*(4), 667–675.

McCormick, M. I., Ryen, C. A., Munday, P. L., & Walker, S. P. (2010). Differing mechanisms underlie sexual size-dimorphism in two populations of a sex-changing fish. *PLoS One*, *5*(5), e10616.

Warner, R. R. (1975). The adaptive significance of sequential hermaphroditism in animals. *The American Naturalist*, *109*(965), 61-82.

Warner, R. R., Robertson, D. R., & Leigh Jr, E. G. (1975). Sex Change and Sexual Selection: The reproductive biology of a labrid fish is used to illuminate a theory of sex change. *Science*, *190*(4215), 633-638.

Warner, R. R. (1988). Sex change and the size-advantage model. *Trends in Ecology & Evolution*, *3*(6), 133-136.

Warner, R. R., & Swearer, S. E. (1991). Social control of sex change in the bluehead wrasse, *Thalassoma bifasciatum* (Pisces: Labridae). *The Biological Bulletin*, *181*(2), 199-204.

Warner, R. R., Fitch, D. L., & Standish, J. D. (1996). Social control of sex change in the shelf limpet, *Crepidula norrisiarum*: size-specific responses to local group composition. *Journal of Experimental Marine Biology and Ecology*, *204*(1-2), 155-167.

West, S. (2009). *Sex allocation*. Princeton University Press.

Wright, W. G. (1989). Intraspecific density mediates sex-change in the territorial patellacean limpet *Lottia gigantea*. *Marine Biology*, *100*, 353-364.

Yasuoka, N., & Yusa, Y. (2016). Effects of size and gregariousness on individual sex in a natural population of the Pacific oyster *Crassostrea gigas*. *Journal of Molluscan Studies*, *82*(4), 485-491.

Zöttl, M., Frommen, J. G., & Taborsky, M. (2013). Group size adjustment to ecological demand in a cooperative breeder. *Proceedings of the Royal Society B: Biological Sciences*, *280*(1756), 20122772.

CHAPTER 7
DISCUSSION

Previous research has established that sex allocation in *Ophryotrocha puerilis* is influenced by local social conditions, such as the presence of larger females (Berglund, 1986) and sex ratio imbalances in higher-density groups (Kegel & Pfannenstiel, 1983). However, these studies primarily focused on sex reversal in adult females, and little was known about whether this sensitivity to social stimuli extended to young sexually immature worms. Specifically, it was unclear how the presence, absence, or number of conspecifics during early life may alter the timing and body size at which the initial protandrous sex change occurs. We found that *O. puerilis* meets the conditions for social mediation of sex change as well as strategic growth modulation (Table 7.1).

7.1 **Social mediation of sex change in juvenile worms**

In Chapter 5, we found that young *Ophryotrocha puerilis* worms exhibit social mediation of sex change. Worms in isolation changed sex early, whereas those exposed to adult worms rarely changed sex. When juvenile worms were paired or grouped into triplets of size-matched individuals, they differentiated into opposite sexes. One worm in each replicate accelerated sex change, practically bypassing the functional male phase, while the other prolonged the male phase for much of the 60-day experiment. This pattern of direct mating-pair formation among juveniles aligns with Müller's (1962) observations in *O. puerilis*. Similarly, in the protandrous false clown anemonefish *Amphiprion ocellaris*, pairing sexually immature juveniles also resulted in direct mating-pair formation (Iwata et al., 2020). The adaptive advantage of early sex change in one individual within pairs and triplets of juveniles is that, in the absence of adults, there are no mating opportunities, making the bypassing of the male phase equivalent to shortening the non-reproductive phase. Furthermore, since *O. puerilis* are bidirectional sequential hermaphrodites, sex change is not final and individuals may revert to the male phase if social conditions change.

Table 7.1. Assessment of conditions for the evolution of socially mediated sex change and strategic growth in *Ophryotrocha puerilis*

	Ophryotrocha puerilis	**Notes**
Conditions for Socially Mediated Sex Change		
Opportunities for social interactions (Brante et al., 2013)	✓	Populations persist over generations (Taboada et al., 2016; 2017) In high population densities, worms form clusters, allowing for social interactions (Sella & Ramella, 1999)
Overlapping sizes in males and females (Collin, 2013)	✓	Highly variable size at sex change as observed in Chapter 5 and also reported by Bacci (1951)
Conditions for Strategic Growth (Buston & Clutton-Brock, 2022)		
Variability in social rank	✓	Given fluctuating densities, a lower rank individual may ascend to higher rank when colonizing a new substrate
Fitness depends on social rank	✓	Reproductive success is higher in the second sex in most sequential hermaphrodites (Roberts et al., 2021) Males may incur higher energetic costs for fertilizing highly fecund females
Ability to assess social status	✓	Individuals are able to assess relative size, which determines sex (Chapter 5 & Chapter 6)
Stable group structure	✓	As mentioned above, populations persist over generations (Taboada et al., 2016; 2017)
Low cost of growth adjustment	?	Similar asymptotic sizes between Rank-1 and Rank-2 in pairs suggests there is no severe cost of alternative developmental trajectories, at least in our experimental condition (Chapter 6)

The prolongation of the male phase past the average age or body size typical for sex change can also be explained by the size-advantage hypothesis. In pairs, for instance, at the time of the female's sex change, the male was, on average, about four segments smaller than the female. This size difference likely stabilized the pair, as the larger female can produce more eggs, thereby increasing the overall fitness of the pair (size-advantage hypothesis; Ghiselin 1969).

Worms in isolation changed sex early and remained female for the entirety of the experiment, investing early in the female function. This aligns with Ghiselin's (1969) suggestion that larvae of protandrous marine invertebrates colonizing a novel environment should grow rapidly and change sex to female early, as the male phase becomes redundant in the absence of larger conspecifics. *Ophryotrocha puerilis* are opportunistic colonizers (Taboada et al., 2016, 2017), and juveniles may frequently find themselves in novel environments due to their capacity for dispersal (Premoli & Sella, 1995).

