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Abstract approved: ______________________
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I examined two aspects of the reproductive behavior of the garibaldi, *Hypsypops rubicundus*, a temperate marine damselfish with male parental care. My primary objective was to determine the relationship between female choice and male parental investment in the care of offspring. In particular, I sought to determine: (1) how the presence and developmental stage of eggs already in the nest influences female spawning site choice; (2) how male investment in current offspring varies with the number and developmental stage of eggs in his nest; and (3) how patterns of mate choice and parental investment contribute to the reproductive success of each sex. Because male courtship and parental care behaviors change during a nesting cycle, a secondary objective was to indentify associated hormonal changes that potentially cause this change in behavior.

Female garibaldi showed a strong preference to spawn in nests with early stage eggs over empty nests or those with predominantly late-stage eggs. Within nests containing eggs in multiple stages of development, females always deposited their eggs among the youngest eggs in the nest. Male garibaldi exhibited behavioral tactics that would increase the mortality of eggs deposited in empty nests (first clutches) or in older broods (late clutches) and thus favor such female preference. These include: (1) cannibalism of single-clutch broods; (2) cannibalism of older eggs early in the brood-cycle; (3) cannibalism of younger eggs late in the brood-cycle; (4) increased attentiveness of larger broods; and (5) increased aggressiveness in defense of larger broods. These behaviors are consistent with the predictions of parental investment theory that males invest in current offspring in a way that maximizes the net (current plus future) benefits of paternal care.

Male garibaldi actively courted females when their nests were empty. However, courtship rates declined as males acquired eggs and as those eggs aged. This was concomitant with an increase in parental egg fanning. Levels of both testosterone and 11-ketotestosterone were positively related to levels of courtship activity and inversely related to male parental egg-fanning.

Reproductive Ecology and Endocrinology of the Garibaldi Damselfish, *Hypsypops rubicundus* (Pomacentridae)

by

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TABLE OF CONTENTS

LIST OF FIGURES

Chapter 2:

Chapter 3:

Chapter 4:

LIST OF TABLES

Chapter 1:

Chapter 2:

Chapter 4:

Reproductive Ecology and Endocrinology of the Garibaldi Damselfish, Hypsypops rubicundus (Pomacentridae)

GENERAL INTRODUCTION

The goal of basic animal behavior research is to understand the ultimate and proximate causes of behavior. In other words, how behavior contributes to the animal's lifetime reproductive success and how it is controlled by external stimuli and physiological processes. Although these two approaches to the study of behavior have been considered as separate disciplines (e.g., behavioral ecology and sociobiology versus neuroethology and behavioral endocrinology), there is increasing appreciation for the contribution that research at each level can make to our understanding of the other (e.g., Alcock 1988). For example, understanding how the animal's behavior enables it to adapt to its biological and physical environment provides insights into the mechanisms that control behavior, and understanding the mechanisms that control behavior provide insights into the constraints under which natural selection on behavior operates. Reproductive behaviors have probably received the most attention at both levels. This is likely due to the fact that reproductive behavior directly affects reproductive success and that reproduction often involves a large repretoire of complex behaviors that are easy to quantify but often difficult to explain.

Damselfishes (Family Pomacentridae) are ideal subjects for ultimate and proximate level studies on reproductive behavior, particularly under natural conditions. They are common in clear, shallow, waters of tropical and warm temperate seas and their reproductive behaviors are conspicuous and easy to quantify. In addition, because eggs are deposited on male defended nest sites, egg hatching success (an important component of reproductive success) can be easily quantified and hence related to behavioral strategies. The goal of my dissertation research was to examine two aspects of the reproductive behavior of the garibaldi damselfish (Hypsypops rubicundus). My primary objective was to determine the relationship among male parental investment strategies, female mate choice, and egg hatching success. This led to a secondary objective, to determine the relationship between male reproductive behavior and hormones thought to control those behaviors.

I Male Parental Investment and Female Mate Choice

Sexually reproducing organisms are faced with multiple, often conflicting, demands each of which influences their lifetime reproductive success. These include securing food and other resources essential for growth and survival (e.g., shelter), avoiding predators and disease, and mating. Moreover, although each sex requires the other to reproduce, the determinants of reproductive success largely differ for each sex. This has led to the so-called conflict between the sexes (Trivers 1972, Alexander 1974, Emlen and Oring 1977). According to current views, natural selection should favor behaviors that maximize an individual's lifetime reproductive success in the face of the constraints imposed by conflicting demands, and the strategies adopted by the opposite sex (e.g., Williams 1966). One of the goals of behavioral ecology is to identify the specific nature and magnitude of these constraints and how they influence reproductive success, and then generate and test predictions about how members of each sex should respond behaviorally.

In many teleost fish species, only males care for the embryos (Breder and Rosen 1966, Blumer 1979). Although paternal care increases the survivorship of embryos (and hence contributes to one component of male reproductive success), parental care can have negative effects on other components of reproductive success. For example, parental care may compromise a male's physical condition through reduced access to food or may increase his chances of being eaten. It may also limit his ability to locate and mate with additional females (Gross and Sargent 1985, Sargent and Gross, 1986). Thus, the problem facing parental males is how much to invest in the care of present offspring. In contrast, although females are able to avoid the costs of care, they must entrust their entire investment in gametes to the care of the male and thus have no control over the welfare of their eggs once deposited in the nest. Consequently, females are faced with the problem of finding a spawning site that will maximize the hatching success of their eggs.

Assuming that each sex is selected to maximize its own lifetime reproductive success, and that males face a tradeoff between investment in the care of current offspring and future reproductive opportunities, Rohwer (1978) generated a series of predictions about how members of each sex should behave in order to maximize the _net_ benefits they receive from paternal care. Rohwer's original ideas have been subsequently refined by other authors and incorporated into the broader context of life history and parental investment theory (Dominey and Blumer 1984, Sargent and Gross 1986, Petersen and Marchetti 1989, Sargent 1992). The predictions of these studies and the principles on which they are based are reviewed in detail in chapters 1-3. Briefly, these studies conclude that the size and age of the brood tended by the male should determine the net benefits of care to males. Thus, brood size and age should influence male investment and consequently egg survivorship and female choice. Rohwer's original analysis also emphasized the reciprocal role of female choice on male parental investment, arguing that the effects of eggs on female choice should in turn influence how brood parameters influence male parental investment.

Empirically, many studies have examined the factors that influence female choice, egg hatching success, and parental investment in fishes with paternal care. Some of these studies have examined the role of brood size and age. However, few studies have examined the relationship among these factors as predicted by Rohwer and others, and to my knowledge, none have examined each component experimentally under natural conditions.

In this study, I used a combination of field observations and experiments to test predictions derived from their analyses. In chapter 1, I examine the influence of the presence and developmental stage of eggs in the male's nest on female spawning site choice in garibaldi. In chapter 2, I describe a novel mechanism by which male cannibalism of older offspring increases the reproductive success of the cannibal, and provide evidence that such behavior is an adaptive response to female preference to spawn among other early-stage eggs. In chapter 3, I examine alternative means by which male parental investment strategies could differentially affect egg hatching success and thus favor female preference for nests with early-stage eggs.

II Hormones and Brood Cycling Behavior

In many paternal-caring fishes, males undergo cyclic or sequential changes in reproductive behavior during the spawning season known as "brood cycles" (van Iersal 1953). Brood cycles consist of a so-called "mating" phase during which males actively court females, followed by a "parental" phase during which males care for offspring but are less receptive and more aggressive toward females. The decline in male receptivity is concomitant with an increase in brood size and age and is thought to be adaptive for two reasons. First, it limits the decline in male physical condition that can result from extended mating and parental care. Second, it limits the access of females to the nest at a time when they are unlikely to spawn but may eat eggs already in the nest (Rohwer 1978, Sargent 1990).

The proximate cause of male brood cycling behavior has received considerable attention. In particular, behavioral endocrinologists have been interested in the hormonal control of the associated reproductive behaviors and how the release of these hormones are triggered by environmental stimuli (Brown 1985). Most studies on hormonal regulation of male reproductive behavior in brood cycling species have been conducted in the laboratory on a few freshwater species, and have involved gonadectomy and hormone replacement. These studies have clearly demonstrated that gonadal androgens can regulate some reproductive behaviors (Liley and Stacey 1983, Stacey and Liley, 1987). However, these studies have several limitations. First, it is not clear what role androgens actually play under natural conditions or precisely what behaviors they control. Second, laboratory conditions often introduce stress effects that inhibit endocrine function and lack appropriate stimuli that may control hormone secretion. Finally, because so few species have been examined, it is unclear to what extent results can be generalized (Liley et al. 1987, Stacey and Liley 1987, Pankhurst 1990). Ideally, laboratory studies should be combined with correlative and experimental field studies that involve measurement of hormone levels under natural conditions.

Over the past decade, there has been an increasing emphasis on the use of field studies to understand the relationship among external stimuli, physiological response, and reproductive behavior (Wingfield and Moore 1987, Wingfield et al. 1990). Because male garibaldi exhibit brood cycling behavior and can be easily observed under natural conditions, I sought to determine the relationship between changes in male reproductive behavior and hormones during the course of the brood cycle. In chapter 4, I describe the changes in male nest tending, courtship, and parental behavior during the brood cycle and how these correlate with changes in plasma levels of two androgen hormones: testosterone and 11-ketotestosterone. These androgens are believed to be involved in the control of reproductive behavior in teleost fishes (Liley and Stacey 1983, Stacey and Liley 1987).

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CHAPTER 1

Egg presence and developmental stage influence spawning-site choice by female garibaldi

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Running Head: Sikkel: Egg presence and female choice

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Abstract. During the spawning season, male garibaldi, *Hypsypops rubicundus* (Pomacentridae), defend algal nests within permanent territories on which females deposit eggs. Eggs in the early stages of development are bright yellow in coloration and turn to grey as development proceeds. Field observations indicated that females preferentially approached nests with eggs in the early stages of development prior to or in the absence of male courtship and were more likely to spawn in such nests than in empty nests or nests with only eggs in the advanced stages of development. Field experiments involving pairwise manipulations of nests with eggs revealed that, between nests, those that retained eggs in the early stages of development were significantly

more likely to receive new eggs before nests that were completely stripped of such eggs or retained only eggs in the advanced stages of development. Within nests containing eggs in both the early and advanced stages of development, females deposited eggs only among other early-stage eggs. These results indicate that the presence of early-stage eggs is at least one factor influencing the observed choice pattern. Female preference to deposit eggs among other eggs in the early stages of development is predicted by theoretical and empirical studies on male care strategies and the sources of egg mortality among fishes with paternal egg-care.

Female choice of mates based on male traits or resources accrued by males is common in many animals and is the subject of much recent theoretical and empirical research (reviewed by Halliday 1983; and Partridge & Halliday 1984). Where choice is possible, females can be expected to show preference for features which are predictive of potential fitness gains (Trivers 1972). The number of females with which a male has recently mated and/or the number and condition of progeny in his charge are factors that may influence how a female chooses her mate (Orians 1969; Pleszczynska 1978; Ridley 1978; Rohwer 1978; Nakatsuru & Kramer 1982; Bradbury & Gibson 1983; Losey et al. 1986). How these factors influence choice should of course depend on system-specific costs and benefits.

The mating system of many fish species is characterized by polygyny and paternal egg-care (Blumer 1979; Perrone & Zaret 1979; Gross & Sargent 1985). Parental care is often primarily restricted to fanning and defence of a centralized brood. This, combined with the extremely small size of offspring relative to the parent, allow males to care for a large number of offspring as easily as a few (Williams 1975; Wittenberger 1981). Thus, the cost of decreased care received by each offspring that may accompany larger broods in other parental care systems (e.g. altricial birds) are greatly reduced or absent. Several benefits have been proposed that should favor female choice for nests already containing eggs in fishes with paternal egg-care. These include: (1) a decrease in per capita egg loss from predation (Rohwer 1978; Ridley & Rechten 1981); (2) an increase in male parental investment with increasing brood size (Coleman et al. 1985); and (3) heritable male attractiveness that may be signalled by egg presence (Ridley 1978). However, the benefits of depositing eggs among other eggs may also be dependent upon the developmental stages of eggs already in the nest. For example, a diluting affect would be maximized by depositing eggs among other eggs in the early stages of development by prolonging the time eggs remain among other eggs. Rohwer (1978) argued that nesting males should be more likely to cannibalize the last clutches of a brood as an investment in future broods. Thus, females may prefer to deposit eggs among other early-stage eggs over eggs in more advanced stages of development.

Numerous observations in the field and laboratory suggest that females of polygynous fish species with paternal egg-care are attracted to males whose nests already contain eggs or spawning females (e.g., Hunter 1963; Barlow 1967; Cummings 1968; Fricke 1980; DeMartini 1987). Under laboratory conditions, Ridley & Rechten

(1981) and Marconato & Bisazza (1986) have provided experimental evidence that the presence of eggs has a positive influence on female choice in threespined sticklebacks (*Gasterosteus aculeatus*, Gasterosteidae) and river bullheads (*Cottus gobio*, Cottidae), respectively. Field experiments have demonstrated preference for nests with spawning females in the carmine triplefin (*Axoclinus carminalis*, Tripterygiidae; C. W. Petersen, personal communication), as well as preference for nests with eggs in the peacock wrasse (*Symphodus tinca*, Labridae; R. R. Warner and E. P. van den Berge personal communication). However, no studies have systematically examined the importance of egg developmental stage on female choice.

Field observations suggest that female garibaldi, *Hypsypops rubicundus* (Pomacentridae), prefer to spawn with males whose nests contain eggs in the early stages of development over nests with only advanced-stage or no eggs (Sikkel 1988). However, these observations alone are not sufficient to determine whether eggs per se play any role (either direct or indirect) in this choice. Here, I present detailed field observations supplemented by field experiments which indicate that both egg presence and developmental stage play an important role in spawning-site choice by female garibaldi. I discuss these results in terms of previously hypothesized benefits and offer additional hypotheses for the adaptive significance of this choice pattern. I conclude by relating female choice to the bright coloration of early-stage eggs in this species.

SUMMARY OF BREEDING BIOLOGY

The garibaldi is a large (up to 36 cm total length) pomacentrid common to southern and Baja California rocky subtidal areas (Limbaugh 1964; Clarke 1970; Sikkel 1988). In the population I studied, both sexes defend year-round territories of approximately 10 - 15 m^2. During the spring-summer spawning season, some males promote the growth of a nest of red algae on an open rock face within the territory (Limbaugh 1964; Clarke 1970; Foster 1972). The same nest site is used repeatedly for as long as the male occupies the territory. Thirty-eight reproductive males measured during this study ranged in size from 16.7 to 21.5 cm standard length ($\bar{X}$ = 9.1, *SD* = 1.2), and occupied nests of 314 to 1770 cm^2 ($\bar{X}$ = 660.2, *SD* = 310.6). Gravid females leave their territories sporadically, displaying erect fins and performing erratic swimming behaviour (Sikkel 1988). This behaviour is hereafter referred to as 'searching behaviour'. Such females are courted by receptive males, and either ignore the males or approach their nests. Females will approach nests in the absence of male courtship, but will usually not enter tended nests unless the male performs a courtship act.

Searching behaviour continues until a nest is selected for spawning, which occurs any time between sunrise and sunset. Both sexes are promiscuous. I have observed some females spawning with different males as often as every other day, and males spawning with as many as five females over a period of several hours.

When first deposited, the elliptical eggs are bright yellow and turn grey after approximately 5 days. Embryos hatch and then disperse as planktonic larvae after approximately 12 to 23 days. I have observed males tending a given brood for as long as 29 days, and as many as four broods per spawning season.

METHODS

Study Area

This study was conducted between 11 May and 30 July, 1987 in Big Fisherman Cove, Santa Catalina Island, California (latitude 33 28'N, longitude 118 29'W). A total of 71 nesting males occupied territories along the rocky perimeter of the cove between the surface and 10 m depth. Five sub-areas were defined within the cove, each separated by 30-100 m of low relief substrate or dense kelp (Macrocystis) where no nests were located.

One area, supporting 37 nesting males was used as the observation site. The other four, supporting 5-11 males each, were used as experimental sites.

Observations

I observed females to determine (1) whether they preferentially approached nests with early-stage eggs, independent of attraction by male courtship; and (2) whether they were more likely to spawn in such nests tended by a receptive male. I surveyed the nests at the observation site once or twice daily by free diving. During surveys, I recorded the presence or absence of eggs, their coloration, and the area they occupied in the nest. I measured nest areas approximately every 14 days (see Sikkel 1988 for methods).

On days in which vertical underwater visibility exceeded 9 m (when the risk of losing a focal animal from sight was minimal), I followed females exhibiting searching behaviour (detectable by their erect fins and erratic swimming) until they either spawned or were lost from sight. During any given sampling period, I followed the first such female encountered, and recorded her behaviour continuously. I also recorded responses of males to passing females. I conducted a total of 173 observations of females which included 124 spawns. Observation times ranged from 1 to 186 min ($\underline{\bar{X}}$ = 32.2, <u>SD</u>

= 37.2). At least 52 different females, identified on the basis of tags, scars, and territory locations were involved.

Analysis of observational data

To compare approach probabilities with nest state, I compared the number of times females directly approached nests containing no eggs, yellow eggs only, yellow and grey eggs, and grey eggs only, prior to or in the absence of courtship, to the number of times expected on the basis of the availability of nests in each state. I estimated nest availability by tallying the number of nests available at the observation site in each state for days on which females were followed. I analysed the data using a G goodness-of-fit test. It should be noted that this analysis only controls for the effects of current male courtship, since a female may have been courted previously by a given male. To compare spawning probabilities with nest state, I used only those cases in which (1) the female was observed to spawn (was not lost) during the observation period; (2) the female approached within 1 m of a receptive male (where eggs were judged to be easily visible to the female) and was not chased (unless she attempted to bite eggs); and (3) sufficient space was available for egg deposition. I estimated the amount of space necessary for egg-deposition from new clutches deposited in empty nests. These clutches occupied an area of from 28.6 to 186.8 cm^2 ($\bar{X}$ = 112.5, SD = 43.3, N = 47). I then performed two analyses using G contingency tests, one for nests having a minimum of 50 cm^2 of available nest space and one for nests having a minimum of 200 cm^2. These space limits approximate the minimum and maximum amount of space required by females depositing eggs in empty nests. These estimates are likely high since females spawning in nests with eggs deposit their eggs in denser clutches among existing eggs, independent of available nest space, and thus use less space (Sikkel 1988, this study).

Experiments

Yellow eggs versus no eggs

If the presence of yellow eggs does not influence female spawning-site choice, then egg presence or absence should not influence the amount of time taken to acquire additional eggs among suitable males or nests. To test this null hypothesis, I performed the following field experiment. I located all nests with only yellow eggs in each of the four experimental sites. When a pair of such nests was found at any site, one nest was selected to have all eggs removed (complete removal) while a circular patch of eggs of approximately 15 cm in diameter was retained in the other (see below for protocol). The patch measuring 15 cm in diameter provided an image easily visible to the observer (and thus presumably to female garibaldi) while providing sufficient space for the deposition of new eggs. The uniformity of this patch facilitated the detection of any additional eggs added by females following the manipulation. Experimental pairs of nests were separated by 3 to 30 m^2 ($\underline{\bar{X}} = 14.1$, $\underline{SD} = 9.8$). Manipulations were performed before 0900 or after 1700 hours. With another scuba diver, I simultaneously removed eggs from each nest using a mascara comb. Since complete removals required more time than partial removals, the diver performing the partial removal combed the nest for the excess time in order to control for any secondary disturbances caused by the manipulation. There was no visible indication of damage to nests as a result of these manipulations.

