

Social control of male sexual maturation in the swordtail characin, *Corynopoma riisei*

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The presence of adult male *Corynopoma riisei* was found to inhibit the maturation of juvenile males. Males in a conspecific community tank took longer to mature and did so at increasingly slower rates compared to isolated controls, providing a positive correlation between size and time of maturational onset. The inhibitory effect increased with greater numbers of adult males. The first males to mature did so at accelerated rates, suggesting positive as well as negative control. Tanks in which adult males were removed as soon as maturation was complete showed an intermediate pattern, with no lengthening of the maturational time course but an increase in standard length. Histological examination revealed immature males with lengths greater than the control range; these appear to represent inhibited animals. Both the testes and pituitary gonadotropic zone of these males appeared undeveloped, arguing for inhibition at least at the hypothalamic level. This phenomenon has also been described in poeciliids. The presence of a similar complex mechanism in two distant groups of fishes suggests convergent evolution. It also suggests that this phenomenon may be physiologically possible for a wide range of fish species.

Key words: social control; sexual maturation; Glandulo-caudinae.

I. INTRODUCTION

A phenomenon exists in some groups of fishes in which their sexual maturation is under social control. This has been well-documented in the Poeciliidae. Borowsky (1973a) described a relationship between size and maturation for the platyfish, *Xiphophorus variatus* (Meek), in which large mature males inhibited the maturation of juvenile males. This inhibition was not absolute, but caused instead a delay in maturation while allowing somatic growth. In this way, within a group of maturing fish, a positive correlation between size and time of maturation was seen.

Inhibition decreases as group size increases (Borowsky, 1973b), and larger numbers may also allow some fish to mature in synchrony (Borowsky & Diffley, 1981). Field studies have confirmed the existence of social control among the Poeciliidae in the wild (Borowsky, 1978; Chapman *et al.*, 1991). Work with other poeciliids in the genus *Gambusia* has shown evidence both for (Hughes, 1985; Campton & Gall, 1988) and against (Yan, 1987) the presence of social control mechanisms. Social maturation control has also been demonstrated in *Pseudolabrus celidotus* (Bloch and Schneider) (Jones & Thompson, 1980) and *Thalassoma duperrey* (Quoy and Gaimard) (Ross, 1987) of the family Labridae, the cichlid fish *Sarotherodon mossambicus* (Peters) (Silverman, 1978),

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and the freshwater prawn *Macrobrachium rosenbergii* (de Man) (Karplus *et al.*, 1991).

Observations of aquarium-reared specimens of the characid fish, *Corynopoma riisei* (Gill), suggested the existence of inhibition of male sexual maturation in this species (S. H. Weitzman, pers. comm.). *C. riisei* is a small (4.5-5.5 cm) carnivorous freshwater fish found in Trinidad and Venezuela. It belongs to the Glandulocaudinae, a group of characid fish genera first recognized by Eigenmann *et al.* (1914). It is a shoaling species, found throughout its range in fairly slow, turbid water (Nelson, 1964). Males in this group usually possess epidermal glandular tissue on both sides of the caudal peduncle, covered by caudal fin scales modified to form a bellows pump (Weitzman & Fink,). A pheromone function has been suggested for this tissue (Atkins & Fink, 1979; Weitzman & Fink, 1985). *C. riisei* is sexually dimorphic. Males possess long unpaired fins, hooks on the anal fin used in internal fertilization (Kutaygil, 1959), and an opercular extension, which is moved in a male's frontal plane perpendicular to its body during courtship to position the female for insemination (Nelson, 1964).

The purpose of this study was to investigate the existence of a social maturational control in *C. riisei*. The neotropical characins are a very large diverse group, separated at the superorder level (Nelson, 1984) from the Poeciliidae. The presence of a similar complex mechanism in such diverse and distant groups could argue for environmental and social constraints playing an important role in their evolution.

II. MATERIAL AND METHODS

The study consisted of two parts. Part 1 was designed to determine the existence of the inhibition phenomenon by observing maturing fish under three social regimes. Part 2 was a histological analysis of immature, mature, and suspected inhibited males.