7.1.1. **The role of male-male competition in sex change decisions**

Among late sex changers, individuals in triplets prolonged the male-phase longer than those in pairs, suggesting that the presence of even one additional conspecific can alter social dynamics and shift the optimal timing of sex change. This may be explained by male-male competition: in protandrous species, male size typically does not confer significant reproductive advantages, as females do not have a strong preference for larger males, and this has been validated in *O. puerilis* (Berglund, 1990, 1991). Consequently, protandry is often found in species that exhibit monogamy or random mating, where males cannot monopolize females. However, in some protandrous invertebrate species, males do compete for access to females, and larger males may dominate reproductive opportunities, creating a male size advantage, as observed in the protandrous pandalid shrimp *Pandalus latirostris* (Chiba, 2007) and snapping shrimp *Athanas kominatoensis* (Nakashima, 1987). A similar male size advantage has been observed in *O. puerilis* (Berglund, 1990), which could explain why males in triplets extended the male phase longer than those in pairs, where male-male competition was absent.

7.1.2. **Plastic responses to conflict over sex roles**

In *O. puerilis*, this male size advantage may be negated by female choice, as females tend to reject larger males, likely to avoid competition over the female role (Berglund, 1990). Berglund (1986, 1990) also showed that competition and conflict over sex roles is not sex-specific: both males and females engage in aggressive interactions with same-sex conspecifics. Extra-pair males sustain injuries while competing for access to females, and extra-pair females sustain injuries presumably due to competition for the female role, with such conflicts leading to one female suppressing reproduction altogether (Berglund, 1990). Therefore, the variation in the timing and body size at sex change in triplets may result from opposing forces: male-male competition, which favours larger males; female choice, which may favour smaller males; and competition over the female role, which may lead males to delay sex change in order to avoid aggressive interactions with females.

The influence of male-male conflict on the timing of sex change is evident in juveniles exposed to adults, where only one individual changed sex out of 20, in clear contrast to the other social environments. This suggests that while the number of conspecifics influences sex allocation (as seen in pairs vs. triplets), the presence of adults has a much stronger influence, as also observed in several other invertebrates, where males accelerate sex change when fewer larger individuals are present and delay sex change in the absence of females or larger conspecifics (Table 3, Chapter 6). In both the pairs and triplets, one worm changed sex early, meaning their male partners were exposed to a female for most of the experiment. However, this did not produce the same "suppression" effect seen in groups, where increased conflict among males may have inhibited sex change. For example, in the protandrous and territorial owl limpet *Lottia gigantea*, high-density groups lead to increased aggressive interactions among conspecifics, delaying sex change (Wright, 1989). In our experiment, while no direct injuries were observed, mortality was highest in these groups (30%), and juveniles (and later small males) were often isolated or physically distant from adults (P.R., personal observation), as previously also noted by Berglund (1986). This isolation may have led to starvation, as smaller males likely avoided conflict, and food tends to settle at the bottom of the bowl. Berglund (1986) reported that individuals kept in groups of three had a lower average feeding rate than isolated individuals, suggesting a potential cost to feeding efficiency associated with the presence of conspecifics. However, the potential stress from reduced feeding efficiency might be minimized in our pair and triplet treatments due to the *ad libitum* feeding regime used.

7.1.3. **Growth-stunting in males**

Influence of competition and energetic costs?

In *O. puerilis*, males grow faster than females due to the high energetic costs associated with egg production in females (Berglund, 1986). However, in our experiment, male partners remained smaller than the female for the duration of the experiment despite this growth-advantage. Additionally, Berglund (1986, 1990) tested the size-advantage hypothesis for *O. puerilis* and found that, as predicted, female fecundity increased with body size, and the size of the male partner did not affect this outcome. However, he also discovered a size advantage for males under conditions of male-male competition for access to females, similar to observations in the protandrous shrimp *Pandalus latirostris* (Chiba et al., 2003). Interestingly, in *O. puerilis*, while larger males won contests, they had lower mating success than smaller males, as females often rejected larger males, sometimes aggressively, possibly to avoid future conflict over sex roles, as large males are closer to changing sex and could become competitors (Berglund, 1990). This might explain why males did not grow rapidly despite having a growth-rate advantage over females (Berglund, 1986).

Another possible explanation for the growth stunting observed in male partners may be the energetic cost of sperm production or associated behavioural demands (Pla et al., 2020). Protandrous males may face considerable energetic demands to fertilize the eggs of highly fecund females, which could hinder growth, particularly in the presence of rival males (Pla et al., 2020). In *O. puerilis*, males in competitive settings (e.g., groups or triplets) showed stunted growth compared to those in monogamous pairs, suggesting that both reproductive investment and competition both likely regulate male size and growth. Males in monogamous pairs eventually grew to a size similar to that of females, possibly because these worms are capable of alternating sex roles, and such alternation would increase the overall fitness of the pair (Berglund, 1986). This further supports the idea that ongoing male-male competition or the presence of an extra-pair individual influences growth and sex allocation decisions.

7.2. **Strategic Growth Among Juveniles**

In Chapter 6, we observed that females were consistently larger than males until the end of the experiment, despite being size-matched at the start of the experiment. This raised several questions: Is the growing size difference a result of i) sex-specific developmental trajectories, ii) a growth spurt triggered by sex change, or iii) competitive growth to establish a size advantage early, as a means of establishing sex roles and facilitate pair formation?

In Chapter 6, we examined the growth trajectories of juveniles exposed to different social environments: isolation, pairs and triplets of size-matched juveniles and groups of adults.

Our findings revealed that *Ophryotrocha puerilis* exhibits context-dependent plasticity in growth trajectories, with variations not only across different social environments but also between individuals within the same environment. These results suggest that individual growth strategies are strongly influenced by social dynamics, first by the intensity of competition over sex roles (pairs vs. triplets vs. groups), and then by an individual's competitive ability (rank-1 vs. lower ranks).

7.2.1. **Alternative hypothesis**

Sex-specific growth

Berglund (1986) found that females grew more slowly than males when producing clutches, which he attributed to the higher energetic cost of egg production. However, when females were not producing clutches, their growth rates were similar to those of males. This conditional growth difference suggests that sex-specific growth is driven by reproductive demands rather than inherent differences between sexes. Thus, if growth differences are observed from the beginning, as was the case in our experiment, it suggests that sex roles and anticipated reproductive demands (e.g., future fecundity) were influencing growth patterns early on, rather than purely sex-specific growth trajectories.

7.2.2. **Alternative hypothesis**

Sex-change induced growth-spurt

On the other hand, if the differences were due to females accelerating growth before sex change to maximize fecundity, we would expect the end of the growth spurt to coincide with the timing of sex change. For instance, in the protandrous coral-inhabiting snail *Coralliophila violacea* as well as in the protandrous slipper limpet *Crepidula norrisiarum* and *C. cf. marginalis*, individuals accelerate growth during sex change (Warner et al., 1996; Chen & Soong, 2002; Collin, 2013). Since female fecundity is size-dependent, it would be advantageous for those on the path to becoming female to grow as large as possible (Chen & Soong, 2002; Collin, 2013).

