Review

Gonadal Development, Social Structure, and Implications of Protandry by Aggressive Dominance in *Amphiprion* Anemonefish

Ivory Jorgenson[1]

[1]Biological Sciences, University of Manitoba, Winnipeg, MB Canada R3T 2N2
Corresponding Author: I. Jorgenson (jorgens6@myumanitoba.ca)

Abstract

Anemonefish of the genus Amphiprion *have developed a mating system involving protandrous sequential hermaphroditism, wherein male sexual maturation occurs prior to female sexual maturation in an individual[1]. This review will summarize changes in the gonad and individual behaviour through the transition from juvenile to male to female, as well as explore the relationship between body size and fecundity in* Amphiprion. *Anemonefish and their eggs obtain protection from predation by living among the tentacles of sea anemones, which are armed to sting most other fish[2]. A sequentially hermaphroditic mating system is advantageous due to the low abundance of host anemones, as it ensures that a migrating anemonefish can find a potential mate in any group it encounters, and the loss of a mate will always trigger a replacement. This strategy ultimately grants* Amphiprion *the ability to withstand unpredictable host abundance and maximize safety and offspring production, encouraging the proliferation of the genus[3].*

Keywords: Anemonefish, Amphiprion, Protandry, Gonad, Aggressive Dominance

1 INTRODUCTION

Anemonefish of the genus *Amphiprion* live in symbioses with host anemones of the order Actiniaria[1]. A large anemone can harbour a maximum of two anemonefish, with up to 10 individuals found in a single anemone colony at one time[3]. *Amphiprion* are sequentially hermaphroditic protandrous fish, meaning that male units of sexual reproduction reach maturity prior to female units[1]. This change is socially controlled by a size hierarchy in anemonefish through aggressive dominance by the sole female, the oldest and largest individual of the group[3,4]. The second-largest individual is the only reproductive male, who acts aggressively towards the smaller subadults, resulting in a monogamous relationship between himself and the female.

The mating pair's domination renders the subadults "juvenile", unable to sexually mature[3]. Upon death or removal of the female from the group, her mate changes sex to become the new female while the alpha-subadult, the most dominant in the group of up to eight subadults, quickly reaches sexual maturity to become the reproductive male[3,4]. Remarkably, a transitioning male anemonefish is able to completely alter its physiology, gonadal morphology, and behaviour in as little as 20 days[5,6]. Only recently have researchers begun to elucidate the complex physiological mechanisms underlying *Amphiprion* sex change, which is largely facilitated by steroid hormones[6,7,8], though it has

long been known that social behaviour plays a foundational role in the process[3,5]. This review will describe the gonadal development of anemonefish through each social role (subadult, male, female), investigate the behavioural role in mechanisms governing protandrous sex change, and explore the relationship between body size and fecundity in *Amphiprion*.

2 DISCUSSION

Aggressive dominance within a social group of *Amphiprion* anemonefish determines the gonadal development of each member. Fricke and Fricke[3] described subadult males as "psychophysiologically castrated," as pressure from high-ranking group members suppresses gonad growth. The gonads, located in the caudal region below the swim bladder, are smaller in subadults than they are in functional males or females[9]. Juvenile gonads are ovotestes lacking clear boundaries between immature ovarian and testicular tissue, wherein sparse spermatocytes remain undeveloped until the individual assumes the breeding male position[4,9]. When subadults become males, considerable gonad growth is accompanied by the redirection of gonadal development so that the ovary assumes testicular characteristics; the male ovotestis incorporates both mature testicular tissue and immature ovarian tissue[4,7,10].

Sex change from male to female involves the degradation of testicular tissues and the growth and maturation of ovarian tissues; this female gonad is slightly larger than the functional male ovotestis[7, 9, 10]. Godwin[4] quantified the proportion of oocytes within *A. melanopus* gametogenic tissue throughout sex change, revealing that nearly 60% of male gametogenic tissue is composed of oocytes preceding sex change and spermatogenic tissue can be completely diminished within a matter of weeks (Table 1). Functional female ovaries harbour oocytes in various maturity stages, with testicular tissue degenerated to a narrow band surrounding the gonads[4, 7]. Since the testicular tissue of *Amphiprion* deteriorates in the sexual transition from male to female, this process is thought to be irreversible in nature[9]. However, a recent study on *A. clarkii* has found that the functional ovary does retain high sexual plasticity; treatment of a female with an aromatase inhibitor (AI) has been shown to regenerate active spermatogenic tissue within the ovary, likely due to depletion of estrogen levels caused by the AI[8].

Table 1: *Oocytes as a proportion of total gametogenic tissue at various points in sex change of* Amphiprion melanopus.