In assigning treatments, three measures were taken to control for the effects of other factors that may influence female choice (e.g. nest quality, nest size, male size). First, pairs used in manipulations were in close proximity to one-another, and received their first eggs within several days of one-another. Thus, both nests (males) should be similar with respect to those factors that influence female choice among empty nests (Sikkel 1988). Second, I randomized treatment assignments (by coin toss) unless one of the members of a pair had received only one treatment, or a treatment reversal could be performed (see below). In the latter case, the order of treatments was randomized. Finally, 19 of the 24 nests used received both treatments (Table I.1). These included eight cases in which previously used pairs were used again, reversing the treatments the second time ($\underline{N}$ = 16 nests and 16 total pairwise manipulations). These treatment reversals provided the most effective means of controlling for other factors. In total, I performed 27 pairwise manipulations using 24 different nests and males. Although some nests were used more than once, no pairwise treatment combination was used more than once.

Following egg removal, I remained near the nest until planktivores feeding on dislodged eggs had dispersed and nesting males resumed normal behaviour (e.g. feeding, nest fanning, courting). For morning manipulations, I checked nests beginning 1 h after egg removal for the presence of eggs and to verify that both males were still courting females and performing normal nest tending behaviour. For evening manipulations, I began such checks the following morning. I considered the first nest of a given pair that received additional eggs to be the preferred nest. I analysed the data using a *G* goodness-of-fit test assuming equal receptivity for males tending partially and completely stripped nests.

Although it would have been ideal to also perform egg addition experiments, my attempts to add real or artificial eggs to empty nests resulted in unsuccesful attachment, or their being eaten or removed, respectively, by the resident male.

Yellow eggs versus grey eggs

Between-nest manipulations. To determine whether egg developmental stage influences female choice, I conducted two additional experiments. The first (between nests) was conducted in a manner identical to the previously described experiment except that a 15 cm diameter patch of eggs was left in both yellow and grey-egg nests. Experimental pairs were separated by 2 to 30 m^2 ($\underline{\bar{X}}$ = 13.0, *SD* = 8.9). I performed a total of 18 pairwise manipulations involving 26 nesting males (Table I.1). In four cases each nest contained both yellow and grey eggs. One in each pair was stripped of all yellow and the other stripped of all grey eggs. In the remaining 14 cases, nests contained only yellow or only grey eggs prior to partial stripping. Although seven nests received both treatments, I did not reverse treatments on any of the pairs.

To increase the number of available grey-egg nests with sufficient spawning space, I also removed eggs from an additional 32 nests containing grey eggs and monitored them for the acquisition of additional eggs. These manipulations were not paired with removals from nests with yellow eggs.

Analysis. I analysed data from the yellow-grey between-nest manipulations using a binomial test. However, unlike the previous experiment, it was not reasonable to assume equal receptivity for males tending only yellow and only grey eggs. Previous observations indicated that males tending only grey eggs were less receptive to females than males tending only yellow eggs. These observations were supported by the observations of females conducted during this study. Males tending only grey eggs were significantly less likely than males tending only yellow eggs to perform courtship displays in the presence of females exhibiting searching behaviour ($G = 79.4$, $P < 0.001$) and were significantly more likely to chase those females that approached close to or entered the nest following courtship displays ($G = 29.4$, $P < 0.001$). A similar pattern has been reported for a variety of other temperate fishes (references cited in DeMartini 1987). Rohwer (1978) argued that such behaviour is an expected consequence of female reluctance to spawn in nests with advanced-stage eggs. It was therefore necessary to incorporate this descrepency in formulating a null hypothesis. I used two methods, using data obtained during observations of females, to estimate relative receptivity. First, for each of those males ($\underline{N}$ = 19) for which I had recorded at least five cases of searching females passing the nest under both yellow-only and grey-only conditions, I multiplied the proportion of times the male performed courtship displays in the presence of females exhibiting searching behaviour by the proportion of times he accepted (did not chase) those females that approached close to or entered the nest. This can be stated mathematically as: $\underline{R} = \underline{ca}$, where $\underline{R}$ = receptivity, $\underline{c}$ = the probability of courting (reflecting the efforts by males to attract females to the nest), and $\underline{a}$ = the probability of accepting (reflecting the proportion of times attracted females were actually allowed to sample the nest). I calculated two $\underline{R}$ values for each male, one for yellow eggs only ($\underline{Ry}$), and one for grey eggs only ($\underline{Rg}$). I then combined the ratios $\underline{Ry/Rg}$, calculated for each male to compute a mean receptivity ratio. The second method for calculating receptivity used a composite of all observations recorded for 41 males under yellow-only and grey-only conditions to compute a single _Ry_ and _Rg_ value. These two methods produced results of males tending only yellow eggs being 3.5 (range = 1.3 - 14.3, _SE_ = 0.70) and 2.3 times as receptive, respectively, as males tending only grey eggs. The larger value for the former estimate was largely influenced by one male that was 14.3 times more receptive when tending yellow eggs. The next highest value was 6.6.

In analysing these data, I attempted to offset this difference in receptivity by allowing nests with grey eggs 5 days from the time of manipulation to acquire more eggs compared to 1 day for those with yellow eggs. Five days was selected since this was the fewest number of days that elapsed until the onset of hatching for any of the manipulated grey-egg nests. The null hypothesis can thus be stated explicitly as follows. If females do not prefer nests with yellow eggs over nests with grey eggs, then the probability of a grey-egg nest receiving eggs within 5 days of manipulation is equal to the probability of a yellow-egg nest receiving eggs within 1 day.

Within-nest manipulations. If females prefer to spawn among yellow eggs rather than grey eggs, then within nests containing both, new eggs should be more likely to be deposited among yellow eggs. This assumes that females are able to select the spawning site within the nest. Observations of spawning females suggest that this is the case. To test this hypothesis, I used 25 full nests, each containing approximately equal amounts of yellow and grey eggs. From each nest, I removed equal areas of eggs, approximately 10 cm in diameter, from both yellow and grey-egg areas. In 16 of these nests I also removed eggs from the yellow-grey interface. After egg removal, I monitored nests until new eggs appeared or all eggs turned grey, and noted the position of any new eggs.

RESULTS

Observations

Females were significantly more likely to approach nests prior to or in the absence of male courtship if the nest contained yellow eggs ($G = 84.6$, $P < 0.001$; Table I.2). The difference between nests containing yellow only and yellow and grey eggs was not significant ($G = 0.67$, $P > 0.25$). Moreover, approaches to nests of receptive males were more likely to result in spawning if the nest contained yellow eggs (Table I.3). This was true for both 50-cm^2 ($G = 118.3$, $P < 0.001$) and 200-cm^2 ($G = 93.6$, $P < 0.001$) space limits (the difference between yellow and yellow/grey for the 50-cm^2 space limit was not significant [$G = 0.20$, $P > 0.50$]).

Three other lines of evidence are consistent with these results. First, in 102 of 116 cases in which yellow eggs appeared in nests (observation and experimental nests combined), the nests received additional eggs while yellow eggs were present. Nests continued to receive eggs as long as nest space adjoining yellow eggs was avilable (up to 14 days in large nests). In contrast, of 81 nests with only grey eggs, only two received eggs while the grey eggs remained. In both cases, nearly all eggs from the previous brood had hatched and the new eggs were deposited at the opposite end of the nest. Furthermore, these two nests were among the few remaining at the end of the spawning season. Second, females spawning in nests with yellow only or both yellow and grey eggs invariably deposited their eggs among the pre-existing yellow eggs. This was independent of how much space remained in the nest. Finally, in the only three cases in which focal-sampled females were observed selecting empty nests, they made previous attempts to approach nests with yellow eggs and were either ignored or chased by the resident males. A similar incident occurred during the 1986 spawning season (Sikkel 1988).

Experiments

Yellow versus empty

Combining all 27 pairwise manipulations, the partially stripped nest received eggs first in 24 cases. The completely stripped nest received eggs first in two cases, and in one case a tie occurred. This difference was highly significant ($\underline{G} = 21.94$, $\underline{P} < 0.001$, tie excluded; Table I.4). All partially stripped nests received new eggs within 1 day of egg removal. Although completely stripped nests received new eggs in 18 of 27 cases, only five did so within 1 day. Among the 19 nests that received both treatments, nests received eggs first when partially stripped in 21 of 23 cases. These same nests never received eggs first when completely stripped (22 cases). Of the 16 cases in which eight pairs were used twice, reversing the treatments the second time, the partially stripped nest received eggs first in 15 cases. The other case represented the tie. This result alone was highly significant ($\underline{P} < 0.001$, binomial test; tie excluded).

Overall, these results do not appear to be confounded by the effects of other factors that influence female choice. The amount of time elapsed until the acquisition of the first clutch of the initial brood, a measure of male/nest attractiveness independent of egg presence, was lower for the completely stripped nest in 13 cases, and the partially stripped nest in 11 cases. Data were not available for the remaining three cases.

Yellow versus grey

Between-nest manipulations. Of the 18 pairwise manipulations, no nests with grey eggs received eggs within 5 days, whereas all nests with yellow eggs received eggs within 1 day ($\underline{P} < 0.001$, binomial test, Table I.4). In fact, of a total of 50 partially stripped grey-egg nests, only one received new eggs while the grey eggs remained. Among the 14 pairs for which data were available, the nests receiving the first clutch of the initial brood were equally divided (7:7) between the two treatment groups.

Within-nest manipulations. Of the 25 spaces created within the yellow-egg portion of 25 nests with both yellow and grey eggs, 15 received new eggs. Three of these nests had also received eggs on the yellow portion of the yellow-grey interface. In contrast, eggs never appeared within the spaces created in the grey-egg portion (Table I.5). This difference was highly significant ($\underline{P} < 0.001$, binomial test comparing yellow and grey).

DISCUSSION

An earlier correlative study (Sikkel 1988) identified the presence of yellow eggs as one of several factors that may influence female choice of spawning site in garibaldi. Although their relative importance cannot be ascertained from this study, the more detailed observations and field experiments presented here indicate that eggs per se do play a role independent of other factors.

In cases where paternal care of offspring is indivisible (sensu Wittenberger 1981) the benefit of care received by each offspring may decrease with increasing offspring number. In nest-tending fishes, paternal care is largely divisible, and thus this cost is greatly reduced. Depositing eggs among those of other females would confer the advantage of reduced predation risk per egg through a diluting effect (Rohwer 1978; Ridley & Rechten 1981), provided predation pressure does not increase proportionally. Numerous studies have shown that eggs of substrate-spawning fish species are subject to predation by conspecific males and females (reviewed by Dominey & Blumer 1984) as well as a variety of heterospecific predators (e.g. Gross & MacMillan 1981; Noltie & Keenleyside 1986). In addition to a per capita reduction in predation risk, spawning in nests with eggs would also be beneficial if male defence of the brood increased with the number of eggs in the nest, thereby decreasing the frequency of predation events (Pressley 1981; Coleman et al. 1985). Several studies have shown that males may consume or abandon broods below a certain minimum size (Barlow 1964; Dominey & Blumer 1984; Ochi 1985; C.W. Petersen personal communication). DeMartini (1987) found a correlation between the number of clutches tended by male painted greenling, <u>Oxylebus pictus</u> (Hexagrammidae) and per capita hatching success.

Ridley (1978) argued that female choice of nests already containing eggs could be favoured by sexual selection. Females that chose males with eggs would be choosing males that have demonstrated their ability to attract other females; this would result in the production of 'attractive' sons. More generally, choosing a nest with eggs may be a means of copying the choice of other females that have selected the male on the basis of superior genetic quality (Bradbury & Gibson 1983; Losey et al. 1986).

Why females should prefer to spawn among early over advanced-stage eggs has been addressed by Rohwer (1978). He argued that males should consume the last remaining eggs of a brood as an investment in future broods. He predicted that females therefore should avoid spawning in nests with advanced-stage eggs since their eggs would be more likely to be consumed. A higher probability of mortality for eggs deposited late in a brood may also exist in the absence of filial cannibalism if male investment in brood care decreases with decreasing egg number, or male physical condition declines with increased time spent in nest-tending (e.g. Dominey & Blumer 1984; Sargent & Gross 1985; DeMartini 1987). Both of these arguments would seem most applicable to those species in which males must remain at the nest for prolonged periods of time and in which an upper limit exists to the number of eggs a male can accomodate. Additionally, any diluting effect would be prolonged if eggs were deposited among others of a similar developmental stage since they would remain among these eggs longer than they would among more advanced-stage eggs. Downhower & Brown (1981) and DeMartini (1987) have both provided evidence that eggs deposited late in a brood suffer greater mortality than those deposited earlier. A diluting effect, among other benefits of aggregating (reviewed by Bertram 1978 and Pulliam & Caraco 1984), could also be extended to post-hatching stages of development. These benefits would likewise increase with increased similarity in developmental stage among embryos, particularly if departure from the natal area is synchronized among members of a cohort.

At least some of the arguments presented above would appear to explain female preference for nests with eggs over empty nests in garibaldi. In more than 10% of all approaches to nests with eggs, the female bit or attempted to bite the eggs. I have also observed several microcarnivorous fish species eating eggs. Although I have not observed nesting male garibaldi eating eggs, in 13 of 14 cases in which no eggs were added to single clutches, all eggs disappeared within 5 days. Disappearance of lone clutches was also observed during the 1986 spawning season (Sikkel 1988). Thus, a diluting effect or the risk of loss to a lone clutch (due to male neglect or cannibalism) may favour female preference for nests with early-stage eggs over empty nests in this system. An additional advantage may be that eggs are less likely to be dislodged by vigorous male fanning (see below) or wave surge if they are packed closely together in large batches.

The extent to which these arguments explain female preference to spawn among early-stage over advanced-stage eggs in garibaldi is less clear. In one of the two cases in which fresh eggs were deposited in a nest containing only grey eggs, the fresh eggs disappeared within 3 days. Male garibaldi change from vigorous fanning with the entire lower portion of the body when only yellow eggs are present, to using only the pectoral fins when all eggs are grey (personal observation). Although the reason for this change is not clear, new eggs deposited among grey eggs may receive inadequate care. Furthermore, yellow eggs deposited among grey eggs may suffer disproportionately high predation since they would contrast more with the surrounding nest. I have no data on male cannibalism or neglect of eggs deposited late during the brood cycle. Depositing eggs among yellow eggs would clearly result in a greater amount of time spent among other eggs than would depositing eggs among grey eggs. It would also result in a larger cohort of larvae entering the water column upon hatching, if hatching was sufficiently synchronized. Unfortunately, the timing of hatching is not known for garibaldi.

Although eggs are clearly visible in the nest, it cannot be unequivocally determined from these data whether they represent the actual cue used by females in selecting nests with early- stage eggs. This is because receptive males may produce signals which vary with nest state and which may influence an approaching female's decision to spawn (e.g. sounds, pheromones). Although females preferentially approached nests with early-stage eggs, prior to or in the absence of a male courtship act, it is not clear whether eggs per se were influential here. Females may be attracted by the eggs.

However, females may also approach certain nests because of recent, previous experience with those nests, independent of the presence of eggs. The preference by females to deposit eggs among other early-stage eggs does suggest however that eggs are at least one important que influencing the choice of an egg deposition-site within nests.

If depositing eggs among those of others is advantageous to female garibaldi, then the highly conspicuous coloration of new eggs may also be advantageous despite the fact that garibaldi nests are typically exposed. The advantage would arise by increasing the detectability of eggs in the nest to other females. This would be particularly important when the density of eggs is low (i.e. when a single clutch is present). In support of this conjecture, in nine cases in which females deposited eggs in nests having an exposed and a concealed portion, the first clutch of eggs was always deposited on the exposed portion where it was most easily seen by the observer.

In conclusion, the findings of this study are consistent with predictions of theoretical and empirical studies on male parental care strategies and the sources of egg mortality among paternal-caring fishes. Furthermore, they complement a growing body of literature which suggests that the presence of eggs already in the nests of parental males is of widespread importance in determining female spawning-site choice among such species. Future experimental work on this and other species should examine the importance of brood size and chronological order of clutch deposition, relative to other factors (i.e. male and nest-site traits), in influencing female choice and egg hatching success.

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Table I.1 The distribution of treatment assignments among nests (males).

Distribution of treatment assignments			
Yellow vs Empty (Y:E)		Yellow vs Grey (Y:G)	
Y:E	N (nests)	Y:G	N (nests)
1:1	8	1:1	5
2:2	3	2:1	1
2:1	2	3:1	1
1:2	2	1:0	8
3:2	1	0:1	11
1:0	4	Total	26
0:1	3		
0:2	1		
Total	24		

Example: Among the 24 nests used in yellow versus empty manipulations, eight nests received each treatment once, three received each treatment twice, two retained yellow eggs twice and were completely stripped once, etc.

Table I.2 The number of times females approached nests prior to or in the absence of male courtship, relative to the number expected on the basis of the availability of nests in each state

	Number of approaches to nest			
	Empty only	Yellow & Grey	Yellow only	Grey
Observed	18	66	20	30
Expected	63.5	29.6	11.0	29.9

Table I.3 The percentage of female approaches to nests of receptive males that resulted in spawning

Available nest space		Empty	Yellow only	Yellow & Grey	Grey only
> 50 cm^2	N (approaches)	172	228	36	46
	% Spawn	1.7	39.0	27.8	0
> 200 cm^2	N (approaches)	172	191	8	18
	% Spawn	1.7	37.2	37.5	0

Table I.4 Results of yellow versus empty* and yellow versus grey between-nest experiments

	Number of times new eggs received first	
	Yellow only	No eggs
Observed	24	2
Expected	13	13
	Yellow only	Grey only
Observed	18	0
Expected	9	9

* Tie excluded from yellow versus empty experiment

Table I.5 Results of the yellow-grey within-nest manipulations*

Distribution of spawns within manipulated nests

	Yellow	Yellow/Grey Interface	Grey
Observed	15	3	0
Expected	6.8	4.4	6.8

* Only the location of the first spawning following manipulation was included.

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CHAPTER 2

Filial cannibalism in a paternal-caring marine fish: The influence of egg developmental stage and position in the nest

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Running Head - Sikkel: Filial cannibalism in garibaldi

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Abstract - Female garibaldi damselfish (Hypsypops rubicundus) selectively spawn with males guarding early stage eggs. Within nests containing eggs in different stages of development, females always deposit their eggs near the youngest eggs. Thus, the position of eggs already in the nest can influence where and how many additional eggs will be deposited in the nest. In field observations and experiments, brood guarding males usually cannibalized the relatively older clutches of eggs during the mating phase if these eggs were exposed to empty nest space. Mating males rarely cannibalized the younger clutches or the older clutches that were surrounded by younger eggs. When exposed older eggs

were cannibalized, the nest space previously occupied by and surrounding the cannibalized eggs was covered with new eggs. When exposed older eggs were not cannibalized, the surrounding nest space remained empty. These data suggest that male filial cannibalism during the mating phase is an adaptive response to female spawning site preference; Selectively cannibalizing exposed older eggs minimizes the amount of empty nest space by ensuring that only younger, more attractive, eggs are exposed to empty nest space. These data provide an explanation for the gradient in the age of eggs observed in garibaldi nests, where the oldest eggs occur at one end of the nest and younger eggs accumulate toward the opposite end.

The killing or abandoning of offspring by parents is a well documented phenomenon among parental caring animals (Hausfater & Hrdy, 1984; Clutton-Brock, 1991). Adaptive explanations suggest that by sacrificing some or all of its current offspring the parent is better able to rear other current offspring or invest in future reproductive opportunities, resulting in a net increase in reproductive success (Rohwer 1978, Hrdy 1979, Dominey & Blumer 1984). Thus, whether or not a parent abandons care of a given offspring or group of offspring should depend on the effect rearing those offspring has on the parent's ability to rear other offspring (current or future) relative to the potential contribution those offspring can make to the parent's current reproductive success (the offsprings' reproductive value). The specific determinants of this tradeoff will vary among species, populations, and individuals. However, among the most important factors are the age and number of offspring, which are positively related to their reproductive value, and the parent's opportunity for future reproduction (Wickler and Seibt 1983; Taborsky 1985; Dominey & Blumer 1984; Mock & Parker 1986; Clutton-Brock 1991). Although this kind of adaptive explanation is receiving increasing acceptance among behavioral and evolutionary biologists, there are still few experimental data on the conditions under which parents kill or abandon care of offspring and how it benefits them.