However, we found that growth spurt ended days before sex change began (i.e., before the first appearance of oocytes in the coelom), suggesting that the observed size differences were not solely attributable to sex-specific variations in growth or growth acceleration during sex change. Moreover, the intensity of the growth spurt correlated with the number of individuals in the social environment (pairs vs. triplets) as well as an individual's rank among conspecifics within that environment (rank-1 vs. lower-ranks). Berglund (1986) also observed that growth rates among isolated females of *O. puerilis* were relatively uniform, but the presence of another female led to increased variation in growth rates. Therefore, growth acceleration is likely a response to conflict intensity.

7.3. **Why establish size-hierarchies?**

7.3.1. **Relative size determines phenotypic sex among same sex individuals**

In sequential hermaphrodites, relative size emerges as a cue most predictive of phenotypic sex (Charnov, 1982; Francis & Barlow, 1993). In protandrous species, when two males are paired, the larger individual typically becomes female, as reproductive fitness is primarily limited by egg production (Warner et al., 1975; Fricke and Fricke, 1977), whereas even smaller males can fertilize a considerable number of eggs, aligning with the size-advantage hypothesis (Ghiselin, 1969; Warner et al., 1975; Charnov, 1982). Conversely, in protogynous species or those living in social groups, when two females are paired, the larger female often becomes male, as larger individuals are better suited for the haremic role (Ghiselin, 1969; Charnov, 1982; Nakashima et al., 1995; Leonard, 2018). In species such as the protogynous saddleback wrasse *Thalassoma duperrey* and the sea goldie *Anthias squamipinnis*, even visual cues of a smaller female can trigger sex change in the larger individual (Shapiro, 1983; Ross et al., 1983; Ross, 1987). Thus, relative size serves as a significant cue for sex change among same-sex individuals.

7.3.2. **Competitive growth to establish size differences among size-matched juveniles**

Establishing size hierarchies early in development may function as a conflict resolution strategy among juveniles, clarifying sex roles. In our study, size-matched juveniles had equal opportunities to assume the female role, leading to competitive growth as individuals modulated their growth in response to the growth of competitors. As size differences grew, the smaller individual conceded the competition for the female role (around day 5, evident by its shorter growth spurt), while the soon-to-be female continued to grow at an accelerated pace until a clear size hierarchy was established. This critical size ratio marks a turning point in social dynamics, representing the threshold at which rank-1 and lower-rank individuals acknowledge their respective roles, leading to a stable hierarchy.

Similar observations have been made in the protandrous clown anemonefish *Amphiprion percula*. When two non-breeders (i.e., sufficiently large but still sexually immature individuals, Rueger et al 2018) are paired, conflict intensity is strongest when the individuals are size-matched, as both individuals have equal opportunity to assume the dominant breeding position. Consequently, they compete by accelerating their growth in response to each other (Wong et al., 2016; Reed et al., 2019). Furthermore, losers in these conflicts (subordinates) strategically regulate their growth (subordinate self-restraint) to maintain a size ratio with the dominant individual, and the establishment of a size hierarchy resolves the conflict (Wong et al., 2016).

In another study, when juveniles *Amphiprion percula* were kept in size-matched pairs, the competition over the breeding position intensified as expected. However, this conflict was resolved when an individual grew faster than the competitor to achieve dominant status (Reed et al., 2019). Interestingly, both competitors grew faster than isolated individuals, suggesting that the presence of a competitor triggers growth modulation in both individuals in a pair, but a more pronounced accelerated growth in one individual until it reaches dominant status (Reed et al., 2019). Therefore, pairing size-matched individuals results in competitive growth, where the presence of a competitor for the breeding position intensifies conflict, triggering a period of accelerated growth by one of the individuals to achieve dominant status (Reed et al., 2019). Strategic growth has also been shown in the daffodil cichlid *Neolamprologus pulcher* (Heg, 2010), the clown anemonefish *Amphiprion percula* (Wong, 2016; Reed et al., 2019; Iwata et al., 2020) and also in meerkats (Huchard et al., 2016).

7.3.3. **Preference for the second sex?**

Since the second sex is typically associated with greater reproductive success in sequential hermaphrodites (Benvenuto et al., 2017; Roberts et al., 2021), pairing size-matched same-sex individuals often escalates conflict over sex roles (or status). In *Ophryotrocha puerilis*, pairing two females led to aggressive interactions (resulting in injuries and amputation of segments from biting), with the “loser” reverting to the male phase (Berglund, 1990). Pfannenstiel (1975) proposed that this sex reversal to male may be influenced by pheromones that inhibit the production of the ootropic hormone in females, leading to resorption of oocytes and subsequent sex reversal. Indeed, both Müller (1962) and Pfannenstiel (1975) found that this pair-culture effect occurs only when individuals are allowed physical contact. When worms are separated by a mesh, both females retain their oocytes.

Similar sex role conflicts have been observed in the protogynous bidirectional bluebanded goby *Lythrypnus dalli* that lives in social groups of a single large male and multiple smaller females (Solomon-Lane et al., 2016). Pairing or grouping same-sex individuals led to aggressive interactions and subsequent sex changes: in male groups, all except the 'winner' changed sex to female, while in female groups, only the 'winner' changed sex to male (Rodgers et al., 2005; 2007). In the protandrous Red Sea clownfish, *Amphiprion bicinctus*, where the dominant female and male are obligately monogamous and inhabit an anemone with several subordinate juveniles, forcibly pairing two females resulted in severe injury or death of the subdominant individual, likely due to the failure to establish sexual roles, as females do not revert to males (Fricke and Fricke, 1977).

7.4. **Juveniles engage in competitive growth to exert preferences over the female role**

In the absence of adult individuals, it would be adaptive for a pair of juveniles to express opposite sexes to minimize the non-reproductive period and initiate reproduction. In line with the size-advantage hypothesis (Ghiselin, 1969), the relatively larger juvenile should express female and the relatively smaller juvenile should express male. However, if juveniles are of equal size, both individuals have equal opportunity to assume the female role. Consequently, competitors are incentivized to grow as rapidly as possible to establish a size difference significant enough to compel the smaller individual to concede and accept the male role. In *Ophryotrocha puerilis*, conflicts are resolved at size ratios between 0.77 - 0.86, depending on the number of individuals in the environment and relative position in the hierarchy (Chapter

6). In the clown anemonefish *Amphiprion percula,* the stabilizing size ratio are reported as 0.92 in laboratory experiments (Wong et al., 2016) and 0.80 in wild populations (Buston and Cant, 2006).