Days After Female Removal	Proportion of Oocytes in Total Gametogenic Tissue
0	0.58
10	0.69
20	0.89
30	0.93
45	1.0

A study by Godwin[5] on *A. melanopus* verified that protandrous sex change is under social control; induced sex change by female removal from the anemone has led to the observation of several sex change-associated social cues. One noted cue was the absence of female aggressiveness towards the male upon her removal. Taking the female from the anemone and leaving behind the male and subadults eliminates the aggressive approaches by the female, which normally total about 150 per day towards the male. This may act as a signal to the male that he can assume dominance once the female oppressor is absent. However, it would be beneficial in future to study whether keeping the female in an enclosure within the anemone would yield the same effect, as it would eliminate only the aggressive approaches and disqualify the possibility that sex change may be controlled by some visual or chemical cue instead.

Nevertheless, Godwin's results concur with Fricke & Fricke[3], who suggested that sex change in males is suppressed by aggressive female dominance. *Amphiprion* sex change

also involves mating pairs bathing, or placing themselves in close contact with host anemone tentacles, near and parallel to one another in a behaviour referred to as visiting. Visiting increases within a day after female removal, with the sex-changing male often visiting the same juvenile to establish which subadult shall sexually mature; this behaviour decreases as the sex-changer characteristically grows more aggressive in the subsequent days. This aggression prevents other individuals within the group from also changing sex to female, securing them as potential mates rather than competitors for mates. Following female removal and initial visiting behaviours, sex-changers increase aggressive approaches on the alpha-subadult compared to other subadults as they attempt to clearly establish their position as the only female[5].

The drastic contrast between the body size of *Amphiprion* sexes suggests the necessity of larger female body size for reproductive success. Fricke and Fricke[3] observed body size in two *Amphiprion* species to differ substantially between sexes; female body length was measured approximately 20 mm longer, while female body weight was roughly double that of male averages (Table 2). Correlations between female body weight and ovary weight have been observed across multiple *Amphiprion* species, indicating that female fecundity increases with body size[1, 3]. A larger female can produce and accommodate more oocytes, but an increase in size would not necessarily affect male reproduction because a small male can still mate successfully with a large female and sperm takes up relatively little space and is continuously produced. These observations led to the hypothesis that sex change direction in *Amphiprion* was decided based on larger female body size being more favourable than larger male body size. An important reason for the anemonefish's tremendous growth when transitioning from male to female may be that male reproductive success depends less on body size, while female reproductive success increases with increasing body size[1].

Table 2: *Mean and standard deviation of body length and wet body weight in samples of* Amphiprion akallopisos *and* Amphiprion bicinctus.

	Amphiprion akallopisos		*Amphiprion bicinctus*	
	Male	Female	Male	Female
Length(mm)	73±3	97±5	113±7	129±6
Weight(g)	7.1±1.1	19.5±2.9	28.0±3.9	46.1±7.6

In groups of *Amphiprion*, females control the reproductive state of every member, restricting the breeding population size and suppressing the sexual maturity of female candidates[3]. The absence of a female results in sex change

in the dominant male by the degeneration of testicular tissue and growth and maturation of ovarian tissue[10], coupled with a newfound aggression in the interest of establishing dominance status amongst their peers. Increases in the body size of sex-changing individuals translates to higher female fecundity, though to what extent remains unexplored. Future studies might venture to ascertain the exact growth-to-fecundity increase ratio, as well as pinpoint the cause of the extreme body size increase in sex-changing anemonefish (the dominant fish might feed more, for example). Nevertheless, the evolutionary advantages of sequential hermaphroditism in *Amphiprion* extend far beyond fecundity.

Considering the low population density of anemonefish and erratic distribution of host anemones, chances of a migrating individual (usually juvenile) encountering an anemone hosting an opposite-sex potential mate would be scant if not for this mating system[3]. A study by Hattori[1] effectively disproved protogyny (females change into males) as a viable option for the anemonefish mating system; one hypothesis suggests that a dominant male would require a harem of smaller females to breed with (polygyny), which would be costly to defend given the low abundance of hosts available. The study was able to mathematically prove the efficacy of protandry over polygyny by determining that reproductive success in various *Amphiprion* species is maximized if the mating system involves monogamous pairing with a dominant female rather than polygyny with a dominant male.

3   Conclusions

Protandry in *Amphiprion* ultimately ensures that there will always be a potential mate available and loss of a mate will always trigger replacement. Once an individual has joined a group, they may never have to leave the safety of their anemone again to find mates[3]. However, it should be noted that larger subadults may pair with each other and move amongst hosts to establish their own territory, securing them a chance to mate if they are outlived by the mating pair in their current anemone[1]. Thus, the evolution of protandrous sequential hermaphroditism has granted Amphiprion the ability to withstand unpredictable host abundance and maximize safety and offspring production in order to promote the proliferation of the genus. The display of aggressive dominance is the foundation of the *Amphiprion* social mating system, suppressing radical changes in the protandrous anemonefish gonad: full transition from juvenile ovotestis to functional male ovotestis to a completely restructured working ovary, contained within a large female capable of producing a plentiful next generation of anemone-inhabiting juveniles.

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