The consumption by parents of their own viable offspring, or "filial cannibalism", is widespread among teleost fishes (e.g., Loiselle 1983; Salfert & Moodie 1985; De Martini 1987; Mrowka 1987; Hoelzer 1988; Petersen & Marchetti 1989; Lavery & Keenleyside 1990; Belles-Isles & Fitzgerald 1991). Because of the ease with which potentially important parameters (e.g., offspring number and age) can be manipulated, fishes offer convenient subjects for experimental studies on the conditions under which parents abandon or kill offspring and how such behavior contributes to their reproductive success.

In fish species with exclusive paternal care, males often simultaneously guard egg clutches that are in different stages of development. Because they are closer to hatching, the relatively older eggs should have higher reproductive value than the younger eggs, while younger eggs should have higher nutritional value (Pressley 1981; Sargent & Gross 1986; Fitzgerald 1991). Thus, it has been predicted that parents with mixed age broods should preferentially cannibalize the younger eggs in the brood (Dominey & Blumer 1984; Petersen & Marchetti 1989; Fitzgerald 1991). This prediction is based on the assumption that the primary benefit to the cannibalizing parent is a gain (or savings) of energy. Indeed, apparent preferential cannibalism of the younger eggs has been documented for some species (Petersen & Marchetti 1989; Petersen 1990). However, female choice can play a significant role in determining the costs and benefits of cannibalizing eggs of different developmental stages. In many paternal-caring fish species, females selectively spawn with males whose nests already contain eggs (Ridley & Rechten 1981; Marconato & Bisazza 1986; Unger & Sargent 1988; Gronell 1989; Knapp & Sargent 1989). As predicted by Rohwer (1978), in at least some species females are most likely to spawn with those males guarding newly deposited eggs and may avoid spawning with males guarding predominantly late stage eggs (DeMartini 1987; Petersen 1989; Petersen & Marchetti 1989; Sikkel 1989). Thus, eggs in the care of a parent represent not only a direct contribution to the parent's current reproductive success, but also contribute indirectly by increasing the parent's chances of receiving more eggs during the same brood cycle. This latter effect may be a decreasing function of egg developmental stage. Rohwer (1978) argued that in species in which females preferentially spawn with males guarding early stage eggs, males should cannibalize clutches of otherwise low reproductive value only after these eggs have reached an age where they no longer increase the attractiveness of the nest. Although his argument was concerned specifically with the abandonment of lone, small clutches, a similar argument could apply to mixed age broods. Cannibalism of early stage eggs could decrease the probability that a male would receive additional eggs, whereas cannibalism of older eggs would not. Of course, whether cannibalism of older eggs would result in a net reproductive advantage would depend on the number and age of eggs eaten, and the number of additional eggs a male could expect.

In the garibaldi damselfish, Hypsypops rubicundus, females selectively spawn with males whose nests contain predominantly early stage eggs over those with empty nests or those guarding predominantly late stage eggs. Moreover, when nest space is available next to eggs of different developmental stages, females always deposit their eggs among or adjacent to the youngest eggs in the nest, and will not deposit their eggs next to other eggs that are older than about 2 days (Sikkel, 1989 and unpub. data). Thus, the position of eggs already in the nest can influence where and how many additional eggs can be deposited (Fig. II.1). During a typical brood cycle, the oldest eggs occur at one end of the nest, and newer eggs accumulate toward the opposite end, such that the youngest eggs are always exposed to available nest space In this manner, males usually continue to receive eggs until the nest is filled to or near its capacity.

In this paper, I present data from field observations which suggest that parental males maintain this gradient in the age of eggs by selectively cannibalizing the oldest eggs in the brood early during the brood cycle. I hypothesize that such behavior is a brood management strategy which enables the male to maximize the number of eggs he receives during a brood cycle, and hence increase his reproductive success. I use these field observations, along with field experiments to test three predictions derived from this hypothesis. These predictions are: (1) Because older eggs have higher reproductive value, males should be more likely to consume older clutches of eggs when these eggs are in a position that could reduce the number of additional eggs the male can receive; (2) When males cannibalize the older clutches, new eggs should be deposited around the remaining eggs and should cover the area previously occupied by the cannibalized eggs. Males should then continue to receive additional eggs; and (3) Early during the mating phase, males should avoid cannibalizing the youngest clutches of eggs. I also provide further experimental evidence that nest space around older eggs that remain exposed in the nest will not receive eggs, and circumstantial evidence that increasing the cost of cannibalism decreases its likelihood.

Although developing eggs are more appropriately termed embryos, I will refer to them here as "eggs". I define a "clutch" of eggs as the cohort of eggs deposited in the nest on the same day, often by more than one female. A "brood" consists of all of the clutches in the nest.

STUDY SPECIES

The garibaldi is a large (up to 36 cm total length) damselfish (Family Pomacentridae) common in rocky subtidal areas of southern and Baja California (Limbaugh 1964; Clarke 1970; Sikkel 1988). Both sexes defend year-round territories, although some non-territorial "floaters" exist. In the population I studied, spawning typically occurs from May through July, although in some years low levels of spawning activity may also occur in late April and early August. During the spring-summer spawning season, some males promote the growth of a nest of red algae on an open rock face within the territory (Limbaugh 1964; Clarke 1970; Foster 1972). When ready to spawn, a female leaves her territory and visits numerous males before selecting one. Spawning occurs throughout the day, every day for the duration of the spawning season. When first deposited, eggs are bright yellow. As development proceeds, dark pigment occludes the yellow yolk sac, until after approximately 5 days the eggs appear gray. Just before hatching, eggs appear silver due to the presence of eye pigment. Hatching occurs after 12-23 days, depending on temperature. As noted above, females show a strong tendency to deposit their eggs in nests that contain early stage eggs, and always deposit their eggs among the earliest eggs in the nest. Thus, a receptive male may take weeks to receive an initial clutch, and some (5-10%) go an entire season without spawning. However, once a male receives an initial clutch of eggs, most (approximately 95%) spawn daily with numerous females over a period of about 5-10 days (up to 20 days in rare cases). This period of active courtship and spawning is referred to as the "mating phase" (e.g., van Iersal 1953) and is followed by a "brooding" phase during which males care for the eggs but do not spawn. Males care for the eggs until they hatch. Males do not care for the planktonic larvae. A complete brood cycle lasts about 20-30 days, and males may complete up to four brood cycles in a season. However, 35-55% of males that spawn complete only one, and 85-95% complete no more than two brood cycles in a season.

FIELD OBSERVATIONS

Methods

I conducted this study in Big Fisherman Cove, located on the west end of Santa Catalina Island, CA (33 28′ N, 118 29′ W). During the spawning seasons of 1987-1989, I monitored a total of 79 nests. I usually monitored nests once daily, although on rare occasion conditions prevented me from monitoring nests for up to two days. For each nest, I mapped the location of eggs on a scale drawing of the nest. In cases where eggs had disappeared, I mapped the resulting gap in the egg mass. Although I have observed males eating apparently viable eggs on at least 13 occasions, I could not unequivocally determine the cause of egg loss in most cases. Thus, I assumed filial cannibalism to be the cause of egg loss in cases in which an area of at least 40 cm^2 had disappeared from a clutch between succesive surveys (usually 1 day). This area is equal to at least 20 garibaldi bites (a single garibaldi bite leaves a gap of 1.5 - 2.0 cm^2). I feel confident that such losses were the result of filial cannibalism for two reasons. First, the perimeter of these gaps had "bite marks", similar in size and shape to a garibaldi mouth. Although conspecific females also eat eggs, I have never observed them taking more than three bites before being chased by the male. In over 2,000 hrs of observation, I have never observed groups of females or neighboring male garibaldi attacking nests. Second, the major heterospecific egg predators are small-mouthed species such as gobies and wrasses that can remove only a few eggs per bite, and do not "raid" nests in groups (in contrast to many tropical species). Such predators would have to remain in the nest for a considerable length of time to remove an area of 40 cm^2. Males are seldom out of sight of the nest for more than a few seconds and violently defend their broods against such egg predators.

Results

Using the above criterium, there were a total of 68 incidents of apparent filial cannibalism in mixed age broods. These are summarized in Tables II.1 and II.2. Most egg loss occurred early during the mating phase. Fifty four of the 68 cases (79.4%) occurred during the mating phase, and 45 of these 54 cases occurred when the nest was less than half full. The remaining 14 of the 68 cases occurred early during the brooding phase. In all 68 cases, egg loss occurred from the perimeter (end) of the brood. During the mating phase, 52 of 54 losses (96.3%) occurred at the oldest end of the brood, while during the brooding phase 11 of 14 (78.6%) losses occurred at the youngest end (Table II.1).

Loss of older eggs during the mating phase was related to their position in the nest relative to empty nest space (Table II.2). In 45 of the 52 cases (86.5%), the cannibalized older eggs were exposed to empty nest space. The mean age of these eggs was 3.70 days (SE = 0.04, range = 1-8). In contrast, there were only seven cases in which older eggs were lost when they were surrounded by younger eggs. The mean age of these eggs was 4.67 days (SE = 0.63, range = 1-8). The disproportional loss of older, exposed eggs was not due to the activities of a few males or the disproportional occurrence of exposed, older eggs during the brood cycle. There were a total of 25 different brood cycles involving 21 different males in which older eggs were exposed during the brood cycle. In 22 of these 25 brood cycles (88%) the older eggs were lost. Eighteen males lost older eggs during each of the 22 brood cycles (1-3 per male) in which older eggs were exposed, while the remaining three males did not lose eggs during the three (one per male) brood cycles in which their older eggs were exposed. In 8 brood cycles, loss occurred 2-10 times during the brood cycle, while in the remaining 14 brood cycles, loss occurred only once. In comparison, there were 149 brood cycles involving 84 males in which the older eggs were not exposed. Of these, the older eggs were lost during only seven (4.7%) brood cycles, involving five males. These five males lost older eggs in only 17% (1 of 6) to 66% (2 of 3) of the 1-6 brood cycles per male in which their older eggs were not exposed.

The most revealing comparison is for the subset of males (n = 20) that had brood cycles in which older eggs were exposed as well as brood cycles in which older eggs were not exposed. Seventeen of these 20 males lost older eggs during each of the 21 brood cycles in which older eggs were exposed, while three did not lose older eggs during any of the three brood cycles in which their older eggs were exposed. Only one of these males lost older eggs during one of the 49 brood cycles in which older eggs were not exposed. Thus, for these males, the proportion of brood cycles in which older eggs were lost was significantly higher when older eggs were exposed than when they were not exposed ($p < 0.001$, sign test).

Males that cannibalized older eggs continued to receive additional eggs. When the cannibalized eggs were exposed, new eggs eventually covered the space previously occupied by the cannibalized eggs. However, in three of the seven brood cycles in which cannibalized eggs were not exposed, the space remained empty. In the three cases in which exposed, older eggs were not cannibalized, no eggs were deposited around the older eggs.

FIELD EXPERIMENTS

Methods

Experiment 1

The above observations suggest that early in the brood cycle, cannibalism of the oldest eggs in the brood is dependent on their position relative to younger eggs and available nest space. In particular, males cannibalize the older eggs when these eggs are not surrounded by younger eggs, and thus are exposed to empty nest space. In 1989, I conducted a field experiment to determine the effects of clutch position on the consumption of older eggs. I assigned 25 males with comparably sized nests to one of two treatments: 1) pigmented (4-5 d old) eggs exposed to available nest space; and 2) pigmented eggs surrounded by younger eggs (control) (Fig II.3). To assign treatments, I divided these males into four groups based on male size and location. I randomly assigned treatments to the first males in each group to receive eggs, such that in two groups the first males received the exposed treatment while in the other two the first males received the control treatment. Thereafter, I alternated treatments within each group. I used four of the 25 experimental males twice, once in each treatment.

To create each of these treatments, on the second day of the brood cycle, I selected an approximately 80 cm^2 circular or elliptical area of eggs to serve as the "target" clutch. I then selectively removed eggs from around the target clutch, leaving a "halo" of low egg density. For the next three days, I removed excess eggs and thinned the area around the target clutch. This insured that the target clutch was continually surrounded by new eggs. On the sixth day of the cycle, I selectively removed surrounding eggs, leaving an approximately 80 cm^2 area of < 2d old eggs adjacent to (exposed treatment) or surrounding (control) the target clutch. There was no significant difference between exposed and control groups in the size of the original brood or the area of eggs removed (and hence added) per day during the five days of manipulation ($p = 0.20$ and 0.49 respectively, t-tests). After the final manipulation, I monitored each nest for the occurrence of cannibalism. I also monitored 13 of the 16 exposed treatment nests and 10 of the 13 control nests for subsequent egg acquisition.

Experiment 2

To further verify that by failing to cannibalize exposed older eggs the nest space around these eggs would remain empty, I conducted an additional, within-nest experiment in 1990. Doing this required that males not cannibalize the older eggs (see results below). Preliminary manipulations indicated that males would not cannibalize the older eggs if the brood size was large enough. I created this condition by allowing nine males to acquire eggs in the normal manner over a period of six days. On the sixth day, I removed eggs from both the oldest and youngest end of the brood, creating an approximatley 400 cm^2 brood with approximately equal areas of empty nest space on each end (Fig. II.3). Thus, the brood was positioned approximately in the center of the nest and consisted of a gradient of 5 d old eggs at one end, and 0 d old eggs at the other end. The number of eggs removed from the old and young end to create empty space was not identical in all cases. However, I controlled for any possible differences in disturbance by combing over an equal area of eggs for a similar amount of time at each end.

Results

Experiment 1

In 13 of the 16 cases in which the target clutch was exposed, at least 75% of the clutch disappeared. In contrast, there was no noticeable loss to the target clutch in 12 of the 13 controls (Table II.3). In the one case where a control nest lost eggs, less than 25% of the eggs were lost. This difference between treatments was highly significant ($G = 14.56$, $p < 0.001$, G-test on > 50% of target clutch lost). All target clutches showed normal development. In three cases, I observed the male eating eggs within 10 minutes of the final manipulation. In three other cases, the eggs disappeared within 20 minutes of being exposed. In the other seven cases in which the target clutch disappeared, loss occurred within 24 hrs. In one of these seven cases, I observed the male releasing "runny", dark yellow feces later on the day I observed the older eggs missing. I have observed this on only one other occasion, also from a male that recently lost a large mass of eggs. In only one of the 29 cases was there any measurable loss from the younger eggs.

All 29 males received additional eggs from females after the final manipulation. Among males that received the "exposed" treatment, males that did not eat exposed target clutches received fewer eggs during the brood cycle in which the experiment was performed, filled a smaller proportion of their nest, and had more empty nest space than males that ate exposed target clutches (Table II.4). However, only the latter two differences were statistically significant ($p < 0.05$, Mann-Whitney tests). As expected, the nest area that remained empty in non-cannibalized cases was the area surrounding the older, target clutch. The spawning success of males that cannibalized exposed clutches was similar to that of control males (Table II.4).

Experiment 2

As expected, there was no loss of exposed older eggs in any of the nine nests used for the within-nest experiment. All nine males continued to receive additional eggs after the manipulation. However, eggs accumulated only at the younger end of the brood until the egg mass reached the margin of the nest, while all of the nest space surrounding the older end remained empty (Table II.5). These results corroborate field observations and results of a previous experiment conducted using a different egg removal scheme (Sikkel 1989).

In this experiment, the cost of cannibalizing exposed older eggs was much greater than in the previous experiment. The total brood size was about 2.5 times greater than in the previous experiment, and the male would have to cannibalize virtually the entire brood to allow the nest area at the older end of the brood to be exposed to the youngest eggs (and hence receive eggs). However, this result cannot be used to determine whether males are actually sensitive to increased costs of cannibalism since the manipulation was performed in a slightly different manner and during a different year than the exposed treatments in the previous experiment.

DISCUSSION

The results of this study corroborate the hypothesis that filial cannibalism by male garibaldi during the mating phase is an adaptive response to female spawning site preference, enabling a male to maximize his reproductive success during a brood cycle. First, in field observations and experiments, males cannibalized the older eggs when these eggs were exposed to available nest space. Second, males rarely cannibalized the younger eggs which attract females to lay more eggs. Third, males received additional eggs only around other newly deposited eggs and nest space around uncannibalized, pigmented eggs remained empty. The few (n = 3) males that did not cannibalize exposed, older eggs in the first (low brood size) experiment realized less of their potential reproductive success during the brood cycle than males that did cannibalize the eggs. A more direct test of this hypothesis would involve selectively preventing males from cannibalizing older eggs under these conditions and comparing their success with males that were allowed to cannibalize such eggs. However, conducting such a test does not seem possible.

It is difficult to make general predictions about how parental investment in offspring should vary with offspring age (Clutton-Brock 1991). However, for paternal-caring fishes, it has been predicted that males should invest more effort in the care of and should be less likely to cannibalize relatively older eggs (see references in introduction). The observation that garibaldi preferentially consume younger clutches early in the brooding phase is consistent with the predictions of other authors, and males may also consume small numbers of younger eggs at other times. However, the observation that all or most of entire, older clutches are selectively cannibalized during the mating phase is clearly inconsistent with these predictions. Males undoubtedly obtain some nutritional benefit from cannibalizing eggs, and female preference to deposit eggs in nests with early stage eggs over those with late stage eggs ("among nest" preference) could increase the benefits of cannibalizing the relatively older eggs. However, only the within-nest preference appears to adequately explain the biased consumption of exposed eggs. Such a within-nest preference has thus far been reported only for garibaldi, although it also appears to occur in two other pomacentrid species (R. Knapp and V. Vredenberg pers. comm.). Why females exhibit this preference is not clear.

The patterns of male filial cannibalism observed in garibaldi during the mating phase have been interpreted here as an adaptive response to female spawning preference for early stage eggs over late stage eggs, particularly within nests. These patterns of filial cannibalism could in turn favor other patterns of female choice. As described earlier, females also prefer to spawn with males guarding early stage eggs over those with empty nests. The tendency of lone (only) clutches to disappear may partially explain this behavior (Sikkel 1988, 1989). However, even if additional eggs are added to the nest, the pre-existing eggs may still be lost, depending on their position. Thus, cannibalism of exposed older eggs could at least reinforce such a preference. One way a female spawning in an empty nest can reduce the risk of her eggs being cannibalized is to deposit them near the edge of the nest, since this would minimize the area of the clutch exposed to empty nest space and would increase the likelihood that subsequent eggs would completely surround the clutch. Although the location of the first clutch could be influenced by a variety of factors (e.g., differences in the quality of the algal substratum within the nest, and the position of egg predators around the nest) first clutches do tend to be deposited near the periphery of the nest. There were 83 clutches in 47 nests that were mapped accurately enough to determine their precise distance from the perimeter of the nest (1988 and 1989 clutches). The modal distance of the margins of these clutches from the nearest margin of the nest was 0 cm (range = 0 - 18 cm): Forty-four of the 83 clutches (53%) were in contact with perimeter of the nest, and 62 (75%) of these clutches were $\leq$ 4 cm from the perimeter. Nests range in size from 300 to 2500 cm^2. The maximum possible distance a clutch could be deposited from the perimeter of the nest is the geometric center. Thus, on a relative level, fifty nine of the 83 clutches (71.1%) were closer to the periphery of the nest than to the center. Assuming these observations are independent, this difference is highly significant ($G = 15.2$, $p < 0.001$).