PART 1—OBSERVATIONS

All fish used were raised in four 57-l stock tanks. These tanks were part of a closed system, each tank draining into a common wet/dry filter and receiving recycled water from this filter. Adults in this system were induced to breed, and the resulting juveniles were collected at standard lengths (S.L.) of 10–20 mm. Because juvenile males and females are indistinguishable externally, the sexes of experimental fish were not known at the time of collection.

Experimental tanks, either 38-l or 3.8-l in volume, were prepared for the juveniles. They were filled with deionized water, with sea salts added to produce a hardness equal to that of a mixture of equal volumes of deionized water and Washington, D.C. city tap water. Water temperature was 25° C, pH between 6.0–6.8, and photoperiod was 12:12 hours. All fish were fed daily with either TetraMin flake food, dried *Euphausia* sp., newly hatched *Artemia* sp. or live wingless *Drosophila* sp. All tanks were kept visually isolated and no water transfer was allowed among tanks. Water quality was maintained by either an undergravel filter for the 38-l tanks or an airstone for the 3.8-l tanks. Partial water changes were done periodically, or when the water quality in any tank was seen to be deteriorating. Once the experimental tanks were well established, juveniles were placed into them according to the following protocol: Group A, 10 juveniles were placed in each of three 38-l tanks; Group B, 10 juveniles were placed in each of two 38-l tanks, and males were removed when they had reached full sexual maturity; Group C, 15 juveniles were placed into individual 3.8-l tanks; this group acted as a control.

Group A was used to test the hypothesis that *C. riisei* exhibits a social control of sexual maturation. Under this hypothesis early maturing males should influence the maturation pattern of later maturing males in the tank. Group B was used to show cessation of inhibition upon removal of the mature males from the tank. Under the above hypothesis maturational inhibition should be absent or decreased in the males from group B. Group C served as a control, where isolated juveniles could provide data on length at maturation and rate of maturation in the absence of potentially inhibiting males.

Once per week, for each group, the fish were removed and anaesthetized with tricaine methanesulphonate (1: 10 000). For each fish, S.L. (mm) and weight (g) were recorded. If a maturing male was discovered, its degree of sexual maturity was recorded by measuring the length of the opercular paddle once its growth was detected. Kutaygil (1959) reported that the paddle is the first external secondary sex character to develop. Each group was terminated when all fish had exceeded the normal length for maturational onset, noted in Kutaygil (1959) as 27 mm. The fish were killed with an overdose of tricaine methanesulphonate, and dissected to confirm sex and determine the degree of sexual maturation.

The S.L. at onset of maturation were calculated for each male by plotting S.L. (ordinate) *v.* paddle length (abscissa). The *Y*-intercept was taken as the S.L. at a hypothetical paddle length of zero. A rate of maturation was defined for each male as the slope of a plot of paddle length (ordinate) *v.* day when that length was recorded (abscissa). The points used were between paddle lengths of 4 and 8 mm. If a male's paddle did not reach 8mm, then the point where steady growth was initiated and the point of greatest length reached were used. Each slope then represents the maximum paddle growth rate for that male, expressed in mm day⁻¹.

For each group and each tank, standard length and paddle length data for each male were analysed. Replicates were pooled for each group and analysed as a whole (group A: *n*= 14; group B: *n*= 11; group C: *n*=6). The mean time required for all males to begin maturation, the mean standard length at maturational onset, and the temporal patterns of standard length at onset and maturational rate were examined for each group. When means were examined, the first data point for each tank in groups A and B, representing the first male to mature, was not included in the analysis. These males were not subject to the same conditions as the others, and the data taken from them did not reflect the group treatment conditions. When analysing temporal patterns, they were included, since they were thought to represent a non-treatment baseline from which deviations could be examined.