Integrating the findings from both chapters, we infer that size-matched juveniles in *Ophryotrocha puerilis* engage in competitive growth to exert preferences over the female role. Consequently, the post-conflict resolution status in the size hierarchy serves as a predictor of phenotypic sex even before the actual sex change occurs. Similar observations of size-matched juveniles establishing a size hierarchy and making subsequent sex changes have been observed in the protogynous bluebanded goby *Lythrypnus dalli* (Solomon-Lane et al., 2016).

In another study it was found that triplets of *A. ocellaris* juveniles first establish social order through dominance interactions, and sex expression is determined by an individual's post-conflict social status. Winners accelerate their growth and follow the female developmental trajectory, second-ranked individuals become males, and subordinates supress growth and remain juveniles (Iwata et al., 2008; Madhu et al., 2010). In *A. percula,* pairing size-matched juveniles also leads to elevated status-related conflict, which is resolved after establishing a size hierarchy, with the winner being significantly larger (Wong et al., 2016). Since post-conflict status is predictive of phenotypic sex in the closely related *A. ocellaris*, it is likely that the winners among pairs of *A. percula* were on a trajectory towards assuming the female role.

7.4.1. **Potential limitation of the study**

As previously mentioned, even a small size difference might trigger sex changes or reversals among same-sex individuals. In the protogynous goby *Trimma caudomaculatum*, removing all females led the smallest of four males to revert to the female phase, despite only a 2.5 mm (~6%) size difference between the two smallest males, highlighting how sensitive sex roles are even to minor size differences (Tomatsu et al., 2018). Similarly, in the protogynous hawkfish *Cirrhitichthys falco*, a size difference of just 4–8 mm (~4–9%) triggered sex reversal to female in the smaller male (Kadota et al., 2012).

In our study, it is possible that even a two-segment initial size difference may have established sex roles immediately upon pairing or grouping, rather than after reaching the conflict-resolving size ratio on day 5. This potential limitation should guide future studies to aim for minimal initial size variation among conspecifics, though achieving such precision may be difficult. Nevertheless, even considering this potential alternative, the larger individual in every pair and triplet consistently adopted the female role, indicating that even slight size differences reliably predict sex roles. Additionally, the stability of the size hierarchy, until the onset of reproduction in pairs, and throughout the experiment in triplets, where male-male competition occurred, despite the potential for males to grow faster than females (Berglund, 1990), suggests that size differences were not arbitrary but were continually maintained by subordinates through downregulating growth presumably to reduce conflict.

7.5. **Social regulation of growth in invertebrates**

Strategic growth implies a sophisticated need for social recognition to monitor and adjust growth relative to competitors. While strategic growth has been primarily documented in group-living vertebrates, a simpler form of this behaviour, in the form of growth limitation in the presence of larger conspecifics as a result of conflict and growth acceleration when alone, has also been observed in several invertebrates.

In the gonochoristic giant river prawn *Macrobrachium rosenbergii*, juveniles exhibit rank-specific growth: some grow rapidly and become large “jumpers”, while others grow slowly and remain small "laggards" (Karplus, 2005). These differences are thought to arise from social interactions, reflecting the establishment of a social hierarchy, as juveniles raised in isolation show more uniform growth rates and do not exhibit this differentiation (Karplus, 2005). Interestingly, exposing a jumper to larger individuals stunts its growth, while exposing a laggard to smaller individuals accelerates it, suggesting growth is adjusted based on relative social rank (Karplus, 2005).

Similarly, in the gonochoristic red claw crayfish *Cherax quadricarinatus*, pairing juvenile males with only slight size differences results in suppressed growth in the relatively smaller individual. However, when the crayfish are prevented from physically interacting, i.e., when opportunity for aggression was reduced, growth suppression diminishes (Barki & Karplus, 2004). Additionally, isolating a juvenile that was previously under such growth inhibition imposed by larger individuals resulted in accelerated growth (Barki & Karplus, 2004), suggesting that direct social interactions are responsible for suppressed growth.

The protandrous slipper limpet *Crepidula cf. marginalis* shows a similar pattern, suggestive of competitive growth: when two size-unmatched snails are paired, the larger snail experiences accelerated growth while the smaller snail's growth is stunted. This effect disappears when the snails are separated by a barrier, indicating that physical interaction triggers competitive growth (Carrillo-Baltodano & Collin, 2015).

Across these examples, a trend emerges: relative size determines social status, and social status regulates growth. The underlying rule is simple: if smaller, grow slowly and stay small; if the largest or isolated, grow rapidly and become large. An added rule in the context of protandrous sequential hermaphrodites might be: if relatively smaller, express male, if relatively larger, express female. If equal size, compete for the female role by establishing size differences, and if successful, continue growing and become female; otherwise remain male.

7.6. **Is it costly to be an early sex changer?**

Early sex changers in *O. puerilis* invested heavily in their female function, practically bypassing the male phase entirely (although even early sex changers may pass through a transient male phase; Müller, 1962). In contrast, late sex changers extended their time as males and either did not change sex or changed sex relatively later. We expected some life-history trade-offs due to this skewed reproductive investment, particularly due to the higher energy demands of both accelerated growth and egg production (Berglund, 1986, 1990). However, our findings in Chapter 6, showing that worms in pairs expressing opposite sexes reached similar asymptotic body sizes, suggests that there may be no significant cost to final body size. However, there may be a trade-off related to lifespan. In the closely related simultaneous hermaphrodite *Ophryotrocha diadema*, individuals that exclusively invested in the male function (functional males) tended to have a longer lifespan compared to those that engaged in both sexual functions (Di Bona et al., 2010). Additionally, previous studies have shown that females that over-invested in the female role experienced reduced fecundity later in life (Berglund, 1986).