Although comparison of the experiment 2 nests with the exposed treatment nests from experiment 1 cannot be considered as a true test of the sensitivity of males to increased costs of cannibalizing older eggs (in this case, the number of eggs that must be cannibalized), the results are suggestive. Cannibalism in natural situations also tends to occur under conditions that would appear to minimize the costs of cannibalizing older eggs. Specifically, although *relatively* older eggs were cannibalized, these eggs were still young; males usually cannibalized older exposed eggs after about 4 days, soon after they could negatively influence the deposition of subsequent eggs. In addition to minimizing the costs of losing older eggs (of higher reproductive value) per se, cannibalizing older eggs early would also minimize the number of eggs cannibalized by precluding the accumulation of a larger number of older eggs.

In conclusion, given the widespread occurrence of filial cannibalism among teleost fishes, it is not surprising to find adaptive patterns of cannibalism unique to individual species or populations. In garibaldi, filial cannibalism during the mating phase appears to be a brood management strategy which reflects the influence of the position and developmental stage of eggs on female spawning site preference. Cannibalism under these circumstances is similar to such tactics as egg stealing and the takeover of nests with eggs that enhance male reproductive success in other species in which egg presence influences female choice (van den Assem 1967; Rohwer 1978; Constantz 1985; van den Berghe 1988; Unger and Sargent 1988; Gronell 1989).

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Table II.1. - The distribution of individual cases of cannibalism in relation to the age of eggs (relative to the median age in the brood) and the phase of the brood cycle.

	Mating Phase		Brooding Phase		Total
Relative Age of eggs lost:	Older	Younger	Older	Younger	
N cases (males)	52 (25)	2 (2)	3 (3)	11 (9)	68 (31)

Table II.2 - Cannibalism of older eggs during the mating phase in relation to their position in the nest (exposed or not exposed to empty nest space). Data in parentheses are for the subset of males that had both brood cycles in which older eggs were exposed to empty nest space and in which older eggs were not exposed to empty nest space. "Total cases" = The total number of times apparent cannibalism occurred, since it can occur more than once during a brood cycle.

	Older eggs exposed			Older eggs not exposed		
Older eggs lost?	Yes	No	%	Yes	No	%
Brood Cycles	22 (21)	3 (3)	88.0 (87.5)	7 (1)	142 (48)	4.7 (2.0)
Males	18 (17)	3 (3)	85.7 (85.0)	5 (1)	78 (19)	6.0 (5.0)
Total Cases	45 (44)			7 (1)		

Table II.3 Results of experiment 1 - 4-5 day old "target" clutch exposed to available nest space or surrounded by younger eggs (control).

	Treatment	
	Exposed	Control
Clutch Eaten *	13	0
Clutch not eaten	3	13

* At least 50% of clutch lost within 24 hrs.

Table II.4 Spawning success of males used in experiment 1 during the brood cycle in which the manipulations were performed. Data are presented as mean (standard error).

	Target Clutch Exposed			Target Clutch Not Exposed
	Eggs eaten		not eaten	
N =	10		3	10
%Full	91.3 (2.5)	*	57.7 (9.2)	95.1 (3.8)
Total Area	757.4 (97.7)	**ns**	533.0 (122.9)	729.8 (37.6)
Area Empty	80.6 (22.6)	*	511.3 (107.1)	46.5 (33.8)

%Full = The percentage of the nest filled with eggs at the end of the mating phase; **Total Area** = The total area (cm) of the nest covered with eggs; **Area Empty** = The area of the nest not covered by eggs by the end of the mating phase. Statistical comparisons were made only between males in the "exposed" treatment. * = Differences significant at $p < 0.05$, Mann-Whitney test. Note: Data not available for three cases in which males cannibalized exposed target clutches and three control nests.

Table II.5 - Results of experiment 2 - Empty nest space created at the oldest and youngest end of the brood.

	New eggs deposited in nest	
	Older end	Younger end
Number of nine nests	0	9

Figure II.1. A - Schematic diagram of the pattern of egg deposition during a typical brood cycle. The solid line delineates the boundary of the algal nest. Stippling and shading indicates different clutches in a brood. Numbers indicate the day of the brood cycle on which eggs were deposited. Thus, day 1 eggs are the oldest and are not exposed to empty nest space. Day 4 eggs are the youngest. Arrow represents the direction of further egg accumulation. **B** - Pattern of egg deposition in a case where the oldest eggs are exposed to available nest space. Note that since day 2 eggs are younger than day 1 eggs, after day 2, additional eggs would accumulate at the younger (right) end of the nest and nest space to the left of day 1 eggs would likely remain empty.
C - Consequences of cannibalizing the older eggs from days 1-3 in case B. Subsequent to day 4, eggs are deposited to the left, opposite of case B.

Figure II.1

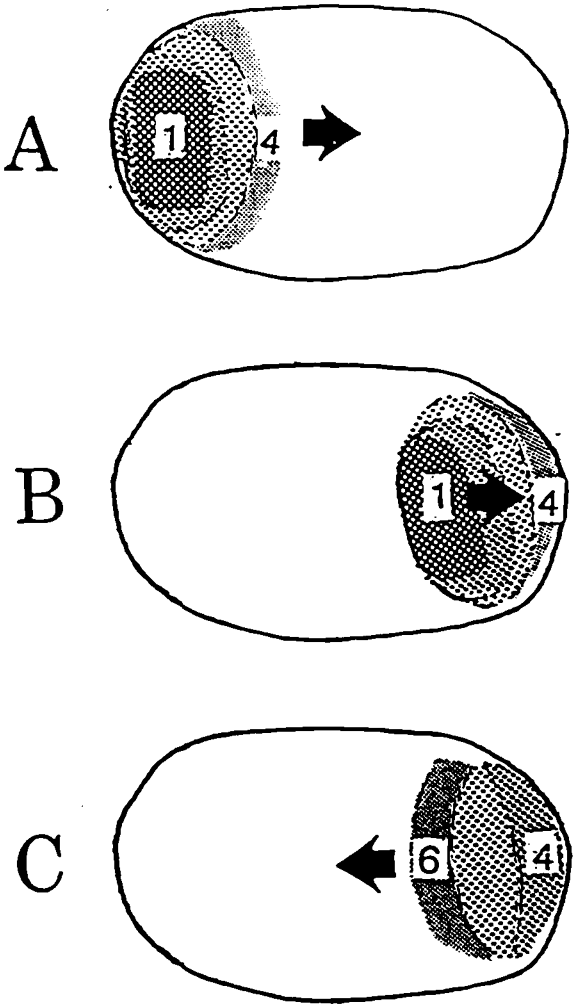


Figure II.2. Schematic diagram of treatments in experiment 1. Gray shading indicates young (0-1 day old) eggs. Elliptical area defined by the solid line within the shaded region indicates the target clutch. Stippling indicates changes in the age of the target clutch. The shaded area outside the dashed line indicates the area of eggs completely removed each day from day 2 through day 5. The area between the dashed and solid lines indicates the area of eggs that was thinned, leaving a low density halo of 0-1 day old eggs. This manipulation insured that new eggs were continually deposited around the target clutch until the final manipulation (day 6). The exact position of the target clutch varied within each treatment, depending on the size and configuration of the initial brood.

Figure II.2

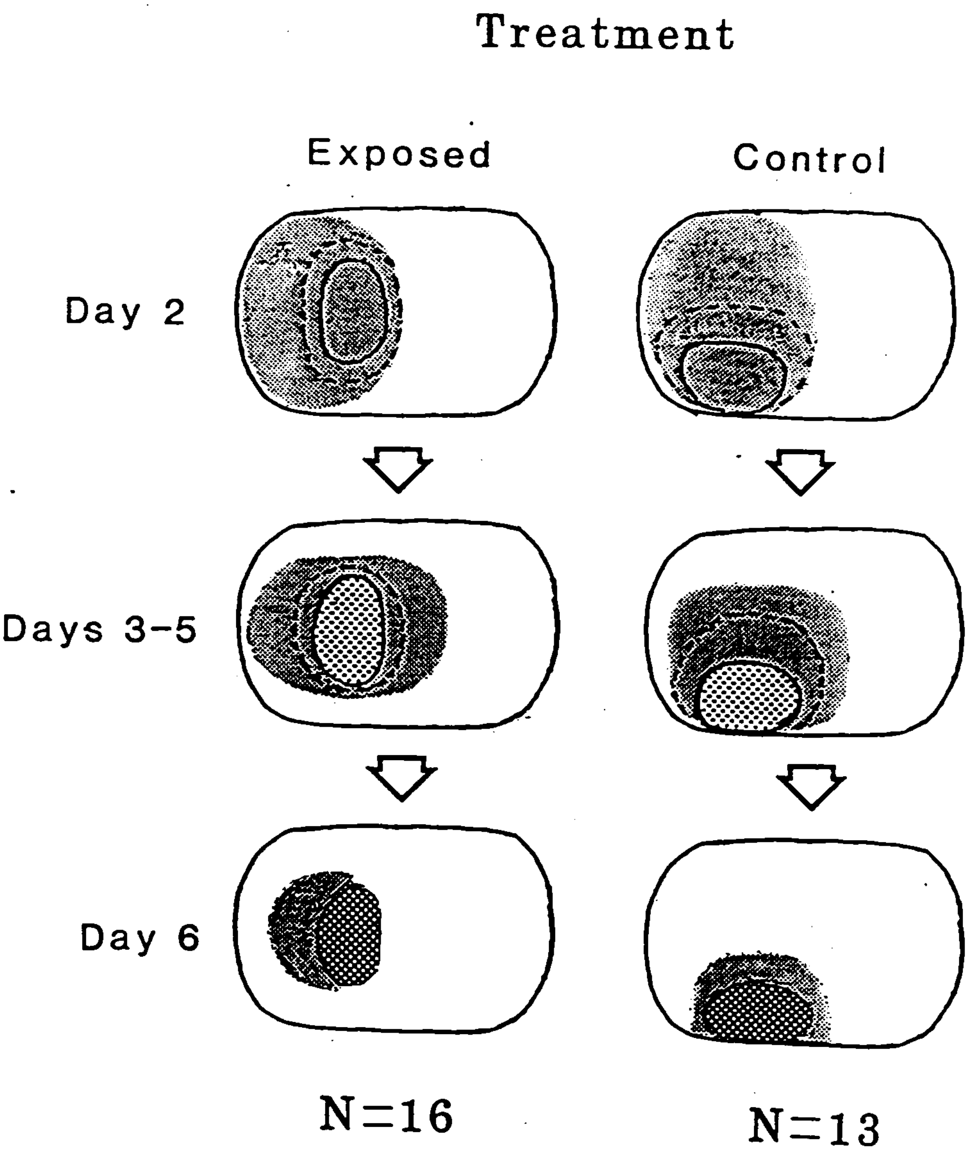
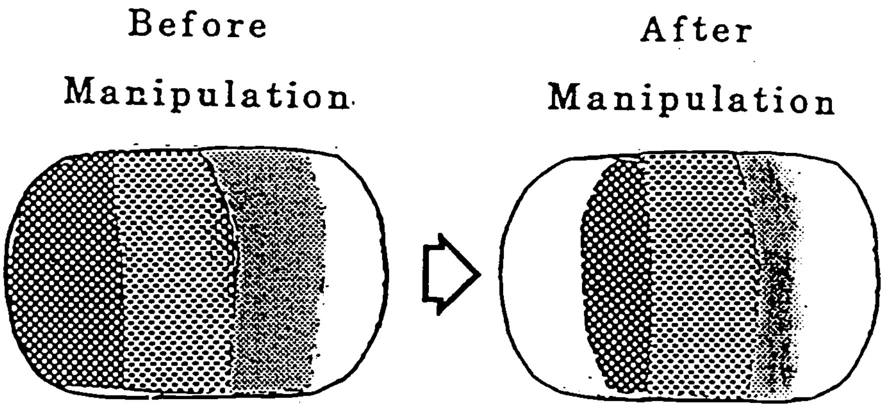


Figure II.3. Schematic diagram of experiment 2. As in previous figures, the solid line delineates the boundary of the nest. Differences in stippling and shading represent eggs in different stages of development. Dark stippling (left side of nest) represents the oldest (5 day old) eggs. Gray shading (right side of nest) represents the youngest (0-1 day old) eggs. White area within the nest boundary represents empty nest space.

Figure II.3



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CHAPTER 3

Male parental investment and female choice in a paternal caring marine fish

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Running Head: Paternal care and female choice

Abstract - Female garibaldi damselfish *Hypsypops rubicundus* strongly prefer to spawn with males guarding early-stage eggs over those guarding empty nests or nests with late-stage eggs. According to the parental investment hypothesis, such female behavior is an adaptive response to male egg-guarding tactics which are dependent on brood size and age, and result in brood-size and age dependent differences in clutch hatching success. In this study, I tested five predictions derived from this hypothesis, along with the underlying assumption, that males experience a trade-off between egg guarding and self-maintenance activities. Feeding rates of nesting males declined when males received eggs and remained low until late in the brood-cycle. Thus, males experience a trade-off between egg guarding and feeding. Males nearly always cannibalized

the entire brood when they received only a single-clutch, and the probability of cannibalism of last clutches increased with brood age. Males were also more attentive of larger broods and guarded them more aggressively. These behaviors would result in higher hatching success of eggs deposited among other early-stage eggs.

In many fish species where males guard demersal eggs, females preferentially spawn with males whose nests already contain eggs (Ridley and Rechten 1981; Marconato and Bisazza 1986; Unger and Sargent 1988; Gronell 1989; Knapp and Sargent 1989; Jamieson and Colgan 1989). In some of these species, such female preference appears to be a decreasing function of egg developmental stage, females tending to spawn in nests with early stage eggs but avoiding those with predominantly late stage eggs (DeMartini 1987; Petersen 1989; Petersen and Marchetti 1989; Sikkel 1989). Why females prefer nests with eggs, particularly early stage eggs, is not fully understood.

Three general hypotheses have been proposed to explain why females prefer nests with eggs over empty nests. These include the phenotypic quality hypothesis, the dilution or selfish herd hypothesis, and the parental investment hypothesis (Sargent 1988). The latter two hypotheses have been extended to explain the more specific question of why females in some species preferentially spawn with males guarding early-stage eggs.

The phenotypic quality hypothesis holds that the presence of eggs in the nest is indicative of male attributes that are independent of the presence of eggs but that may accrue a survivorship or other advantage to the offspring of the choosy female (Ridley 1978). Some females may choose males directly on the basis of such attributes, while other females may use egg presence to copy the choice of these "smart" females (Losey et al. 1986; Pruett-Jones 1992).

According to the dilution hypothesis, if egg predators are not capable of consuming the entire brood during an attack, then the per capita risk of predation should decrease with the number of eggs in the nest (Rohwer 1978; Ridley and Rechten 1981; Constanz 1985; Unger and Sargent 1988). Any diluting effect due to large brood size would be maximized with increased brood synchrony because a newly deposited clutch would be associated with other early-stage eggs longer than it would with older eggs which would soon hatch (Sikkel 1989). Moreover, egg predators may preferentially consume the younger eggs in mixed-age broods. Thus, eggs deposited in nests with older eggs may suffer disproportional predation.

The parental investment hypothesis has probably received the most attention (Rohwer 1978; Dominey and Blumer 1984; Sargent 1988; Petersen and Marchetti 1989). In contrast to the phenotypic quality hypothesis, the parental investment hypothesis holds that differences in the quality of male parental care (and hence expected survivorship of offspring) are both signalled by <u>and</u>

determined by brood parameters such as brood size and age. The underlying assumption of this hypothesis is that providing parental care results in restricted male feeding opportunities, increased expenditure of energy, and possibly increased risk of injury or predation, all of which decrease the male's probability of mating in the future. Thus, male investment in present offspring should depend on how caring for offspring would contribute to the male's current reproductive success (benefit) relative to the negative effect rearing present offspring will have on future reproductive success (cost) (Sargent and Gross 1986).

Rohwer (1978) first used these considerations to explain why females of some species should specifically spawn with males guarding early-stage eggs. His argument addressed those species in which males undergo prolonged brood cycles (described below) and emphasized the role of "filial cannibalism", whereby the parental male consumes some of its own viable offspring. Rohwer's original arguments have been refined by other authors and incorporated into the broader context of life history and parental investment theory (Dominey and Blumer 1984; Petersen and Marchetti 1989; FitzGerald 1991; Sargent 1992). Based on Rohwer's original arguments and subsequent studies, there are three proposed mechanisms by which male filial cannibalism could favor female choice for early-stage eggs in brood-cycling fishes:

(1) Males with large broods may offset the cost of care by cannibalizing a fraction of the brood. However, if males are guarding mixed-age broods, they should preferentially cannibalize the younger eggs of higher nutritional value. This would better enable them to care for the older eggs of higher reproductive value. Cannibalism of later clutches should increase with the age of the brood, as the male becomes more energy depleted and as the age differential of eggs increases.

(2) Because unusually small broods do not warrant the cost of care, males may cannibalize small broods remaining at the end of the brood cycle as an investment in future broods. Thus, the most recently deposited younger eggs would be cannibalized once the older eggs have hatched.

(3) Similarly, if males can expect to receive multiple clutches during a spawning bout (due to female choice or episodic spawning activity), males may cannibalize small broods early if they receive an unusually small number of eggs.

The first and second mechanisms would decrease the survivorship of eggs deposited in nests with late-stage eggs, whereas the third mechanism would decrease the survivorship of eggs deposited in empty nests. Although Rohwer (1978) emphasized the role of filial cannibalism, parental males may adjust their investment in current offspring in other ways that would increase the survivorship of eggs deposited among other early-stage eggs. First, males may devote less time to caring for smaller broods and devote more time to feeding or other self-maintenance activities (Sargent 1988, 1992; Lavery and Keenleyside 1990). Second, males may expend less effort or assume less risk in repelling egg predators from the nest (Pressley 1981; Carlisle 1982; Coleman et al. 1985; Ridgeway 1986). Thus, small broods may suffer increased risk of predation from other egg predators. As with cannibalism, declines in male attentiveness or aggression may occur if males receive small broods or if small broods result from hatching or prior loss of eggs. Of course, these hypotheses are not mutually exclusive and any combination of mechanisms may exist in a given population. For example, per capita predation rates may be a function of brood size in systems where predators attack the nest. However, male parental investment tactics may determine whether or not predators (including the male himself) gain access to eggs in the first place.

STUDY SPECIES AND OBJECTIVES

The garibaldi (*Hypsypops rubicundus*:Family Pomacentridae) is a large (up to 36 cm SL) damselfish found in rocky subtidal areas of southern and Baja California (Limbaugh 1964; Clarke 1970). Both sexes defend year-round territories that include invertebrate prey, shelter, and nest sites for certain males. During the spring/summer spawning season, males culture red algal nests on exposed rock faces on which females deposit eggs (Limbaugh 1964; Clarke 1970; Foster 1972; Sikkel 1988). Nests range in size from about 300 to 3000 cm^2. Males care for the eggs by fanning and defending them until they hatch as planktonic larvae. Hatching requires approximately 2-3 weeks, depending on temperature. Female garibaldi strongly prefer to spawn with males whose nests contain early-stage eggs over those guarding empty nests or mostly late-stage (pigmented) eggs (Sikkel 1989). Within mixed age broods, females always deposit their eggs among the youngest eggs and will not deposit eggs among other eggs older than about 2 days (Sikkel 1989, in press *a*).