PART 2—HISTOLOGY

Since all males in Part 1 were allowed to reach the size of full sexual maturity before removal or termination of the trials, additional tanks were set up to provide data on external morphology and the histology of the pituitaries and testes of males at varying stages of sexual maturation. Ten juveniles were placed in each of two, 38-1 tanks. In one, all fish were removed simultaneously after they had reached adult size but had not necessarily matured. In the other tank, males were removed at the onset of sexual maturation.

All fish from Parts 1 and 2 were fixed in Bouin's solution and decalcified. Heads and bodies were separately dehydrated, embedded in paraffin and sectioned at 5 μ m. Sagittal sections of the head and transverse sections of the body were stained with Masson's trichrome (Schreibman, 1964). Testes were examined for degree of maturity, as determined by the presence of the latest spermatogenic stages. Pituitaries were examined for the degree of development of the presumptive gonadotropic zone in the proximal pars distalis.

III. RESULTS

PART 1—OBSERVATIONS

Mean differences were analysed using one-way analyses of variance, with further examination of differences between groups using Tukey's HSD test

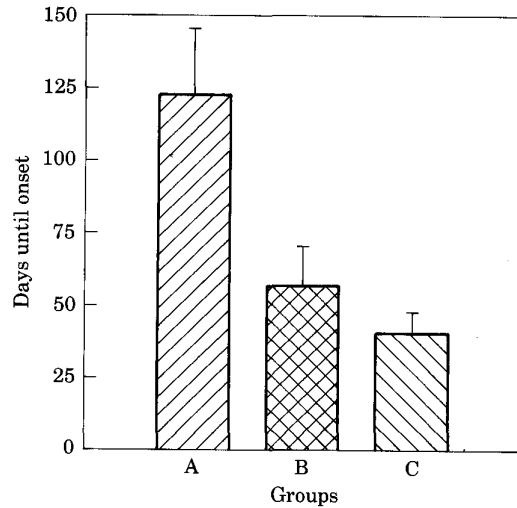


FIG. 1. The mean number of days required for males to commence sexual maturation in each experimental group. Bars represent standard error. Group A, no removal; Group B, removal; Group C, isolated controls.

(Jaccard, 1983). The males in each group began maturing at different times ($F=6.56$, $P<0.05$) (Fig. 1). Group A males took significantly longer to begin maturation than those in the control group C ($CD=62.58$, $P<0.05$), or in group B ($CD=62\sim 58$, $P<0.05$). No significant difference was found between groups B and C ($CD=62\sim 58$, $P>0.05$). The mean s.L. at which males began maturation (Fig. 2) differed between groups ($F=9.51$, $P<0.05$). Groups A and B both were significantly longer than the control group C ($CD=3.06$, $P<0.05$; $CD=3.06$, $P<0.05$). There was no significant difference between the means of groups A and B ($CD=3.06$, $P>0.05$).

Male size at maturational onset was further examined in each experimental group by plotting the standard length for each male against the day at which sexual maturation began (Fig. 3). Significant positive Pearson's correlations were found for both group A ($r=0.68$, $P<0.05$) and group B ($r=0.71$, $P<0.05$), while no significant correlation was evident for group C ($r=0.31$, $P>0.05$). Late maturing males in groups A and B also commenced maturation at lengths greater than those of the control group C (Fig. 3).

Rates of sexual maturation were determined by measuring rates of paddle growth. For each male and group, rates of paddle growth were plotted against the day when that growth began (Fig. 4). Group A males showed a significant negative correlation ($r=-0.82$, $P<0.05$), indicating that late maturing males did so at increasingly slower rates. Neither group B ($r=-0.48$, $P>0.05$) nor group C ($r=0.68$, $P>0.05$) males showed significant correlations.