Alternatively, it may be costly for the male partners to prolong the male phase. Pla et al. (2020) suggest that males of protandrous species may incur high energetic costs to effectively fertilize eggs of a highly fecund female. As mentioned previously, *O. puerilis* females can lay upto 350-400 eggs in a single clutch, and produce one clutch every week (Berglund, 1986, 1990). This may be a significant cost to the males and may explain the growth stunting seen in the male partners. However, only males in triplets and groups, where potential for male-male conflict was present, remained smaller than the female until the end of the experiment, whereas males in pairs, under conditions of monogamy, were nearing the body size of females by the end of the experiment, suggesting that inter-individual conflict regulates growth.

7.7. How might *Ophryotrocha puerilis* assess relative body size?

Ophryotrocha puerilis lacks advanced visual organs; their simple photoreceptors are primarily designed for light detection (Rhode, 1990). So how might individuals assess relative body size? Müller (1962) and Pfannenstiel (1975) found that chemical signals alone were insufficient to facilitate pair-culture effect and that tactile cues were necessary. However, the possibility of a contact pheromone secreted through the prostomia cannot be ruled out (Pfannenstiel, 1977). In *Crepidula cf. marginalis*, the social mediation of sex change relies on direct tactile stimuli from conspecifics (Carrillo-Baltodano & Collin, 2015). Similarly, in protandrous clownfish *Amphiprion percula*, both relative size and mechanosensory cues are crucial for establishing social hierarchy (Wong et al., 2016; Desrochers et al., 2020; Iwata et al., 2020).

Adult *O. puerilis* frequently engage in rubbing behaviors (Müller, 1962; Pfannenstiel, 1977; personal observation), which may potentially aid in assessing competitors' body size through mechanosensory cues, as suggested by the presence of collar receptors with mechanoreceptive functions (Schlawny et al., 1991). Additionally, many *Ophryotrocha* species secrete a mucous-like substance (Thornhill et al., 2009), potentially also serving as a source of pheromonal cues (Pfannenstiel, 1975, 1977, 1978, Marchionni & Rolando, 1981; Berglund, 1990). Similar pheromone-induced prolongation of the male phase has been observed in other protandrous species (Hoagland, 1978; Richter & Luque, 2004).

7.8. **Possibility of social sex determination?**

Jaccarini et al. (1983) drew parallels between socially induced sex reversal in the pair-culture effect in *Ophryotrocha puerilis* and social sex determination in the gonochoristic green spoonworm *Bonellia viridis*. Social sex determination occurs when individuals develop as male or female in response to social interactions, usually to complement the sex of the individual in their environment (Beukeboom & Perrin, 2014; Collin, 2018). For example, in the bidirectional goby *Gobiodon erythrospilus*, isolated juveniles do not mature sexually; however, in the presence of an adult, a juvenile develops into the complementary sex to that adult (Hobbs et al., 2004). In the protogynous Midas cichlid *Cichlasoma citrinellum*, dominance interactions and the relative size of juveniles can predict their phenotypic sex even before sexual maturity (Francis & Barlow, 1993).

In *B. viridus*, undifferentiated larvae develop into females in isolation, whereas in the presence of a female, they develop into males, permanently living as sexual parasites within her (Berec et al., 2005). Here, the ‘masculinizing stimulus’ is the female's presence. Interestingly, in the absence of a female, similar to observations in Chapter 5 of direct pair formation among juveniles in *O. puerilis*, paired undifferentiated larvae also express opposite sexes and form breeding pairs (Jaccarini et al., 1983). Similarly, in the immobile snail *Quoyula monodonta*, the sex of planktonic larvae settling in a patch is determined by the composition of the social environment: juveniles develop as males if a female is already present, but if they are the first to arrive, they grow rapidly and mature directly as females (Soong & Chen, 2003; Collin, 2018). The adaptive significance of early sex change in isolated larvae is that the male-phase is ineffective in the absence of a female making it more beneficial to bypass the male stage and develop directly into females. *O. puerilis* similarly changes sex to female in isolation or in the absence of larger conspecifics and prolongs the male phase in the presence of larger conspecifics, and pairs of juveniles develop into complementary sexes, indicative of sexual lability at the juvenile stage.

Pfannenstiel & Grünig (1990) found evidence of 'bisexual potential' in the gametogenesis of *Ophryotrocha puerilis*, as the early stages of oocyte and sperm development are identical. The authors suggest that the eventual differentiation into male or female pathways are guided by social factors, such as the presence of a female. This raises the possibility that sex differentiation in this species is labile, indicating potential for socially determined sex expression at the juvenile stage. Indeed, it is possible that the bipotential gamete of *O. puerilis* may be shifted to the male or female developmental trajectory depending on the composition of the social environment (Mueller et al., 2015).

Such social sex determination has been reported in the gonochoristic sibling species of *Ophryotrocha puerilis*, *O. labronica*, *O. costlowi, O. robusta* and *O. macrovifera* (called sex inducibility; Rolando, 1984). Juveniles tended to develop as males in the presence of females and as females in the presence of males, and interestingly, juveniles raised in pairs developed into opposite sexes (Rolando, 1984). In a later study, Meconcelli and colleagues (2015) confirmed the finding that juveniles tend to develop as the sex complementary to the adult in their social environment and that this lability is limited to the juvenile stage.

Social sex determination is thought to evolve from sequential hermaphroditism through a heterochronic shift, and may be thought of as a form of socially mediated sex expression at the juvenile stage (Oldfield et al., 2005). It is possible for a species to express both social sex determination, as well as sequential hermaphroditism (Hoagland, 1978). However, whether *O. puerilis* exhibit social sex determination requires further investigation to determine if the male phase is merely transient or if early sex changers entirely bypass the male stage by altering developmental pathways.

7.9. **Adaptive Significance of Reaction Norm Plasticity in *O. puerilis***

The asynchronicity in the timing and body size at sex change, its sensitivity to social cues, and the ability to modulate growth in response to competition intensity in the social environment suggest that *Ophryotrocha puerilis* has evolved finely tuned reaction norms for life-history traits that are highly responsive to social dynamics. This plasticity is highly adaptive, allowing the worms to maximize fitness in socially unpredictable environments by adopting the appropriate sex in response to the presence or absence of conspecifics, as well as the sex of conspecifics. The ability to change sex bidirectionally further enhances their capacity to rapidly adjust phenotypic sex in varying social contexts. This flexibility is especially advantageous for "miniaturized metazoans" like *O. puerilis*, which face limited mobility and resource constraints due to their small size (Clark, 1978).