Spawning in the garibaldi population at Santa Catalina Island off southern California occurs throughout the day, every day during the course of the spring/summer spawning season. However, individual males undergo cyclic or sequential changes in reproductive activity known as brood cycles (Sikkel in press _b_). A "brood cycle" consists of an initial "mating phase" (sensu van Iersal 1953), during which males spawn with females, and is followed by a "parental" or "brooding" phase, during which males care for the eggs but do not spawn again until most or all of the previous eggs have hatched. Typically, once a male receives an initial clutch of eggs, he continues to receive eggs every day for about 5-10 days (up to 20 days on rare occasions) until the nest is filled to or near capacity. However, in rare cases, a male will fail to receive additional eggs after receiving an initial clutch. A complete brood cycle lasts approximately 3-5 weeks, after which a male may begin another cycle.

At my study site, there are three major sources of egg predation. First, male garibaldi sometimes cannibalize their own viable offspring (Sikkel in press _a_). Second, when males move away from the nest (e.g., while feeding) eggs are sometimes eaten by other fishes, primarily the goby _Lythrypnus_ _dalli_ which lives in crevices around garibaldi nests. Third, females will sometimes eat eggs opportunistically after they enter the nest of a courting male.

The goal of this study was to test predictions derived from the parental investment hypothesis to determine whether brood size and age-dependent parental behaviors could increase the hatching success of eggs deposited in nests with other early-stage eggs, and thus provide an adaptive explanation for some of the female preference patterns observed in this species. In an earlier paper (Sikkel in press a), I presented evidence that late during the mating phase or early during the brooding phase, males that cannibalize eggs preferentially cannibalize the younger eggs in the nest (#1, above). Here, I expand on these findings and test six additional predictions of the parental investment hypothesis as proposed by Rohwer (1978) and others, as they apply to garibaldi. These are:

(1) Males experience a trade-off between caring for eggs and feeding (the foundation of Rohwer's original hypothesis);

(2) Male cannibalism of last clutches in mixed-age broods increases with the age of the brood when the last clutch was deposited;

(3) Males cannibalize small broods remaining at the end of the brood cycle;

(4) Males cannibalize small (single-clutch) broods when additional eggs are not added;

(5) Males are more attentive of larger broods; and

(6) Males defend larger broods more aggressively.

The effects of male attentiveness on egg hatching success should depend on the response of egg predators to the eggs exposed in the male's absence. Thus, as a corollary of prediction 5, I also determined whether per capita egg predation in the male's absence varied with the number and age of the eggs in the nest. These data also enabled me to test predictions from the dilution hypothesis.

METHODS

Study system and dates

I conducted this study during the spawning seasons of 1987-1992 on a population of garibaldi in and around Big Fisherman Cove, Santa Catalina Island, California (33 28'N, 118 29'W). Spawning in this population occurs from late April or early May, through late July or early August. I conducted all observations and experiments using snorkel or scuba, assisted by other divers.

Terminology

I define a "clutch" as all eggs deposited in a nest on a given day (between consecutive 24 hr surveys). A "multiple-clutch brood" consists of at least five clutches, and a complete "brood cycle" refers to the accumulation and subsequent hatching of those clutches.

I define a "single-clutch brood" as a clutch of eggs deposited in an empty nest to which no additional eggs are added. As described above, in multiple-clutch broods, the addition of subsequent eggs begins within 24 hours after the first clutch has been laid.

Prediction 1: Changes in foraging activity during the brood cycle

To determine whether egg guarding results in decreased foraging activity, I conducted 12 min activity budgets for nine males at five phases of the brood cycle: (1) when the nest was empty ("empty"); (2) two days after the first clutch was received ("early mating"); (3) when the last clutch was received ("late mating"); (4) 2-5 days after the last clutch was received ("early brooding"); and (5) 7-9 days after the last clutch was received ("late brooding"). I conducted these activity budgets during the 1991 spawning season, between 1130 and 1430 hrs. At 10 sec intervals, I recorded the male's position, and estimated his distance from the nest to the nearest 0.5 m. I also recorded the number of feeding bites taken from the substratum which was used to calculate a feeding rate. I compared the male's average distance from the nest and feeding rates among phases of the brood cycle using Friedman's test, with males as blocks. I then used Wilcoxon's tests to determine which egg-guarding phases differed from the non-guarding (empty) phase.

Male filial cannibalism (Predictions 2-4)

Field observations

To assess egg loss due to filial cannibalism, I monitored 79 nests during the 1987-89 spawning seasons on a daily or near daily basis and recorded the area and location of gaps in the brood where eggs had disappeared. I assumed that the disappearance of entire clutches or a large fraction of the brood ($\geq$ 40 cm^2) from one area was the result of male filial cannibalism. I believe that this assumption is valid for the following reasons. First, when a large fraction of the brood disappears from one area, the area is rimmed by bite marks that are the size and shape of a garibaldi gape (approx. 2 cm^2). Second, in the over 16 cases where I have observed males cannibalizing eggs in natural and experimental conditions, males took multiple bites from the same area of the brood. Third, among 21 guts examined from egg-guarding males, two contained a stomach full of viable eggs, while the remainder contained no more than 10 eggs which were likely eaten incidental to egg care (a single bite removes approximately 340 eggs). This observation suggests that males either consumed entire clutches or large fractions of a clutch or consumed no eggs at all. Fourth, females are chased out of the nest as soon as they take a bite of eggs. I have never observed a female taking more than three bites before being chased out of the nest, and females do not "raid" nests in groups as occurs in some fish species (e.g., Whoriskey and Fitzgerald 1985). Also, I have never observed other males or immature garibaldi attacking nests. Finally, the major heterospecific predators are small-mouthed species that can remove only a few eggs per bite and I have not observed such predators attacking nests in groups.

Prediction 2: The effect of brood age on cannibalism of last clutches

There were 11 cases between 1987 and 1989 in which last clutches were cannibalized in mixed-age broods. To determine whether the likelihood of such cannibalism was greater in older broods, I conducted two analyses using data from nest monitoring records. First, I used a Mann-Whitney test to compare the brood age at the time the clutch was deposited for broods in which the last clutch was cannibalized with that of broods in which the last clutch was not cannibalized. Because 8 of the 11 cases occurred in one year (1988), I restricted this analysis to data from that year. To control for between-male differences in the tendency to cannibalize last clutches, I conducted a second analysis using 8 males that completed 2 brood cycles in a single season and had cannibalized the last clutch during one of them. I used a Wilcoxon test to compare the age of the brood in which the last clutch was cannibalized with the age of the brood in which the last clutch was not cannibalized.

Prediction 3: Cannibalism of small broods at the end of the brood cycle

To determine whether males would cannibalize a small brood remaining at the end of a brood cycle, I conducted the following field experiment. On the basis of body size, brood size, and brood age, I paired 18 males that were guarding late-stage eggs. I then randomly assigned one male in each pair to one of two treatments: "small" brood (80 cm^2) or "large" brood (250 cm^2 control). When the oldest clutch in the brood appeared silver (due to the presence of eye pigment) and was thus close to hatching, I removed the older eggs in the nest, leaving a brood of approximately 80 cm^2 (small) or 250 cm^2 (large) of the youngest eggs. Because small broods require more time to create, I controlled for differences in disturbance by combing the large brood in each pair for the excess time. Following the manipulation, I monitored nests 2-3 times each day until they hatched. At the time of the experiment, the younger eggs had not yet developed obvious eye pigment, and were not ready to hatch. If males cannibalize small broods at the end of the brood cycle, then small broods should be more likely to disappear prematurely (before hatching) than large broods.

Prediction 4: Cannibalism of single clutch broods

If early abandonment of entire broods by cannibalism is dependent on how many eggs a male receives, then lone clutches should be more likely to be cannibalized than complete broods or first clutches to which other eggs are subsequently added. To test this prediction, I performed a goodness of fit test (G-test) using data obtained during the 1987-89 nest surveys. Because males with lone clutches may have been more likely to cannibalize eggs in any case, subsequent females may avoid spawning with them. In other words, the fact that these males are probable filial cannibals may be the reason why they have lone clutches (e.g., Kraak and van den Berghe 1992). The appropriate experiment would involve manipulating brood sizes early in the brood cycle by selectively preventing randomly selected males from acquiring additional eggs after the initial clutch, while allowing others to obtain more eggs. Unfortunately, I was unable to prevent males from acquiring additional eggs and thus could not maintain fixed brood sizes early in the brood cycle. As an alternative, I conducted an additional analysis in which I used only those males (n = 13) that had one single-clutch brood and one multiple-clutch brood during the same spawning season.

Prediction 5: Male attentiveness in relation to brood size

To determine whether male attentiveness of the brood varies with brood size I conducted two field experiments, one in 1989, and one in 1991. In 1989, I randomly assigned 19 males to one of three brood sizes: "large" (control, > 220 cm^2 of eggs), "medium" (120-200 cm^2) and "small" brood size (50-100 cm^2). To create each treatment, on the morning of the fourth day of the brood cycle (ca 1000 hrs), I reduced broods (small and medium) or maintained them at the original brood size (large). To control for disturbance effects, I combed large broods in a manner similar to that used to remove eggs from the other two treatments. In the late afternoon on the same day (1600 - 1700 hrs), I conducted a 15 min focal observation. During each observation, at 10 sec intervals, I recorded the male's approximate distance from the nest to the nearest 0.5 m, whether or not he could see the nest from his position, and the number of bites he took from the substratum (to determine feeding rate). Beginning the day after the initial manipulation, I reduced each brood each day to the designated size by removing additional eggs until the male stopped receiving eggs. I then conducted an additional focal observation on the ninth day of the cycle, when the male had entered the brooding phase.

The designated brood sizes represent the brood sizes at the time of the activity budget. Because males continued to receive eggs after the first removal, mean brood sizes during the brood cycle for each male were actually larger. However, mean brood sizes maintained their relative differences among groups (large > medium > small). There were no significant differences among treatment groups in the size of the original brood (before the first brood reduction), the number of days in which eggs were removed, and the total area of eggs removed (and hence added) after the initial reduction ($p > 0.41$ for all variables, Kruskal-Wallis test). I used a Kruskal-Wallis test to compare the data among groups for each of three variables: mean distance from the nest, feeding rate (bites/15 min), and the proportion of 10 sec intervals the male was out of sight of the nest ("% Out of Sight"). I also compared these variables between the early and late observations using a Wilcoxon matched-pairs test.

For the 1991 experiment, I used the same 18 nests that I used in the experiment to determine whether males cannibalized small broods at the end of the brood cycle (see prediction 2, above). Thus, this experiment specifically examined whether males would be less attentive of small clutches at the end of the brood cycle. To verify that pairs of males were similar in behavior prior to manipulation, I conducted one 12 min activity budget immediately prior to brood reduction. I then conducted a second activity budget two days after brood reduction. All activity budgets were run between 1130 and 1430 hrs. To determine whether males with small broods were less attentive and fed more than males with large broods, I compared data from the second activity budget using a Wilcoxon matched-pairs test.

To determine whether predation by small predators in the male's absence varied with brood size, I performed the following observations. In both 1989 and 1991, a diver kept the nesting male away from the nest while I counted the number of bites taken by goby egg predators during a 1 min period. I also recorded the amount of time elapsed until the first goby attacked the nest ("attack latency"). In 4 cases, I repeated this procedure the following day after reducing the brood size by 50%. In 9 other cases, I repeated the procedure

1-2 days later after the brood size had increased by approximately twofold. I analyzed attack rates for these 13 nests as the number of bites per min per cm of eggs. I compared attack rates and attack latencies for large versus small broods using a Wilcoxon matched-pairs test.

To determine whether these predators were equally likely to attack early-stage and late-stage eggs, I performed a similar procedure in 1991 and 1992. Using 17 broods that were nearly full and contained eggs in multiple stages of development, I recorded whether any given bite occurred on the older or younger half of the brood for a period of 2-5 min. I analyzed these data using a Wilcoxon matched-pairs test.

Prediction 6: Male aggressiveness in relation to brood size

To determine whether brood size influences male aggressiveness toward heterospecific nest predators, I added potential egg predators to male territories in 1991. I placed two juvenile Semicossyphus pulcher (a large wrasse which occasionally preys on garibaldi eggs) in a large plastic bag weighted by a small lead weight and attached to a piece of monofilament line. Over a 1.5 hr period on 4 July, I presented the bag to each of 24 males that differed in the size and age of the brood they guarded. I also presented the bag to 6 males that had previously completed brood cycles but were currently maintaining and guarding empty nests. I lowered the bag from the surface to approximately 1m directly in front of the nest and recorded the number of times the fish bit or rammed the bag. Preliminary trials indicated that males would not attack empty bags. I analyzed these data using a multiple regression analysis, with brood size and brood age as independent variables.

RESULTS

Prediction 1: Changes in foraging activity during the brood cycle

Both the male's mean distance moved from his nest and his feeding rate showed significant changes during the course of the brood cycle (T = 19.8, $p = 0.005$; T = 10.5, $p = 0.03$, respectively; Fig. III.1). Both were highest during the pre-spawning empty-nest phase. Mean distance moved during the empty phase was significantly higher than during each of the egg-guarding phases ($p < 0.001$). Feeding rate during this phase was significantly higher than during the early and late mating phase ($p \leq 0.02$). However, the differences were not significant when compared with the two brooding phases ($p = 0.09$, and $p = 0.26$ for early and late brooding phases, respectively).

Prediction 2: The effect of brood age on cannibalism of last clutches

There were 11 mixed-age broods, involving 9 males, in which last clutches were cannibalized. All cases the clutches were cannibalized within nine days after they were deposited ($\overline{X}$ age = 4.6, SE = 0.66). In 1988, the brood age at the time the last clutch was deposited was significantly greater for the 8 broods in which the last clutch was cannibalized than the 35 broods in which the last clutch was not cannibalized ($\overline{X} \pm SE = 12.3 \pm 2.0$ versus 8.1 ± 0.53, Median = 11 versus 7, $Z = 2.21$, $p = 0.03$, Fig. III.2). There was no significant difference between these groups in brood size ($Z = 0.11$, $p = 0.91$). This pattern also held for each of the eight males that cannibalized last clutches in one of the two brood cycles they completed in a season ($Z = 1.99$, $p = 0.05$; $Z = 1.48$, $p = 0.14$ for brood age and brood size, respectively).

Prediction 3: Cannibalism of small broods at the end of the brood cycle

In none of 18 nests was there any evidence of premature disappearance of the brood. Both artificially created large and small broods continued to show normal color change of eggs associated with development, and all broods disappeared at night 6 - 8 days after the manipulation. This result strongly suggests hatching rather than cannibalism as the cause of disappearance.

Prediction 4: Cannibalism of single-clutch broods

There were 22 single-clutch broods, involving 18 males, during the three spawning seasons of 1987 - 1989 (11, 5, and 5, respectively). In 21 of these 22 cases, the entire clutch was lost within 5 days after it was deposited; in only one case did the male rear the clutch to hatching. In 15 of the 21 losses, the eggs disappeared suddenly (between 24 hr surveys). In the remaining six cases, loss occurred gradually over a 3-4 day period. The mean age of eggs at the time of their dissappearance was 3.33 days (SD = 1.19). In all 19 cases where cannibalism occurred after the eggs were 1 day old, the eggs showed normal color change, suggesting they were viable prior to being eaten. During these same spawning seasons, of 174 broods involving multiple clutches, first clutches were cannibalized in 29 cases, while entire broods were cannibalized in only four cases. This difference among clutch types was highly significant ($G = 27.4$, $p < 0.001$, Fig. III.3). Similar results were obtained for the subset of 13 males that lost single-clutch broods but completed a second brood cycle during the same spawning season. For these males, the complete brood cycle began 1-29 days after loss of the lone clutch ($\overline{X}$ = 12.1, SD = 8.9). In only one case was the first clutch of the second brood cannibalized, and there were no cases in which the entire brood was cannibalized.

Prediction 5: Male attentiveness in relation to brood size

In the 1989 experiment, the percentage of observations a male spent out of sight of his nest, the mean distance he moved from his nest, and his feeding rate all increased significantly during the brooding phase relative to the mating phase ($Z = 3.51$, $p = 0.0005$, for both mean distance and % out of sight, and $Z = 3.10$, $p = 0.002$ for feeding rate). Using the mean values for each male, all parameter values were highest for males with small broods (Fig. III.4). The differences were significant for % out of sight and feeding rate ($T = 10.0$, $p = 0.007$; $T = 7.42$, $p = 0.03$, respectively) but not for mean distance ($T = 3.61$, $p = 0.16$). Because the results for medium and large broods were clearly similar, I limited post hoc comparisons to small versus medium and small versus large broods. For both % out of sight and feeding rate, differences were significant for both pairwise comparisons ($p \leq 0.02$).

In the 1991 brood-size reduction experiment, pre-manipulation levels of all behavioral variables were similar regardless of their assigned treatment ($p > 0.26$,). However, post-manipulation mean distance and feeding rate were significantly higher for small brood males ($Z = 3.1$, $p = 0.002$; and $Z = 2.43$, $p = 0.02$, respectively; Fig. III.5). The % out of sight was also higher for small broods, but the difference was not significant ($Z = 1.62$, $p = 0.11$).

Within nests, the rate per unit brood area at which heterospecific egg predators ate eggs when the male was chased away by a diver was higher for smaller broods. However, the difference was marginally non-significant ($Z = 1.88$, $p = 0.06$). There was no significant difference in the amount of time elapsed until the first attack ($Z = 0.05$, $p = 0.95$). Finally, in the older, mixed-age broods, there was no significant difference in the number of bites taken from the younger versus the older half of the brood ($Z = 0.05$, $p = 0.95$).

Prediction 6: Male aggressiveness in relation to brood size

There was a significant, positive relationship between brood size and the attack rate of males toward introduced egg predators ($r^2 = 0.37$, $p = 0.001$; Fig III.6a). There was no significant relationship between attack rate and brood age (Fig. III.6b) and no correlation between brood age and brood size ($r^2 = 0.0009$, $p = 0.89$; and $r = 0.10$, $p = 0.62$, respectively). When combined in a multiple regression analysis, only brood size was a significant predictor of attack rate ($t = 3.60$, $p = 0.002$).

Males with the smallest broods were either beginning or ending brood cycles in which their nests were filled to or near capacity. Thus, it is highly unlikely that the positive relationship between brood size and male attack rate was due to more aggressive males receiving more eggs.

DISCUSSION

Restricted foraging and the costs of parental care

The data reported in this study corroborate five of six predictions of the parental investment hypothesis for garibaldi. Feeding rates of parental males declined to about 25% of non-guarding levels once males received eggs. Feeding rates increased during the brooding phase, but they did not reach non-brooding levels. Thus, male garibaldi appear to experience a trade-off between feeding and tending the brood (prediction 1).

I have no data on changes in male physical condition. However, because male activity levels are also higher when guarding eggs, it is likely that reduced foraging also results in a decline in male condition. Reduced male foraging and/or condition have been well documented in other paternal caring fishes (e.g., DeMartini 1987; FitzGerald et al. 1989; Petersen 1990; Robertson et al. 1991).

I have no explanation for why foraging rates increased during the brooding phase; this occurred in males with both unmanipulated (1991) and manipulated (1989) broods. One possibile explanation is that male condition declined during the mating phase and males required additional energy to continue rearing the brood. Whatever the cause, increased time away from the nest while foraging could increase the exposure of eggs to predators during the brooding phase.

Nesting males of some species may incur other costs when caring for eggs. Specifically, males may suffer increased risk of predation or injury. I did not examine these costs in garibaldi. However, few predators are capable of eating or injuring adult garibaldi. The only predators present at my study site were pinnepeds which are primarily nocturnal. Because male shelter holes are usually near nests and males remain in front of nests at night, egg care probably does not increase predation risk. Male garibaldi may incur some small increase in risk of injury when repelling a large, persistent egg predator.