PART 2—HISTOLOGY

The degree of male sexual maturation was determined externally by observing the length of the opercular paddle and the colour and development of the unpaired fins. Histological examination of the testes and pituitaries was carried

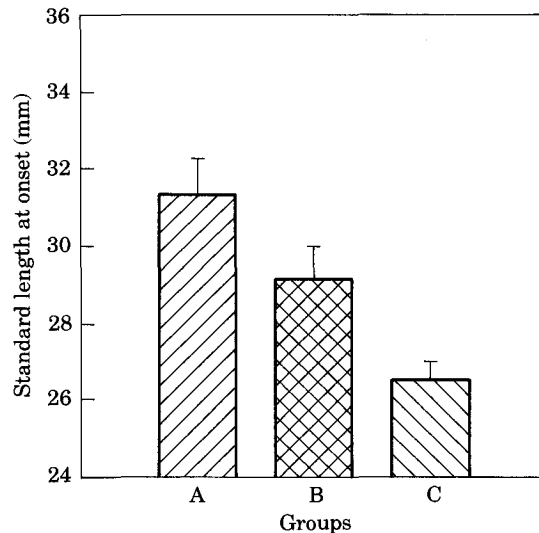


FIG. 2. Mean male standard lengths at the onset of sexual maturation for each experimental group. Bars represent standard error. Group A, no removal; Group B, removal; Group C, isolated controls.

out to confirm the actual stage of gonadal maturation. The testes of all specimens whose opercular paddles were fully grown contained spermatozoa in the testicular ducts. The posterior portion of mature testes appeared to serve mainly for storage of spermatozoa. The pituitaries of mature males possessed a large ventral zone in the proximal pars distalis whose cells stained intensely with aniline blue. In other groups of fishes, such as the poeciliids (Schreibman & Margolis-Kazan, 1979), this has been shown to be a gonadotropic zone. Intense staining of this zone is consistent with the presence of mature testes in this experiment.

Since immature and sexually delayed or inhibited males are externally indistinguishable from equivalent sized females, confirmation of sex required examination of the gonads. In this way a number of males were identified whose s.l. (29—31 mm) were outside the range of maturational onset seen in control males, but whose testes were undeveloped. Histological examination of these testes revealed the presence of only early spermatogonia. The proximal pars distalis of these males contained only a few isolated cells stained with aniline blue. These males were raised in a setting similar to that of group A, save that all fish were removed simultaneously once they had surpassed the normal size for maturational onset. Since the sizes of these specimens were greater than those of any maturing control males, they appear to represent males whose maturation was inhibited through social mechanisms. The lack of a developed gonadotropic region in the pituitary points to a central control for the inhibition of sexual development.

Several specimens whose opercular paddles were just beginning to elongate had testes containing cysts of cells in more advanced stages of spermatogenesis (late spermatogonia to spermatids), indicating that sexual maturation was proceeding. The aniline blue-staining cells of the proximal pars distalis of these specimens were much more abundant than in the sexually undeveloped males,