Such adaptability facilitates opportunistic strategies critical to the success of colonizing species (Clark, 1978; Prevedelli et al., 2006). For example, an initial colonizer might develop as a female, while later arrivals assume male roles, maximizing reproductive potential and promoting population establishment (Ghiselin, 1969; Collin, 2013). Although little is known about the mating system of *O. puerilis* in natural conditions, several studies describe the species as monogamous (Müller & Müller, 1997; Mueller et al., 2015; Yasuoka & Yuka, 2017; Hitchcock & Gardner, 2023). This idea likely stems from Berglund's (1986) suggestion that pair-culture would be adaptive in low-density populations where mate searching is costly. However, at the time, little was known about colonizing behaviors in the wild (Berglund, 1990).

Taboada (2016, 2017), however, reported high population densities at certain times of the year and population persistence over multiple generations. This raises the question of whether *O. puerilis* is truly monogamous. Furthermore, Premoli & Sella (1995) reported low population densities in winter months, where numbers could drop to single digits. Thus, Berglund's proposal of pair-culture facilitating pair formation in very low-density conditions seems plausible, as bidirectional sex change would be advantageous when only two conspecifics are present in such sparse populations.

The causes of extreme fluctuations in population densities remain unclear. Still, it is generally thought that invertebrate species with direct development (those lacking a planktonic larval phase) tend to experience greater population variability (Eckert, 2003). For instance, in the marine copepod *Tigriopus californicus*, both disappearance and recolonization processes are sensitive to environmental stress (Eckert, 2003). Taboada (2017) reported that populations of *O. puerilis* are among the first to colonize substrates and are often abundant, though they can be displaced by other species. Thus, while *O. puerilis* is an efficient colonizer and populations persist over several generations, their population densities can fluctuate significantly. Therefore, reaction-norm plasticity in sex allocation and growth, in response to fine-tuned changes in the social environment, would allow individuals to maximize fitness in these temporally variable environments.

7.11. **Conclusion**

This work demonstrates that *Ophryotrocha puerilis* exhibits sophisticated responses to social cues including modulating sex allocation based on relative size, negotiating sex roles even in the absence of adults, establishing size hierarchies to exert preferences over sex roles as well as maintaining size differences in response to male-male competition. Such reaction-norm plasticity in life-history traits is highly adaptive, allowing individuals to maximize fitness in environments that are often ephemeral, unpredictable, and socially variable. Therefore, this study reveals the significance of subtle conspecific cues in shaping reproductive strategies in *O. puerilis*, revealing that they are anything but puerile.

References

Bacci, G. (1951). Existence of true males and females in a hermaphrodite population of *Ophryotrocha puerilis*. *Experientia*, *7*(6), 222-224.

Barki, A., & Karplus, I. (2004). Size rank and growth potential in redclaw crayfish (*Cherax quadricarinatus*): are stunted juveniles suitable for grow-out?. *Aquaculture Research*, *35*(6), 559-567.

Benvenuto, C., Coscia, I., Chopelet, J., Sala-Bozano, M., & Mariani, S. (2017). Ecological and evolutionary consequences of alternative sex-change pathways in fish. *Scientific Reports*, *7*(1), 9084.

Berec, L., Schembri, P. J., & Boukal, D. S. (2005). Sex determination in *Bonellia viridis* (Echiura: Bonelliidae): population dynamics and evolution. *Oikos*, *108*(3), 473-484.

Berglund, A. (1986). Sex change by a polychaete: effects of social and reproductive costs. *Ecology*, *67*(4), 837-845.

Berglund, A. (1990). Sequential hermaphroditism and the size-advantage hypothesis: an experimental test. *Animal Behaviour*, *39*(3), 426-433.

Berglund, A. (1991). To change or not to change sex: a comparison between two *Ophryotrocha* species (Polychaeta). *Evolutionary Ecology*, *5*, 128-135.

Beukeboom LW, Perrin N. (2014). The diversity of sexual cycles. In *The evolution of sex determination*, pp. 18–36. Oxford, UK: Oxford University Press.

Brante, A., Vilches, C., & Quiñones, A. (2012). Role of population traits and local social interactions in sex-change plasticity of the protandric marine gastropod *Crepidula coquimbensis*. *Marine Ecology Progress Series*, *458*, 123-132.

Buston, P. M., & Cant, M. A. (2006). A new perspective on size hierarchies in nature: patterns, causes, and consequences. *Oecologia*, *149*, 362-372.

Buston, P., & Clutton-Brock, T. (2022). Strategic growth in social vertebrates. *Trends in Ecology & Evolution*, *37*(8), 694-705.

Carrillo-Baltodano, A., & Collin, R. (2015). *Crepidula* slipper limpets alter sex change in response to physical contact with conspecifics. *The Biological Bulletin*, *229*(3), 232-242.

Charnov, E. L. (1982). *The Theory of Sex Allocation* (Vol. 18). Princeton University Press.

Chen, M. H., & Soong, K. (2002). Estimation of age in the sex-changing, coral-inhabiting snail *Coralliophila violacea* from the growth striae on opercula and a mark–recapture experiment. *Marine Biology*, *140*, 337-342.

Chiba, S., Goshima, S., & Shinomiya, Y. (2003). Male-male competition selects for delayed sex change in the protandrous shrimp *Pandalus latirostris*. *Marine Biology*, *142*, 1153-1157.

Chiba, S. (2007). A review of ecological and evolutionary studies on hermaphroditic decapod crustaceans. *Plankton and Benthos Research*, *2*(3), 107-119.

Clark, W. C. (1978). Hermaphroditism as a reproductive strategy for metazoans; some correlated benefits. *New Zealand Journal of Zoology*, *5*(4), 769-780.

Collin, R., McLellan, M., Gruber, K., & Bailey-Jourdain, C. (2005). Effects of conspecific associations on size at sex change in three species of calyptraeid gastropods. *Marine Ecology Progress Series*, *293*, 89-97.

Collin, R. (2013). Phylogenetic patterns and phenotypic plasticity of molluscan sexual systems. *Integrative and Comparative Biology*, *53*(4), 723-735.

Collin, R. (2018). Transitions in sexual and reproductive strategies among the Caenogastropoda. *Transitions between sexual systems: understanding the mechanisms of, and pathways between, dioecy, hermaphroditism and other sexual systems*, 193-220.

Desrochers, L., Branconi, R., Schlatter, E., Dent, B., & Buston, P. (2020). Sensory cues underlying competitive growth in the clown anemonefish (*Amphiprion percula*). *Behavioural Processes*, *181*, 104276.