Filial cannibalism

Among the ways parents can decreaase their investment in current offspring in the face of the costs imposed by care, filial cannibalism is the most extreme. Of the three patterns of male filial cannibalism proposed to favor female choice for early-stage eggs (see Introduction), garibaldi appear to exhibit two. At the end of the mating phase, male garibaldi preferentially cannibalize the younger eggs in the nest. These eggs were cannibalized when they were still in a relatively early stage of development. This pattern is consistent with the interpretation that male cannibalism of younger eggs represents an investment in older eggs of the current brood (Sargent 1992). In the year when the most incidents of such cannibalism occurred, the probability that the last clutch would be cannibalized was higher in older broods (prediction 2). Thus, the last clutches deposited in older broods would be more prone to cannibalism than the last clutches deposited in younger broods. However, the fact that most cases of cannibalism did occur in only one of the three years suggests that the tendency of males to cannibalize late clutches in mixed-age broods varies among years. The reason for this variation is not clear, but may be due to interannual differences in the abundance of invertebrate prey or other factors that affect male condition.

Males did not cannibalize small broods at the end of the brood cycle (prediction 3). However, males did cannibalize small broods (single clutches) when additional eggs were not added (prediction 4). Cannibalism of unusually small broods is probably the most widely reported form of cannibalism in paternal caring fishes (reviewed by Petersen and Marchetti 1989; FitzGerald and Whoriskey 1992). Male garibaldi consumed single clutches when the eggs were about 3 days old, approximately the age when females will no longer deposit new eggs around them. Males will also consume clutches of this age early in the brood cycle when the eggs are part of a mixed-age brood and are positioned between younger eggs and empty nest space (Sikkel in press _a_). This accounted for 22 of the 29 cases in which first clutches of multiple-clutch broods were cannibalized. Combined, these two patterns of cannibalism accounted for 73% of the filial cannibalism incidents observed in garibaldi between 1987 and 1989 and would greatly increase the mortality of eggs deposited in empty nests. Thus, male filial cannibalism appears to be both an adaptive male response to and an ultimate cause of female preference for early-stage eggs, as Rohwer (1978) hypothesized.

Why do males cannibalize small broods early but not late in the brood cycle? One hypothesis is that, late in the brood cycle, the remaining older eggs have higher reproductive value. Because daily predation by heterospecific predators results in loss of a very small fraction of the brood compared to cannibalism (which results in 100% loss), and because eggs will soon hatch, late in the brood cycle males may recover much of their energetic costs by increased foraging (see below) while still hatching most of the remaining offspring. In contrast, increased foraging (and hence decreased attentiveness) at an earlier stage would result in greater cumulative loss of eggs to heterospecific predators, since eggs would require much longer to hatch. Thus, males may benefit more by cannibalizing the eggs early rather than losing them to other predators. Of course, the relative costs and benefits of cannibalism versus other parental investment options may vary among years or individuals, depending on such factors as food availabilty, male condition, the density of egg predators, and the probability of future mating (Sargent 1992).

Male attentiveness and aggressiveness

Males fed more and were less attentive of the nest when guarding smaller broods (prediction 5). This occurred when small broods were created by either a single removal of the older eggs late in the brood cycle (1991 experiment) or repeated removals of eggs during the mating phase (1989 experiment). This result suggests that small broods may suffer increased exposure to predators and/or less care, regardless of whether they result from hatching of earlier eggs (i.e., late clutches) or from low egg acquisition by the male (i.e., clutches deposited in empty nests). Of course, whether increased exposure to predators actually results in higher mortality rates depends on whether, first, predators eat late stage eggs (e.g., eggs remaining at the end of a brood cycle), and second egg predation rates in the male's absence do not decrease in proportion to brood size. Goby predators, the most frequent egg predators at my study site, ate unguarded eggs independent of developmental stage. In addition, the amount of time it took for them to attack the brood in the male's absence, as well as their per egg attack rates, did not decrease significantly with brood size. In fact, there was a nearly significant increase in per capita predation rates for smaller broods. Thus, increased absence of a male from his nest indeed appears to result in increased predation rates by heterospecific predators, independent of egg developmental stage.

These results suggest that a dilution effect may complement the effects of male attentiveness but that differential predation of early-stage eggs does not explain why females avoid nests with late-stage eggs (see Introduction). However, it must be emphasized that different patterns of heterospecifc predation may occur in areas where other predators predominate (e.g., wrasses).

Male aggressiveness toward egg predators also increased with brood size (prediction 6). The two predators used in this experiment were about one half the size of an adult garibaldi, so it is likely that even minimal effort would suffice to repel such predators. Thus the results of this experiment are probably best interpreted as a measure of a male's "readiness" to defend his nest. For example, males that are away from the nest may return more rapidly in response to a predator when guarding larger broods. Males with larger broods may also assume greater risk when defending the brood against larger predators that could injure the male (e.g., male sheephead wrasses), although I have never seen such predators attacking guarded nests. Because male aggressiveness was independent of brood age, small broods that result from low egg aquisition or hatching of older eggs would both be at greater risk of predation.

Other effects of male parental investment

One source of egg predation that was not examined in this study is cannibalism by females. In garibaldi, predation by females can also be considered a consequence of male parental investment tactics, since females only enter nests when they are permitted by males. Male receptivity to females declines over the course of the brood cycle, although the relative effects of brood size and age are not known. However, female visitations to nests may increase with brood size. Nest monitoring records indicate that the number of bites taken by females does not increase proportionally with brood size (Sikkel unpub. data), suggesting that eggs deposited among other young eggs would also benefit from a dilution effect with respect to occasional female predation.

Conclusion

Male parental investment tactics have been invoked as a major determinant of female choice in paternal-caring fishes. However, few field studies have examined alternative male brood-care tactics in populations where eggs have been shown to influence female choice. Male garibaldi appear to experience a trade-off between present and future reproductive investment and employ behavioral tactics which are consistent with predictions of parental investment theory. Whatever the selection factors responsible for the evolution of female preference for spawning near young eggs, these male parental investment tactics appear capable of maintaining or reinforcing this preference in garibaldi.

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Figure III.1. - (a) The relationship between the mean distance a nesting male moved from his nest during a 12 min activity budget and the phase of the brood cycle (see text for definitions). (b) The relationship between the feeding rate of these males and phase of the brood cycle. Data presented as median ± range.

***** = Significantly different from the empty phase (at $p < 0.05$, determined by Friedman's test, followed by Wilcoxon tests)

+ = Difference marginally non-significant ($0.05 < p > 0.10$.)

Figure III.1

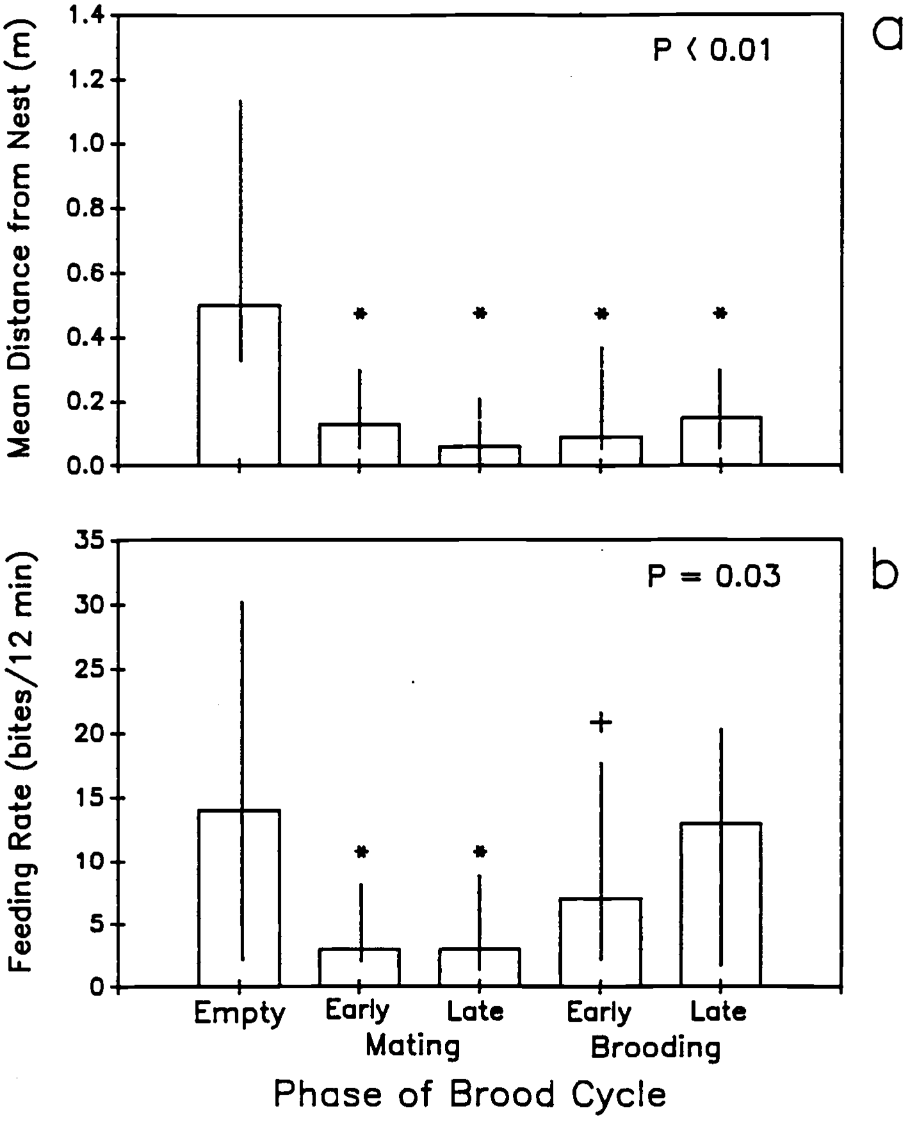

Mean Distance from Nest (m)
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
P < 0.01
a
*
*
*
*
Feeding Rate (bites/12 min)
35
30
25
20
15
10
5
0
P = 0.03
b
*
*
+
Empty
Early
Late
Mating
Early
Late
Brooding
Phase of Brood Cycle

Figure III.2. - Median age of the brood at the time the last clutch was laid for broods in which the last clutch was cannibalized and not cannibalized. Data presented as median ± range. Significance determined by Mann-Whitney test.

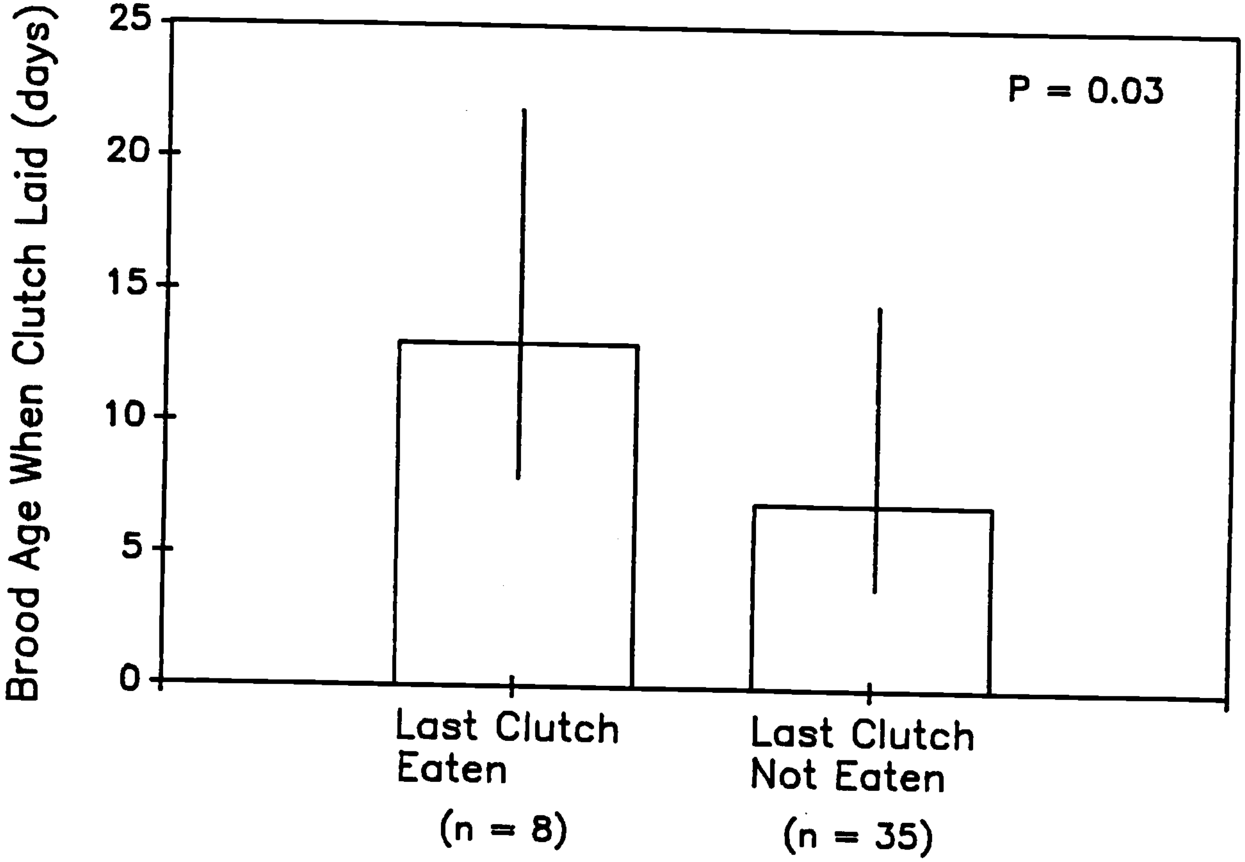

Brood Age When Clutch Laid (days)
25
20
15
10
5
0
P = 0.03
Last Clutch Eaten
(n = 8)
Last Clutch Not Eaten
(n = 35)

Figure III.2

Figure III.3. - Comparison of the probability of cannibalism for single-clutch broods versus first clutches of multiple-clutch broods versus entire multiple-clutch broods (see text for definitions). Significance determined by G-goodness-of-fit test.

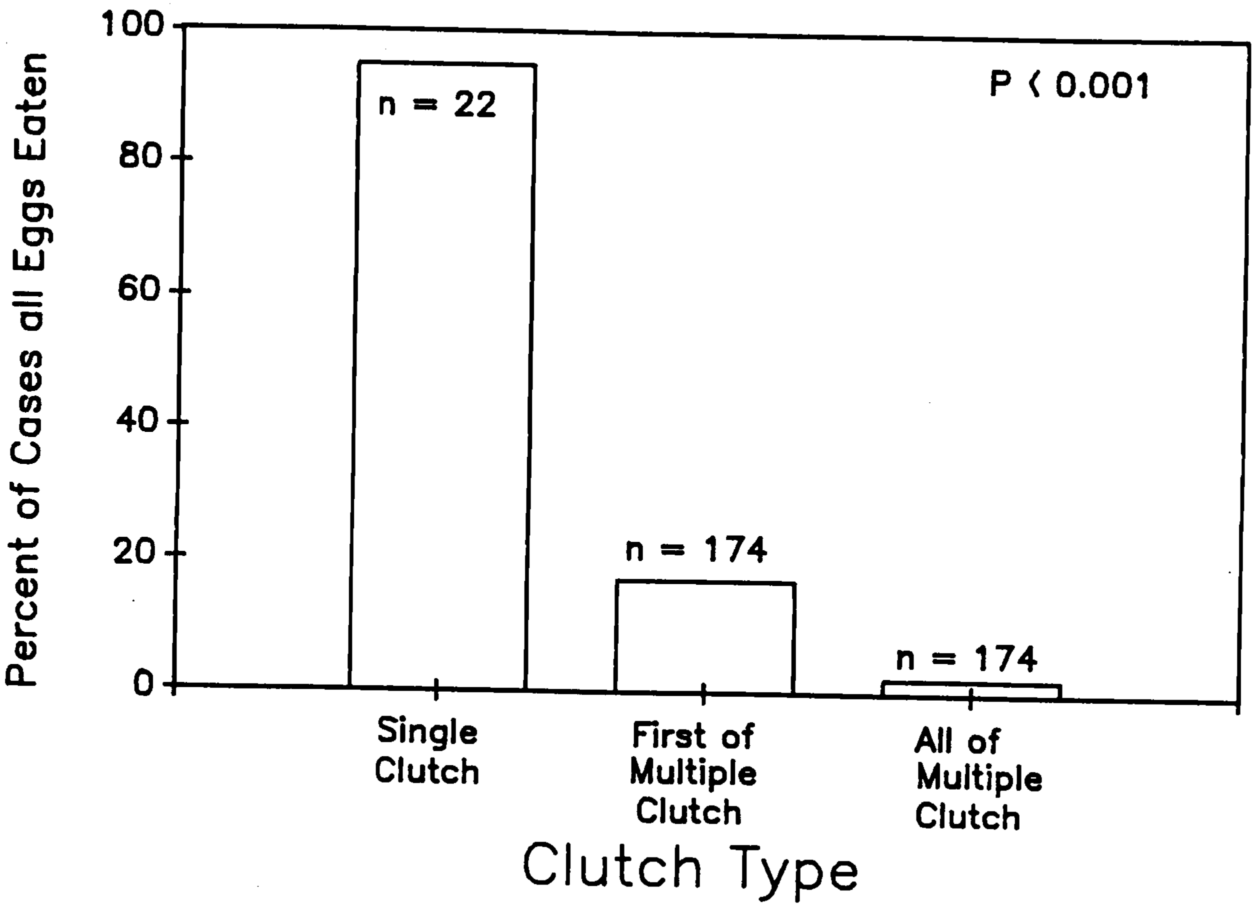

Percent of Cases all Eggs Eaten
100
80
60
40
20
0
P < 0.001
n = 22
n = 174
n = 174
Single Clutch
First of Multiple Clutch
All of Multiple Clutch
Clutch Type

Figure III.3

Figure III.4. - The results of the 1989 brood-size reduction experiment, showing the relationship between brood size and: (a) the percentage of 10-sec observation intervals a male was out of sight of the nest; (b) the mean distance he moved from his nest; and (c) his feeding rate. For each parameter, each data point represents the mean of two 15 min activity budgets conducted on each male. Data are presented as median ± range. * = different from other groups at $p < 0.05$ as determined by Kruskal-Wallis test followed by Mann-Whitney tests.