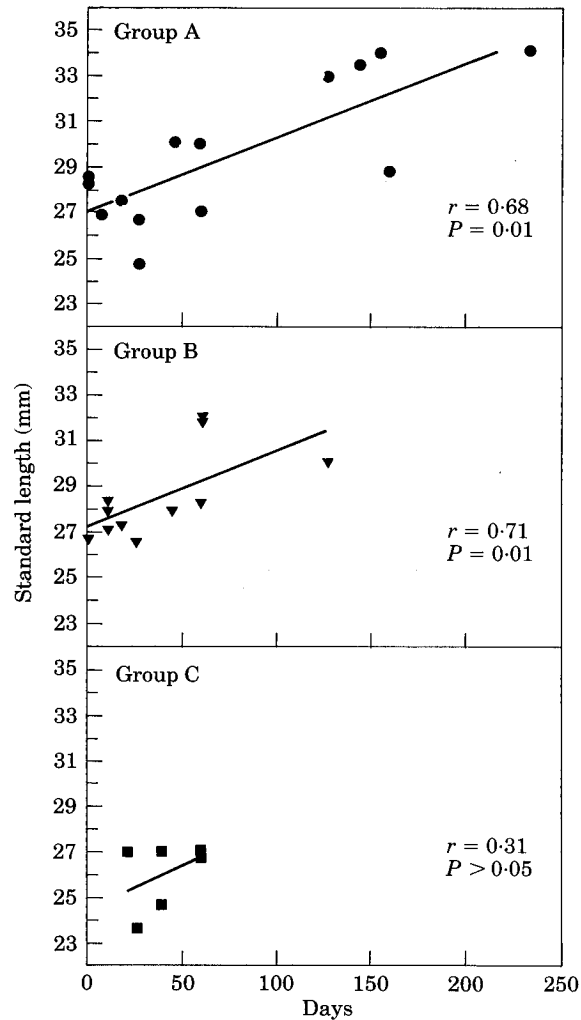


FIG. 3. Plot of the number of days after being placed in the experimental tank when sexual maturation began v. the standard length at which that maturation occurred. Each point represents one male. Lines represent linear regressions. Group A, no removal; Group B, removal; Group C, isolated controls.

and formed a distinct zone. The correlation of the development of this zone with testicular maturation provides further evidence identifying it as a gonadotropic zone. Opercular paddle length thus proved to be a reliable external indicator of the degree of gonadal maturation.

IV. DISCUSSION

C. riisei did exhibit a social control of male sexual maturation. In the presence of adult males, maturing juvenile males delayed maturational onset while continuing somatic growth, resulting in progressively larger fish. The last males

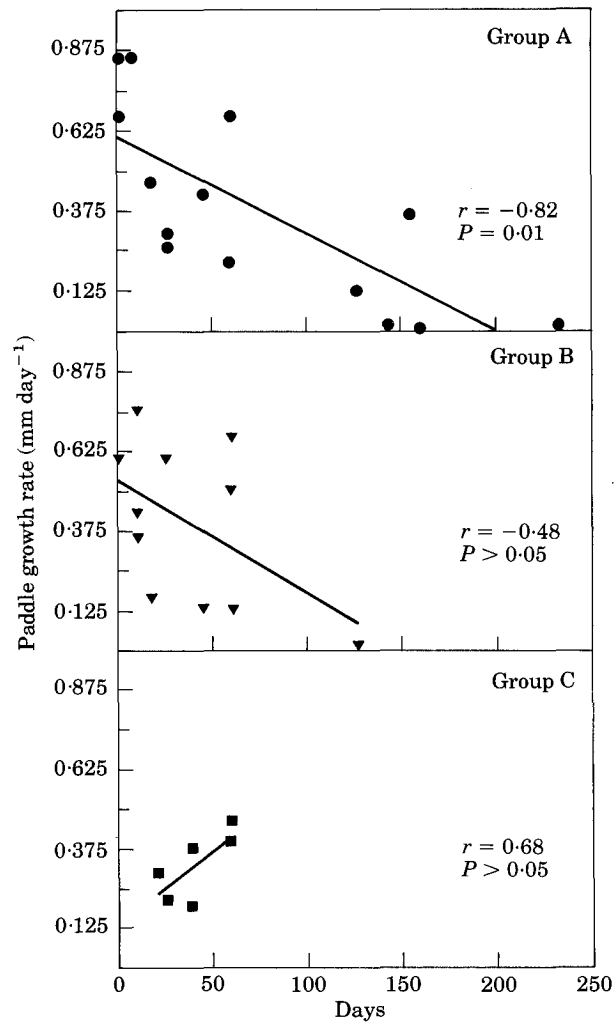


Fig 4. Plot of the number of days after being placed in the experimental tank when sexual maturation began v. paddle growth rate. Each point represents one male. Lines represent linear regressions. Group A, no removal; Group B, removal; Group C, isolated controls.

to begin maturation did so at very slow rates, a few not fully maturing after a year. This implies that the inhibitory effect produced by mature males may be additive.

When sexually mature males were removed, as in group B, a partial pattern of inhibition was seen. Removal of the males did prevent a lengthening of the maturational time course (Fig. 1), but evidently some inhibition took place, since the lengths of males from group B were elevated above those of the controls (Figs. 2 and 3). When a mature male was removed from group B, the next male to mature tended to do so quickly. This is reflected in Fig. 4, where the correlation for group B was not significant. This group exhibited some but not all, of the inhibition seen in group A.