Di Bona, V., Lorenzi, M. C., & Sella, G. (2010). Functional males in pair-mating outcrossing hermaphrodites. *Biological Journal of the Linnean Society*, *100*(2), 451-456.

Eckert, G. L. (2003). Effects of the planktonic period on marine population fluctuations. *Ecology*, *84*(2), 372-383.

Francis, R. C., & Barlow, G. W. (1993). Social control of primary sex differentiation in the Midas cichlid. *Proceedings of the National Academy of Sciences*, *90*(22), 10673-10675.

Fricke, H., & Fricke, S. (1977). Monogamy and sex change by aggressive dominance in coral reef fish. *Nature*, *266*(5605), 830-832.

Ghiselin, M. T. (1969). The evolution of hermaphroditism among animals. *The Quarterly Review of Biology*, *44*(2), 189-208.

Heg, D. (2010). Status-dependent and strategic growth adjustments in female cooperative cichlids. *Behavioral Ecology and Sociobiology*, *64*, 1309-1316.

Hitchcock, T. J., & Gardner, A. (2023). Sexual antagonism in sequential hermaphrodites. *Proceedings of the Royal Society B*, *290*(2011), 20232222.

Hoagland, K. E. (1978). Protandry and the evolution of environmentally mediated sex change: a study of the Mollusca. *Malacologia* 17: 365–391.

Hobbs, J. A., Munday, P. L., & Jones, G. P. (2004). Social induction of maturation and sex determination in a coral reef fish. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *271*(1553), 2109-2114.

Huchard, E., English, S., Bell, M. B., Thavarajah, N., & Clutton-Brock, T. (2016). Competitive growth in a cooperative mammal. *Nature*, *533*(7604), 532-534.

Iwata, E., Nagai, Y., Hyoudou, M., & Sasaki, H. (2008). Social environment and sex differentiation in the false clown anemonefish, *Amphiprion ocellaris*. *Zoological Science*, *25*(2), 123-128.

Iwata, E., Yoshida, T., Abe, T., Takahata, K., & Masuda, K. (2020). Behavioural differences between breeding and nonbreeding pairs of protandry monogamous false clown anemonefish *Amphiprion ocellaris*. *Journal of Fisheries*, 8, 808– 816.

Jaccarini, V., AGius, L., Schembri, P. J., & Rizzo, M. (1983). Sex determination and larval sexual interaction in *Bonellia viridis* Rolando (Echiura: Bonelliidae). *Journal of Experimental Marine Biology and Ecology*, *66*(1), 25-40.

Kadota, T., Osato, J., Nagata, K., & Sakai, Y. (2012). Reversed sex change in the haremic protogynous hawkfish *Cirrhitichthys falco* in natural conditions. *Ethology*, *118*(3), 226-234.

Karplus, I. (2005). Social control of growth in *Macrobrachium rosenbergii* (De Man): a review and prospects for future research. *Aquaculture Research*, *36*(3), 238-254.

Kegel, B., & Pfannenstiel, H. D. (1983). Evaluation of the pair-culture effect in *Ophryotrocha puerilis* (Polychaeta: Dorvilleidae). I. Pair-culture effect and sex ratio. *Helgoländer Meeresuntersuchungen*, *36*, 205-213.

Leonard, J. L. (2018). The evolution of sexual systems in animals. *Transitions between sexual systems: understanding the mechanisms of, and pathways between, dioecy, hermaphroditism and other sexual systems*, 1-58.

Madhu, R., Madhu, K., & Venugopal, K. M. (2010). Sex change of hatchery produced *Amphiprion ocellaris*: Influence of mating system removal on gonad maturation and nesting success. *Journal of the Marine Biological Association of India*, *52*(1), 62-69.

Marchionni, V., & Rolando, A. (1981). Sex reversal in *Ophryotrocha puerilis puerilis* (Polychaeta, Dorvilleidae) induced by ethereal extracts of female phase individuals. *Italian Journal of Zoology*, *48*(2), 91-96.

Meconcelli, S., Lorenzi, M. C., & Sella, G. (2015). Labile sex expression and the evolution of dioecy in *Ophryotrocha* Polychaete worms. *Evolutionary Biology*, *42*, 42-53.

Mueller, W. A., Hassel, M., Grealy, M., Mueller, W. A., Hassel, M., & Grealy, M. (2015). In Preparation for New Life I: Sex Determination and Sexual Development. *Development and Reproduction in Humans and Animal Model Species*, 215-240.

Müller, H. (1962). Über die Sexualität des Polychaeten *Ophryotrocha puerilis*, ihre Determination und ihren Einfluss auf Drüsentätigkeit und auf Apparatenentwicklung. *Zeitschrift für Morphologie und Ökologie der Tiere, 52*(1), 1-32.

Müller, W. A., & Müller, W. A. (1997). Sex and the Single Gene. *Developmental Biology*, 301-309.

Nakashima, Y. (1987). Reproductive strategies in a partially protandrous shrimp, *Athanas kominatoensis* (Decapoda: Alpheidae): sex change as the best of a bad situation for subordinates. *Journal of Ethology*, *5*(2), 145-159.

Nakashima, Y., Kuwamura, T., & Yogo, Y. (1995). Why be a both-ways sex changer?. *Ethology*, *101*(4), 301-307.

Oldfield, R. G. (2005). Genetic, abiotic and social influences on sex differentiation in cichlid fishes and the evolution of sequential hermaphroditism. *Fish and Fisheries*, *6*(2), 93-110.

Pfannenstiel, H. D. (1975). Mutual influence on the sexual differentiation in the protandric polychaete *Ophryotrocha puerilis*. In *Intersexuality in the animal kingdom* (pp. 48-56). Berlin, Heidelberg: Springer Berlin Heidelberg.

Pfannenstiel, H. D. (1977). Experimental analysis of the 'Paarkultureffekt'in the protandric polychaete, *Ophryotrocha puerilis* Clap. Mecz. *Journal of Experimental Marine Biology and Ecology*, *28*(1), 31-40.

Pfannenstiel, H. D. (1978). Endocrinology of polychaete reproduction and sexual development. *Italian Journal of Zoology*, *45*(2), 171-188.

Pfannenstiel, H. D., & Grünig, C. (1990). Spermatogenesis and sperm ultrastructure in the polychaete genus *Ophryotrocha* (Dorvilleidae). *Helgoländer Meeresuntersuchungen*, *44*, 159-171.