Figure III.4

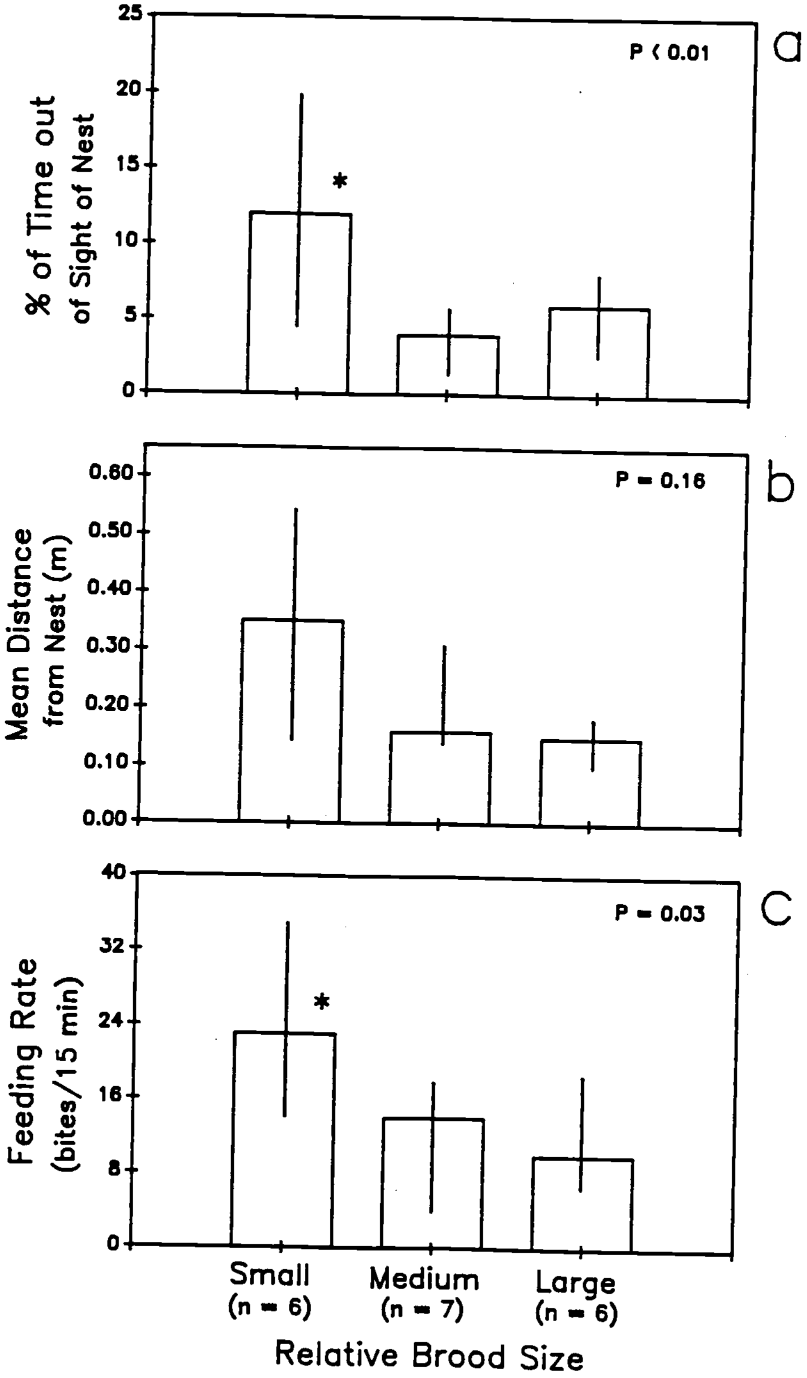

% of Time out of Sight of Nest
25
20
15
10
5
0
P < 0.01
*
a
Mean Distance from Nest (m)
0.60
0.50
0.40
0.30
0.20
0.10
0.00
P = 0.16
b
Feeding Rate (bites/15 min)
40
32
24
16
8
0
P = 0.03
*
c
Small (n = 6)
Medium (n = 7)
Large (n = 6)
Relative Brood Size

Figure III.5. - Results of 1991 brood-size reduction experiment. Variables and symbols as in Fig. III.4. Data are from single 12 min activity budgets for each male. Significance determined by Mann-Whitney test.

Figure III.5

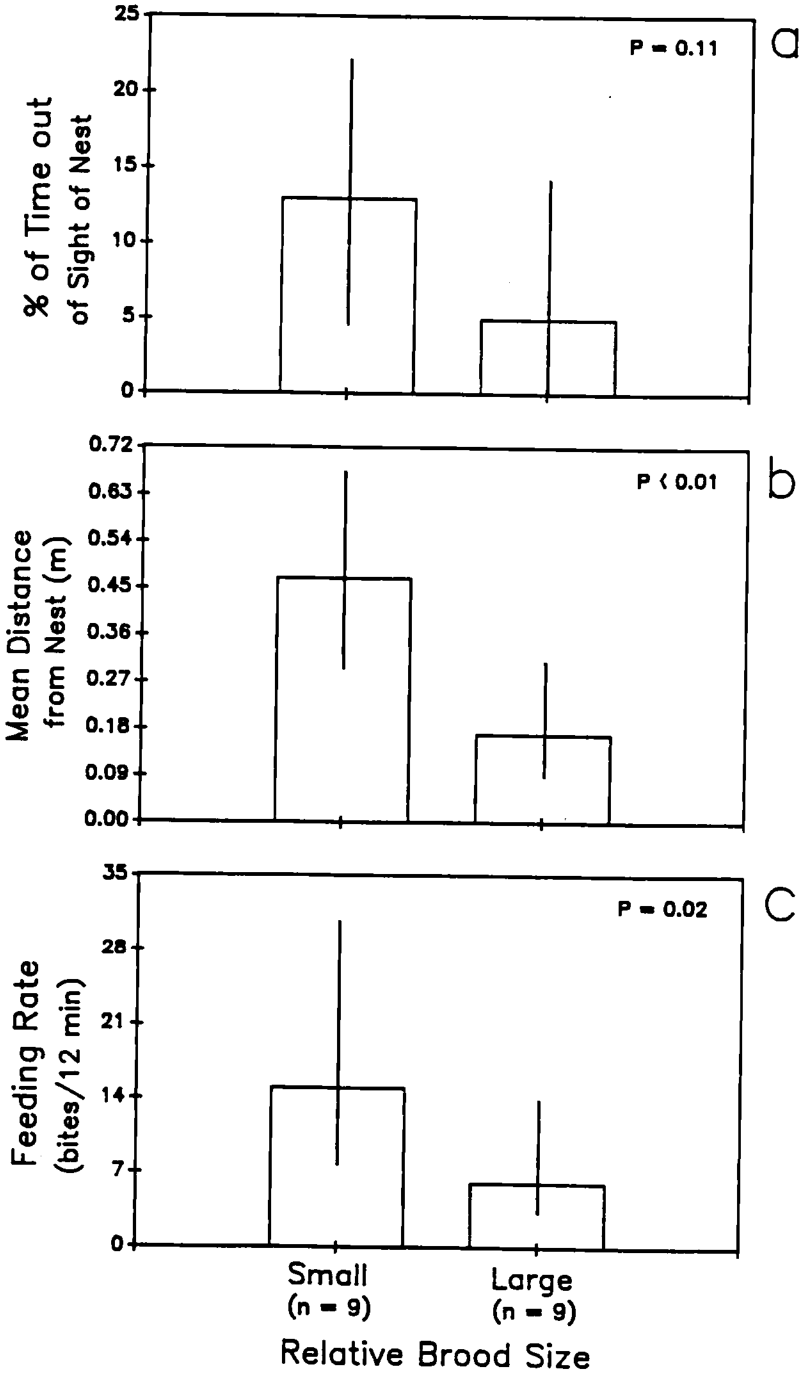
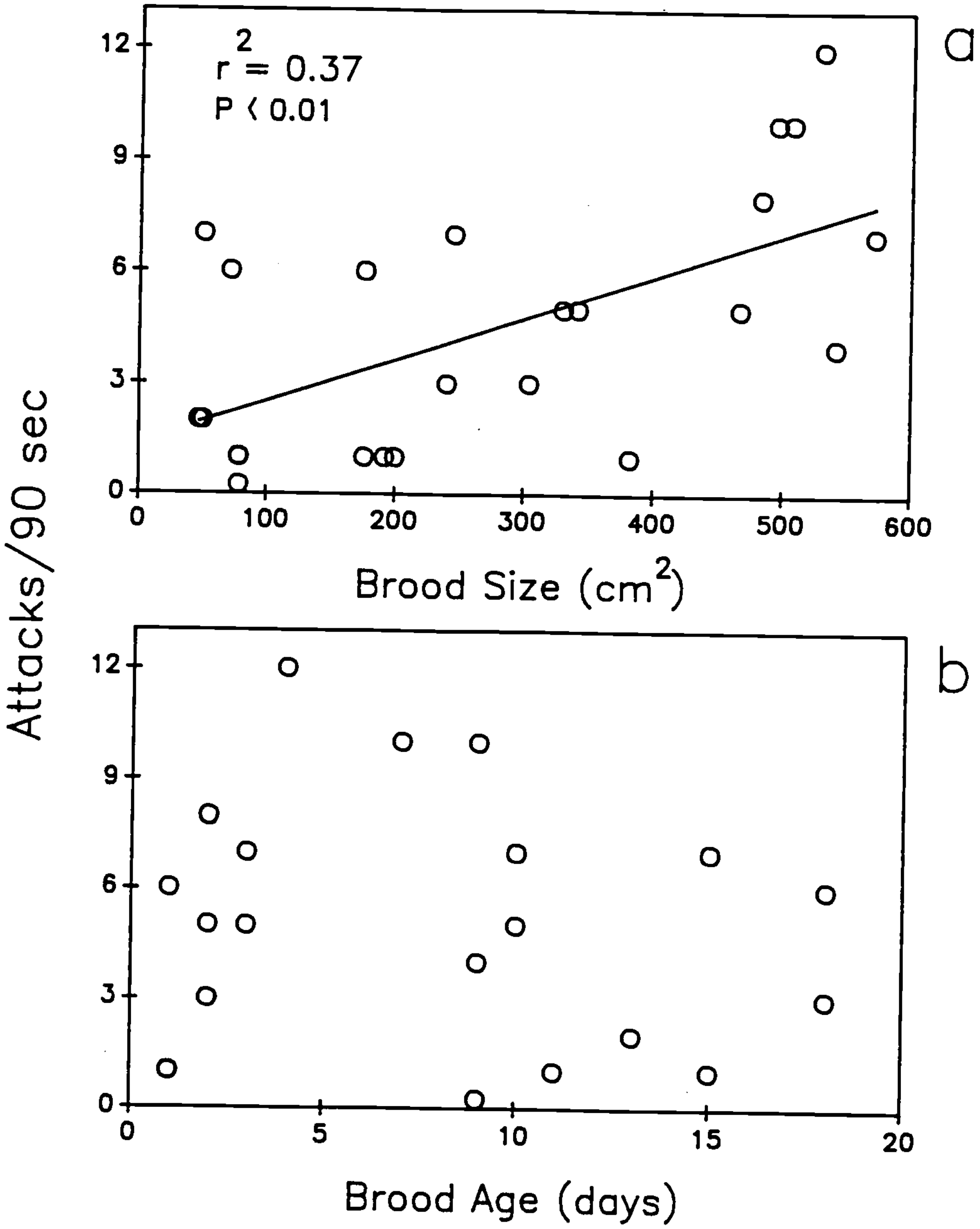

% of Time out of Sight of Nest
25
20
15
10
5
0
P = 0.11
a
Mean Distance from Nest (m)
0.72
0.63
0.54
0.45
0.36
0.27
0.18
0.09
0.00
P < 0.01
b
Feeding Rate (bites/12 min)
35
28
21
14
7
0
P = 0.02
c
Small (n = 9)
Large (n = 9)
Relative Brood Size

Figure III.6. - (a) Simple linear regression fitted to the size of the brood and the rate at which the male attacked a bag containing two juvenile wrasses. (b) The relationship between the age of the brood and attack rate. N = 24 males.

Figure III.6



a
$r^2 = 0.37$
P < 0.01
Attacks/90 sec
12
9
6
3
0
0
100
200
300
400
500
600
Brood Size (cm^2)
b
12
9
6
3
0
0
5
10
15
20
Brood Age (days)

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CHAPTER 4

Changes in pasma androgen levels associated with changes in male reproductive behavior in a brood cycling marine fish

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Running Head: Androgens and male fish brood cycles

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Abstract Like many paternal-caring fishes, male garibaldi (*Hypsypops rubicundus*) exhibit changes in reproductive behavior during the course of a brood cycle. The purpose of this study was to compare plasma levels of testosterone (T) and 11-ketotestosterone (11-KT) among males in different phases of the brood cycle. These phases are characterized by differences in male courtship, nest maintenance, and egg fanning behavior. Blood samples were taken from wild-caught males and plasma androgen levels were measured using radioimmunoassay techniques. Androgen levels among males in different phases of the brood cycle were concordant with differences in courtship behavior, and also showed a general, positive correspondence with nest

maintenance behavior. Levels were nearly inversely related to egg fanning rates. These relationships were independent of seasonal or population level environmental effects. These data suggest a strong three-way relationship among androgen levels, reproductive behavior, and stimuli associated with the progressing brood cycle.

Paternal care of offspring is widespread among oviparous vertebrates (Clutton-Brock 1991). In many species, parental males undergo sequential or cyclic changes in sexual and parental behavior, resulting in so-called brood cycles (e.g., van Iersel 1953; Townsend 1986; Wingfield et al. 1990). The initial period of courtship, mating, and in some species nest establishment, has traditionally been referred to as the "mating" phase. The subsequent period of parental care, during which little or no sexual activity occurs, has been referred to as the "brooding" or "parental" phase. The behavioral changes that occur during the course of a brood cycle have attracted considerable attention from behavioral and reproductive endocrinologists. For parental males, the role of androgens has probably received the most attention (reviewed by Brown 1985).

Over the past decade, increasing emphasis has been placed on the use of field studies to understand the role of androgens and other hormones in controlling reproductive behaviors (Liley et al. 1987; Wingfield et al. 1990). The vast majority of field work on the endocrinology of brood cycling has focused on birds (e.g., Oring, et al. 1989; Wingfield et al. 1990). Most work on the relationship between androgens and behavior in brood cycling fishes has been conducted in the laboratory on a few freshwater species (Brown 1985;

Liley and Stacey 1983; Stacey 1987). To my knowledge, appropriate field data are available for only two species; the bluegill sunfish, *Lepomis macrochirus* (Kindler et al. 1989), and the demoiselle, *Chromis dispilus* (Pankhurst 1990). In addition, since so few species have been examined and all have very similar mating systems (e.g., temporary territories, short mating phases), it is unclear to what extent these results can be generalized.

Here I report the results of a correlative field study designed to assess the relationship between gonadal androgens and changes in reproductive behavior in a brood cycling marine fish, the garibaldi (*Hypsypops rubicundus*). The specific goal of this study was to compare changes in male courtship, nest maintenance, and parental care behavior during the brood cycle with changes in plasma levels of testosterone and 11-ketotestosterone, two androgens known to influence male reproductive behavior in teleost fishes (Liley and Stacey 1983; Matty 1985; Liley et al. 1987).

SUMMARY OF BREEDING BIOLOGY

The garibaldi is a large (up to 36 cm total length) damselfish (Pomacentridae) found in rocky subtidal areas of southern and Baja California (Clarke, 1970). Garibaldi differ from the two other brood cycling fishes examined in the field (C. dispilus and L. macrochirus) in that: 1) both sexes defend year-round territories that include food, shelter, and nest sites for males (a mat of red algae on a boulder); and 2) spawning in a local breeding population occurs daily throughout the spring/summer spawning season and thus is not restricted to synchronized breeding episodes (Sikkel 1988, 1989, this study).

Prior to spawning, a female garibaldi leaves her territory, exhibiting erect fins and swimming erratically. I have referred to this elsewhere as "searching behavior" (Sikkel 1989). Such females also tend to have visibly swollen abdomens. Females exhibiting searching behavior elicit courtship displays as they pass by the nests of receptive males. These females typically enter the nests of numerous males before spawning with one. Females that do not exhibit this behavior are ignored by males or are chased if they pass through the male's territory.

A brood cycle begins when a male receives an initial clutch of eggs. Males typically continue to spawn with numerous females over a period of about 5 to 10 days (up to 20 days in rare cases). Precisely what determines how long a male spawns during a brood cycle is not clear. However, males continue to spawn until the nest has reached at least 70% of its capacity, and at least 30% of the eggs are gray (a developmental color change that occurs after approx. 5 days). Males never spawn when more than 80% of the eggs are gray and fewer than 10% spawn when more than 70% are gray. The brood cycle is complete after three to five weeks, when all eggs have hatched. A given clutch requires approximately 2-3 weeks to hatch, and males do not care for the planktonic larvae. Males may complete up to four brood cycles in a given season.

Typical of brood cycling species, males exhibit changes in certain behaviors during the course of the brood cycle (Sikkel 1989, this study; Fig. IV.1). The most conspicuous changes are in the tendency of males to court females that exhibit searching behavior (courtship readiness), the tendency of males to allow females into the nest after courtship (admittance), the rate of algal fanning, and the rate of egg fanning. When there are no eggs in the nest, male courtship readiness and admittance are high. Both decrease as the brood cycle progresses, although courtship readiness begins to increase as the eggs approach hatching. Algal fanning, performed with the anal fin, is a nest maintenance behavior and occurs only prior to and during the mating phase. In contrast, egg fanning, performed with the pectoral fins, occurs almost exclusively during the brooding phase.

METHODS

Behavior

I conducted the field portion of this study at the west end of Santa Catalina Island (33 28′N, 118 29′ W) located approximately 38 km off Los Angeles, CA. I collected data on the reproductive behavior of garibaldi in and around Big Fisherman Cove over a six year period (1986-1991).

To illustrate the changes in courtship, nest maintenance, and parental behavior during the brood cycle, and to compare levels of these behaviors with androgen levels, I used data from 1991 that I collected for 23 males that completed at least one brood cycle. I selected these data because they included observations for the greatest number of males throughout the brood cycle. However, they are consistent with my observations for the previous five spawning seasons. I collected data on nest and egg tending behaviors during timed focal observations of males. I collected data on male courtship by following females that exhibited searching behavior (described above), and recording the responses of nesting males to the focal female (see Sikkel 1988, 1989 for details). I recorded only those cases where the female passed by the male′s nest while he was facing (and thus presumably saw) her. I used data for five different phases of the brood cycle which correspond to those phases for which I measured androgen levels (see below). I have defined these phases as follows: 1) Premating, no eggs in the nest; 2) Early mating, nest approximately half full, all eggs yellow (< 5 d old); 3) Late mating, nest > 60% full, 10 - 50% eggs gray (5-10 d old); 4) Early brooding, > 70% full, > 70% eggs gray; and 5) Late brooding, nest > 70% full, all eggs silver (> 10 d old, close to hatching).

For each behavior, I used data from only one observation per phase per male. Where more than one observation was available for a given phase of the brood cycle (courtship behaviors), for the premating phase I used the observation closest to the day the male received his first clutch of eggs. For other phases, I used the first observation.

Collection of blood samples

I collected blood samples from 37 males that occupied territories in and around Big Fisherman Cove. I collected samples from 29 reproductive males between 27 May and 27 June 1990, approximately the peak of the spawning season at Santa Catalina Island. The males collected were in one of five phases of the brood cycle, as determined by the presence and developmental stage of eggs and whether or not they were algal fanning (described above). I also collected samples from eight males that had stopped nesting between 17 June and 14 July, 1990. To control for possible diel effects, I collected all samples between 1000 and 1400 hours.

The 37 fish sampled were opportunistically selected by a scuba diver who gave lowest capture priority to males in phases that had most recently been collected. The diver captured each fish using a monofilament landing net. He then transferred it to a free diver who swam the fish to a skiff where I immediately anaesthetized it by placing it in a bucket containing 500 mg/L of tricaine methanosulfate (MS-222, SIGMA) for 30 sec. I then drew 2 to 5 ml of blood from the caudal artery using a heparinized syringe. The time elapsed between capture and immersion in the anaesthesia ranged from 20 to 90 sec., and bleeding required 3 to 9 min. After drawing the sample, I transferred the blood to a sealed plastic culture tube and stored it on ice until I returned to the laboratory (1 - 3 hrs) where I immediately centrifuged it at approximately 766g for 10 min. I then removed the plasma and stored it at -80 C. I later shipped the samples in dry ice and again stored them at - 80 C.

Steroid measurement

I measured plasma levels of T and 11-KT using radioimmunoassay (RIA). The reagents and protocol used in the extraction and assay of samples were those of Sower and Schreck (1982) as modified by Fitzpatrick et al. (1986). I validated the assays for garibaldi by determining parallelism between a standard curve and a five point serial dilution of extracted plasma. Extraction efficiency was 70% for T and 82% for 11-KT. I corrected steroid concentration estimates accordingly. Minimum detection limits were 0.27 ng/ml for T and 0.91 ng/ml for 11-KT. To eliminate interassay variation, I processed all samples at the same time. The distribution of plasma androgen levels did not meet the assumptions of parametric analyses of variance or t-tests, even after transformation. Thus, I used non-parametric analogues to compare differences in androgen levels. For Kruskal-Wallis tests, I used the multiple comparison methods of Marascuilo and McSweeney (1967) as described by Zar (1984).