The first males to mature in groups A and B did so at lengths within the control range, but matured at rates higher than any seen in the control tanks (Fig. 4). Males appear to mature more quickly in social groups than in isolation. It may be that the presence of females or juvenile males accelerates those males that have commenced maturation, or that isolation imposes a deceleration. Male sexual maturation in a school of *C. riisei* may be controlled by a combination of positive and negative social cues.

Large, sexually undeveloped males were found that were outside the size range for mature males seen in controls. These males came from group tanks in which a mature male was present and are taken to represent inhibited specimens. The testes and the presumptive gonadotropic zone were less developed than those seen in smaller males that had just begun maturation. Inhibition, then, seems to act at least at the hypothalamic level. Davis & Fernald (1990) discussed a social control system in *Haplochromis burtoni* Gunther regulated by growth of gonadotropin-releasing hormone immunoreactive neurons; this may occur also in *C. riisei*. Examination of males that had just begun maturation showed that the opercular paddle and the testes grow and mature at the same time. This is at odds with Kutaygil (1959), who reported that the testes were fully mature before the paddle or any external sexual characters developed.

A mechanism for this phenomenon has not been determined. Dominance may play a role, since dominant male *X. maculatus* (Gunther) do inhibit maturation of subordinates (Sohn, 1977). However, since dominance and size are correlated (Constanz, 1975), either or both factors may be important. Dominance and large size may produce agonistic behaviour towards smaller conspecifics, and Borowsky (1987) suggested that this behaviour results in inhibition of maturation through stress in the recipient. It has been shown (Scott & Currie, 1980) that dominant status can be correlated with lower adrenocortical activity, and Pickering *et al.* (1987) have demonstrated that in *Salmo trutta* L. high stress and circulating blood cortisol levels can inhibit plasma testosterone and 11-ketotestosterone levels. Using *Betta splendens* Regen, Leitz (1987) observed that the testes of community-housed males produced less 11-oxy- and more 5, β -reduced steroids. These data support Borowsky's proposal.

If agonistic behaviour plays a role in inhibition, then visual cues may be important. *C. riisei* seems to use vision during courtship. Males display before females, and females move towards the opercular paddle when it is extended and shaken, it may well be that there is a visual component in inhibition of maturation. However, Weitzman & Fink (1985) have suggested the presence of a pheromone pump in male *C. riisei*, and Nelson (1964) described a behaviour, called 'dusting', which may produce pheromone release during courtship. Pheromones have been shown to alter reproductive state in other species of fish (van den Hurk & Lambert, 1983; Sorensen, 1992), small mammals (Massey & Vandenbergh, 1980; vom Saal, 1989), and crustaceans (Gleeson *et al.*, 1984; Atema & Cowan, 1986). Pheromones may also play a role in inhibition of maturation. They could reinforce visual cues, while these cues provide a context within which the pheromone signal is interpreted. Work needs to be done to separate and determine the visual and chemical components of this phenomenon.

Social control of sexual maturation is present in a wide range of phylogenetically diverse groups. Its presence in *C. riisei* and the genus *Xiphophorus* may

represent an example of convergent evolution. Fishes from both genera live under similar environmental conditions and display conspicuous male courtship rituals involving competition for females (Nelson, 1964; Sohn, 1977). If fully mature, courting males are subject to greater predation pressure, as suggested by Borowsky (1978), then it would be a sound strategy for smaller males to delay sexual development until a larger size is attained. When the sexually active males are removed by predators, or when the inhibited males obtain a size that enables them to compete successfully, they may then mature and court females. This increases individual fitness for these males (Borowsky, 1973a). Mature males would benefit by inhibiting juvenile males, resulting in less competition among males for females. This system also produces continuous reproduction, where courtship and breeding can proceed even as sexually mature males are removed (Borowsky, 1978).

The occurrence of a similar social control pattern in several distant species suggests that there may be a common physiological mechanism. Much further work is needed to document this phenomenon in other species, and to determine the mechanism by which behavioural or chemical external cues can influence reproductive states. These systems may be common in social organisms, and as such are crucial to our understanding of their behaviour and physiology.

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