Pla, S., Benvenuto, C., Capellini, I., & Piferrer, F. (2020). A phylogenetic comparative analysis on the evolution of sequential hermaphroditism in seabreams (Teleostei: Sparidae). *Scientific Reports*, *10*(1), 3606.

Premoli, M. C., & Sella, G. (1995). Sex economy in benthic polychaetes. *Ethology Ecology & Evolution*, *7*(1), 27-48.

Prevedelli, D., N'siala, G. M., & Simonini, R. (2006). Gonochorism vs. hermaphroditism: relationship between life history and fitness in three species of *Ophryotrocha* (Polychaeta: Dorvilleidae) with different forms of sexuality. *Journal of Animal Ecology*, *75*(1), 203-212.

Reed, C., Branconi, R., Majoris, J., Johnson, C., & Buston, P. (2019). Competitive growth in a social fish. *Biology Letters*, *15*(2), 20180737.

Rhode, B. (1990). Eye structure of *Ophryotrocha puerilis* (Polychaeta: Dorvilleidae). *Journal of Morphology*, *205*(2), 147-154.

Richter, A., & Luque, Á. A. (2004). Sex change in two mediterranean species of Coralliophilidae (Mollusca: Gastropoda: Neogastropoda). *Journal of the Marine Biological Association of the United Kingdom*, *84*(2), 383-392.

Roberts, B. H., Morrongiello, J. R., Morgan, D. L., King, A. J., Saunders, T. M., & Crook, D. A. (2021). Faster juvenile growth promotes earlier sex change in a protandrous hermaphrodite (barramundi *Lates calcarifer*). *Scientific Reports*, *11*(1), 2276.

Rodgers, E. W., Drane, S., & Grober, M. S. (2005). Sex reversal in pairs of *Lythrypnus dalli*: behavioral and morphological changes. *The Biological Bulletin*, *208*(2), 120-126.

Rodgers, E. W., Earley, R. L., & Grober, M. S. (2007). Social status determines sexual phenotype in the bi-directional sex changing bluebanded goby *Lythrypnus dalli*. *Journal of Fish Biology*, *70*(6), 1660-1668.

Rolando, A. (1984). The sex induction hypothesis and reproductive behaviour in four gonochoristic species of the genus *Ophryotrocha* (Annelida Polychaeta). *Monitore Zoologico Italiano-Italian Journal of Zoology*, *18*(4), 287-299.

Ross, R. M. (1987). Sex-change linked growth acceleration in a coral-reef fish, *Thalassoma duperrey*. *Journal of Experimental Zoology*, *244*(3), 455-461.

Ross, R. M., Losey, G. S., & Diamond, M. (1983). Sex change in a coral-reef fish: dependence of stimulation and inhibition on relative size. *Science*, *221*(4610), 574-575.

Rueger, T., Barbasch, T. A., Wong, M. Y., Srinivasan, M., Jones, G. P., & Buston, P. M. (2018). Reproductive control via the threat of eviction in the clown anemonefish. *Proceedings of the Royal Society B*, *285*(1891), 20181295.

Schlawny, A., Grünig, C., & Pfannenstiel, H. D. (1991). Sensory and secretory cells of *Ophryotrocha puerilis* (Polychaeta). *Zoomorphology*, *110*, 209-215.

Sella, G., & Ramella, L. (1999). Sexual conflict and mating systems in the dorvilleid genus *Ophryotrocha* and the dinophilid genus *Dinophilus*. *Hydrobiologia*, *402*, 203-213.

Shapiro, D. Y. (1983). Distinguishing behavioral interactions from visual cues as causes of adult sex change in a coral reef fish. *Hormones and Behavior*, *17*(4), 424-432.

Solomon-Lane, T. K., Shvidkaya, P., Thomas, A., Williams, M. M., Rhyne, A., Rogers, L., & Grober, M. S. (2016). Juvenile social status predicts primary sex allocation in a sex changing fish. *Evolution & Development*, *18*(4), 245-253.

Soong, K., & Chen, M. H. (2003). Sex expression of an immobile coral-inhabiting snail, *Quoyula monodonta*. *Marine Biology*, *143*, 351-358.

Taboada, S., Bas, M., Leiva, C., Garriga, M., Sardá, R., & Avila, C. (2016). Life after death: shallow-water Mediterranean invertebrate communities associated with mammal bones. *Marine Ecology*, *37*(1), 164-178.

Taboada, S., Leiva, C., Bas, M., Schult, N., & McHugh, D. (2017). Cryptic species and colonization processes in *Ophryotrocha* (Annelida, Dorvilleidae) inhabiting vertebrate remains in the shallow-water Mediterranean. *Zoologica Scripta*, *46*(5), 611-624.

Thornhill, D. J., Dahlgren, T. G., & Halanych, K. M. (2009). Evolution and ecology of *Ophryotrocha* (Dorvilleidae, eunicida). *Annelids in Modern Biology*, 242-256.

Tomatsu, S., Ogiso, K., Fukuda, K., Deki, M., Dewa, S. I., Manabe, H., Sakurai, M., Shinomiya, A., & Sunobe, T. (2018). Multi-male group and bidirectional sex change in the gobiid fish, *Trimma caudomaculatum*. *Ichthyological Research, 65*(4), 502-506.

Warner, R. R., Robertson, D. R., & Leigh Jr, E. G. (1975). Sex Change and Sexual Selection: The reproductive biology of a labrid fish is used to illuminate a theory of sex change. *Science*, *190*(4215), 633-638.

Warner, R. R., Fitch, D. L., & Standish, J. D. (1996). Social control of sex change in the shelf limpet, *Crepidula norrisiarum*: size-specific responses to local group composition. *Journal of Experimental Marine Biology and Ecology*, *204*(1-2), 155-167.

Wong, M. Y., Uppaluri, C., Medina, A., Seymour, J., & Buston, P. M. (2016). The four elements of within-group conflict in animal societies: an experimental test using the clown anemonefish, *Amphiprion percula*. *Behavioral Ecology and Sociobiology*, *70*, 1467-1475.

Wright, W. G. (1989). Intraspecific density mediates sex-change in the territorial patellacean limpet *Lottia gigantea*. *Marine Biology*, *100*, 353-364.

Yasuoka, N., & Yusa, Y. (2017). Direct evidence of bi-directional sex change in natural populations of the oysters *Saccostrea kegaki* and *S. mordax*. *Plankton and Benthos Research*, *12*(1), 78-8.