RESULTS

For both androgens, the lowest levels for nesting males (early brooding phase) were significantly higher than levels for post reproductive males ($p = 0.02$ and

0.03 for T and 11-KT respectively, Mann-Whitney test). For 11-KT, plasma levels were below the detection limit for four of eight post reproductive males. For these four cases, I used the detection limit for analysis.

For nesting males, plasma concentrations of both androgens varied significantly among phases of the brood cycle ($p = 0.02$ and 0.003 for T and 11-KT respectively, Kruskal-Wallis test). Levels were highest for males with empty nests (premating phase) and decreased for males in successive phases of the brood cycle until the early brooding phase (Fig. IV.2). Levels then increased from the early to the late brooding phase, although the increase was not statistically significant.

Changes in androgen levels over the five phases of the brood cycle showed the same pattern as changes in male courtship readiness. There was also a general, positive, correspondence between androgen levels and both admittance and algal fanning. However, trends in the changes of these behaviors were opposite trends in androgen levels between the early and late brooding phases and premating and mating phases, respectively. Egg fanning showed a general inverse relationship to androgen levels, with the only deviation being the tendency for both to increase between the early and late brooding phases.

I did not collect samples from males in each phase of the brood cycle on each collecting day. However, there are three lines of evidence that differences in androgen levels among males in different phases of the brood cycle were associated with reproductive activities of individual males, and were not due solely to seasonal or population level factors. First, in Big Fisherman Cove, males in each phase of the brood cycle were present on each collecting day (I did not systematically survey the other collecting sites; Table IV.1). Second, for "blocks" of 2-3 collecting days (yielding data for 3-5 phases per block), relative androgen levels were consistent with the pattern for pooled data, and hence consistent with changes in courtship readiness (Fig. IV.3). Finally, for both androgens, this relationship held for three of four cases in which I collected males in two phases of the brood cycle from the same location on the same day (3, 6, and 10 June, but not 16 June). For 11-KT, this relationship also held in both cases in which I collected males in three different phases of the brood cycle from the same location on the same day (7 and 8 June). For T, in both cases, two of the three pairwise comparisons showed the expected relationship (Fig. IV.3).

DISCUSSION

The data presented here suggest a close relationship between changes in behavior and changes in androgen levels in male garibaldi over the course of a brood cycle. A positive relationship between courtship activity and testosterone, and a negative relationship between these and parental care behavior has been found in correlative and experimental field studies on a number of bird species (e.g., Wingfield 1984; Oring et al. 1989; reviewed by Wingfield and Moore 1987). Few field data exist for other vertebrates. Townsend and Moger (1987) reported that total androgen was highest for calling and amplectant males of a paternal-caring frog, Eleutherodactylus coqui. Levels declined sharply within 12 hours after the male fertilized the eggs, and remained low until hatching, at which time levels increased. However, administration of T during the parental phase did not produce calling behavior (Townsend et al. 1991). In the only fish studies, Kindler et al. (1989) found increasing levels of T and 11-KT for bluegill sunfish, Lepomis macrochirus, up until the day of spawning. Levels then dropped by approximately two (T) and four (11-KT) fold the day after spawning and remained low during the brooding phase. Similarly, Pankhurst (1990) reported that males of the demoiselle, Chromis dispilus with no eggs or very young (0-2 d old) eggs had the highest levels of T and 17a,20B dihydroxy-4-pregnen-3-one. Levels then declined for males with 2-4 day old eggs, but increased again for males with 4-5.5 day old eggs.

In this study, levels of T and 11-KT showed similar changes during the course of the brood cycle, although 11-KT declined more sharply from the premating to the early brooding phase. The relative effects of these androgens on reproductive behavior is unclear. 11-KT is thought to be a more potent regulator of male sexual behavior in a variety of teleosts, including brood cycling species (Liley and Stacey 1983; Mayer et al., 1990). In field experiments, Kindler et al. (1991) showed that 11-KT had a greater effect than T in eliciting a prespawning behavior in nesting male L. macrochirus.

It is not clear in garibaldi or any fish system studied thus far what stimuli trigger the changes in androgen levels during the course of the brood cycle. Nor is it clear how the effects of these stimuli on androgen levels might be mediated. In fishes and other vertebrates, androgen levels are known to be influenced by a variety of social and environmental factors (e.g., Liley et al. 1986; Orchinik et al. 1988; Cardwell and Liley 1991). In birds, prolactin levels appear to increase in response to the presence of offspring (Oring et al. 1986; Lea and Sharpe 1991, reviewed by Brown 1985), and may have an antiandrogen effect (El Halawani et al. 1984). In brood cycling fishes, androgen levels in parental males could be influenced by such factors as the number and or age of offspring (eggs), the availability of mates and/or competitors, and environmental factors such as temperature or phase of the lunar cycle. In *L. macrochirus* and *C. dispilus*, spawning occurs in temporary breeding colonies during synchronized spawning episodes. Thus, in these species, changes in androgen levels may be determined by environmental or population level factors. In garibaldi, spawning occurs daily (during the spawning season) within permanent breeding colonies, and brood cycles are much less synchronized. Thus, while population level or environmental factors may have some effect on androgen levels in male garibaldi, it appears that stimuli associated with the brood may also play a role. Determining the cause and effect relationships among these stimuli, androgens, and reproductive behavior will require experimental study.

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Table IV.1. The distribution of males (n = 40) in Big Fisherman Cove during 1990 that were in five phases of the brood cycle or had stopped nest-tending ("post") on dates when blood samples were collected from reproductive males. Late brooding phase includes nests where egg hatching had begun.

Date	Phase of Nesting Cycle					
		Mating		Brooding		
	Pre	Early	Late	Early	Late	Post
27 May	26	3	3	4	4	0
3 June	22	5	4	3	6	0
4 June	22	6	5	3	4	0
5 June	22	6	3	5	4	0
6 June	23	6	3	5	3	0
7 June	21	6	3	5	5	0
8 June	20	4	6	5	5	0
10 June	19	5	6	5	4	1
16 June	12	8	4	4	10	2
17 June	12	9	2	5	10	2
27 June	10	5	2	8	7	8

Figure IV.1 - Changes in behavior of male garibaldi during the course of the brood cycle (see text for definition of brood cycle phases): a) the percentage of males that courted a focal female they saw exhibiting searching behavior (see text for description); b) the percentage of courting males that allowed females into their nest following courtship; c) rates of algal fanning; d) rates of egg fanning. All data were taken from a population of 23 males. Numbers in parentheses indicate the subset of these males for which data were available. For each behavior, data represent repeated measures of the same males, except that not all males began a second cycle or were monitored for a given behavior during a second cycle. Where shown, bars represent standard errors. Overall differences among phases (excluding the second premating phase) are significant at $p < 0.05$ for each behavior (Cochran's Q for courtship behaviors, and Friedman's tests for fanning behaviors).

Figure IV.1

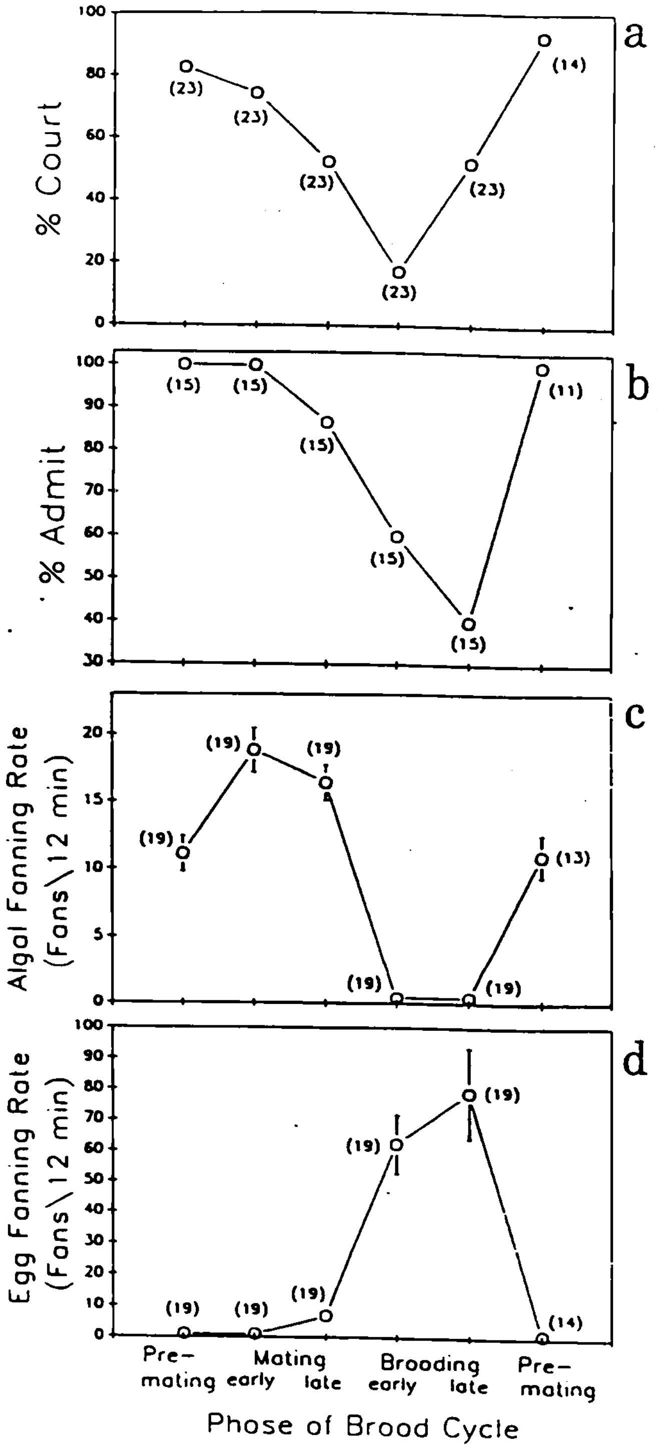

a
% Court
100
80
60
40
20
0
(23)
(23)
(23)
(23)
(23)
(14)
b
% Admit
100
90
80
70
60
50
40
30
(15)
(15)
(15)
(15)
(15)
(11)
c
Algal Fanning Rate
(Fans\12 min)
20
15
10
5
0
(19)
(19)
(19)
(19)
(19)
(13)
d
Egg Fanning Rate
(Fans\12 min)
100
90
80
70
60
50
40
30
20
10
0
(19)
(19)
(19)
(19)
(19)
(14)
Pre-mating
Mating
early
late
Brooding
early
late
Pre-mating
Phase of Brood Cycle

Figure IV.2 Mean (± SE) plasma concentrations of testosterone (T) and 11-ketotestosterone (11-KT) for 37 male garibaldi. Dashed lines indicate medians where distinguishable from means. Sample sizes are shown in parentheses. Identical letters indicate lack of significance between pairs. Note that the mean and median for 11-KT in post reproductive males were estimated using the assay detection limit for cases (n = 4) where plasma concentrations were below the detection limit (see text). Hence, no standard error is shown. Compare with Figure 1.

Figure IV.2

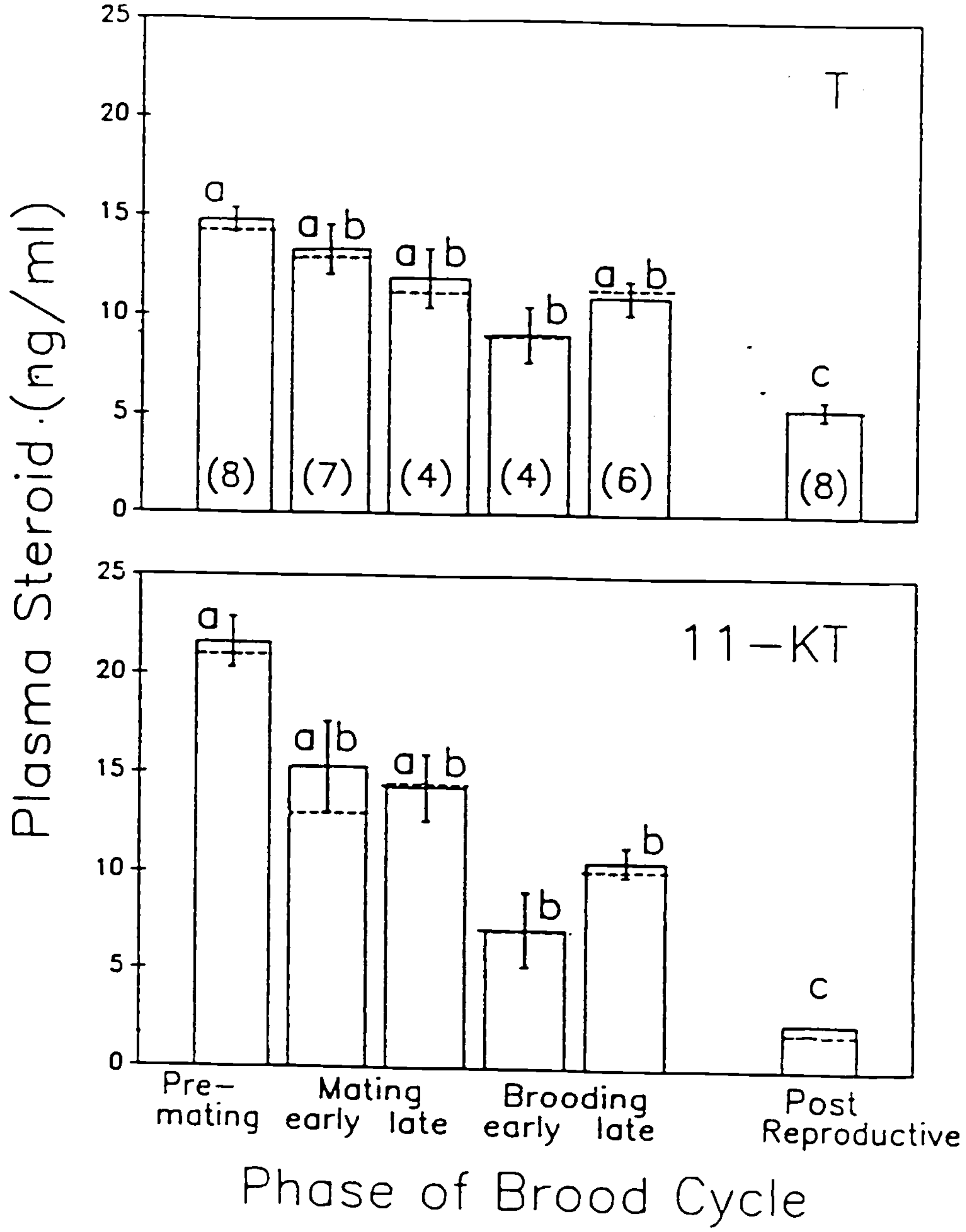
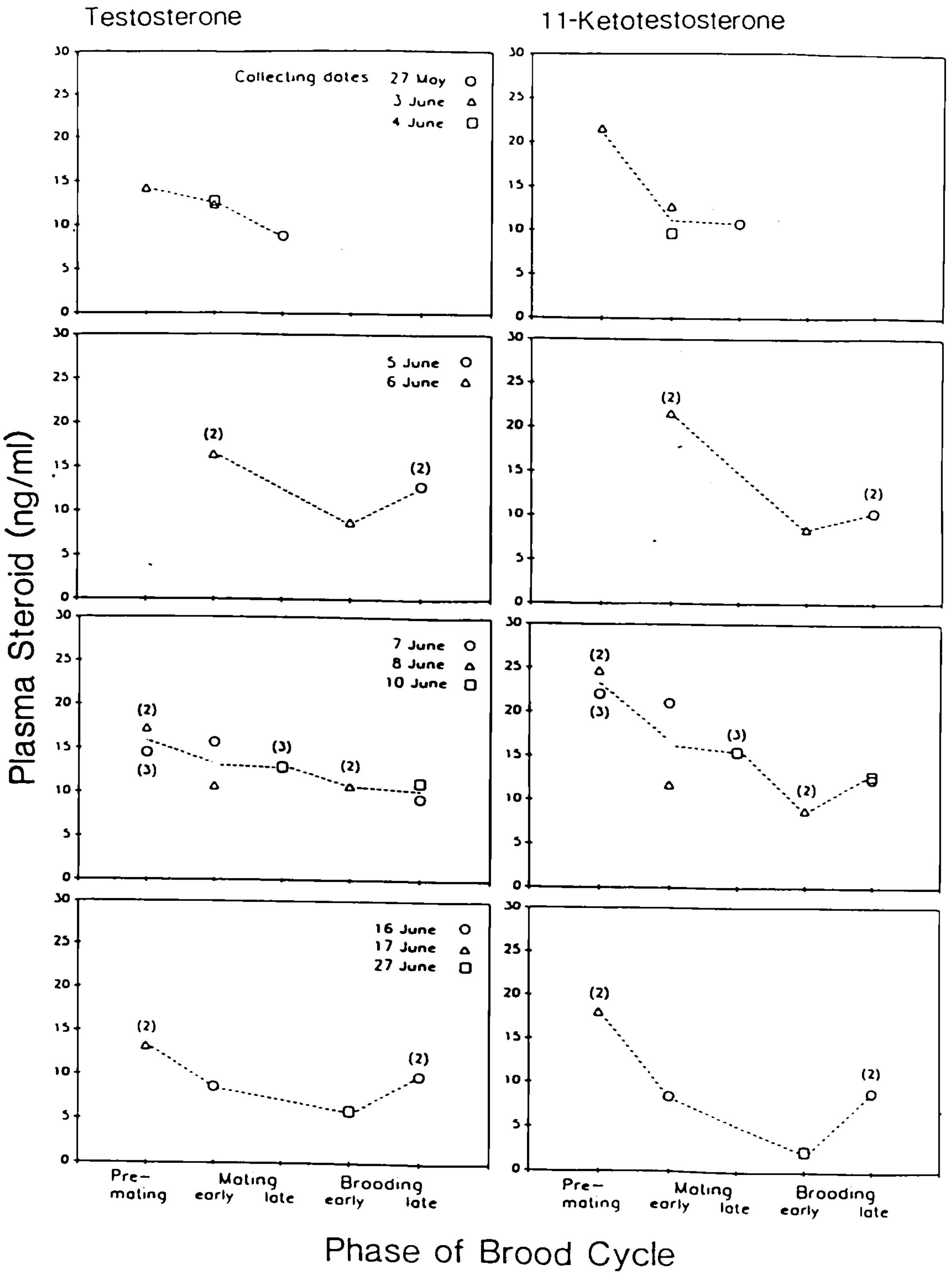

Plasma Steroid ·(ng/ml)
T
11–KT
0
5
10
15
20
25
a
a b
a b
b
a b
c
(8)
(7)
(4)
(4)
(6)
(8)
Pre–
mating
Mating
early
late
Brooding
early
late
Post
Reproductive
Phase of Brood Cycle

Figure IV.3. - Changes in plasma concentrations of T and 11-KT for reproductive males shown for blocks of 2-3 consecutive collecting dates. For each block, different symbols are used for different collecting dates. Means are used where more than one observation was available for a given phase on a given date. In these cases, sample sizes are shown in parentheses. For illustrative purposes, lines in each block are plotted through the overall mean for a given phase.

Figure IV.3


Testosterone
11-Ketotestosterone
Collecting dates
27 May ○
3 June △
4 June □
5 June ○
6 June △
7 June ○
8 June △
10 June □
16 June ○
17 June △
27 June □
(2)
(3)
0
5
10
15
20
25
30
Plasma Steroid (ng/ml)
Pre-mating
Mating early late
Brooding early late
Phase of Brood Cycle

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