Reproductive response to photoperiod affects corticosterone and immunoglobulin G concentrations in prairie voles (*Microtus ochrogaster*)

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Abstract: Nontropical rodent species display prominent breeding seasons mediated by photoperiod. Nonreproductive functions also exhibit seasonal changes; for example, fluctuations in adrenal activities may affect immune function and, ultimately, drive seasonal fluctuations in survival rates. The effects of photoperiod on adrenal and splenic masses and serum concentrations of corticosterone and immunoglobulin G (IgG) were evaluated in male prairie voles (*Microtus ochrogaster*). In one experiment, photoperiodic effects on adrenal and splenic masses and serum corticosterone and IgG levels were assessed in males that maintained "summer-like" reproductive systems after 8 weeks of short-day exposure. In a second experiment, the same parameters were examined in males in which testicular regression occurred after 8 weeks on short days. Voles that maintained reproductive organ size on short days failed to display other photoperiod-mediated differences in body, splenic, or adrenal masses or in serum corticosterone or IgG concentrations. In contrast, voles that underwent reproductive regression in response to short days decreased absolute adrenal mass and body mass compared with long-day animals, and also *increased* serum corticosterone concentrations and decreased IgG levels compared with their long-day counterparts. Taken together, these data indicate that reproductive responsiveness to day length may be linked to seasonal fluctuations in nonreproductive adaptations.

Résumé : Hors de la zone tropicale, les rongeurs ont des saisons bien définies de reproduction reliées à la photopériode. Des changements saisonniers de fonctions non reliées à la reproduction se manifestent également, e.g., les fluctuations de l'activité surrénale peuvent affecter le système immunitaire et, éventuellement, causer des fluctuations saisonnières des taux de survie. Nous avons étudié les effets de la photopériode sur la masse des surrénales et de la rate et sur les concentration de corticostérone sérique et d'immunoglobuline G (IgG) chez des mâles du Campagnol des Prairies (*Microtus ochrogaster*). Dans une expérience, l'effet de la photopériode sur la masse des surrénales et de la rate et les concentrations de corticostérone sérique et d'immunoglobine G ont été estimés chez des mâles ayant des systèmes reproducteurs de type « été » après 8 semaines d'exposition à des jours courts. Dans une seconde expérience, les mêmes variables ont été estimées chez des mâles ayant subi la régression de leurs testicules après 8 semaines d'exposition à des jours courts. Les mâles qui ont gardé des organes génitaux de taille normale après l'exposition aux jours courts n'ont pas subi non plus de variations de leur masse totale, de la masse de leur rate ou de leurs surrénales, ou de leurs concentrations de corticostérone sérique et d'immunoglobine G. En revanche, les campagnols qui ont subi une régression de leur système reproducteur après exposition aux jours courts ont subi aussi une réduction de la masse absolue de leurs surrénales et de leur masse totale et également subi une *augmentation* de leurs concentrations de corticostérone sérique et une diminution de leur immunoglobine G. La combinaison de ces données indique que les réactions du système reproducteur à la longueur des jours sont peut-être reliées aux fluctuations saisonnières d'adaptations non reliées à la reproduction. [Traduit par la Rédaction]

Introduction

Animals inhabiting temperate and boreal habitats confront seasonal changes in energy availability and requirements. For most nontropical species, energy requirements for thermoregulation are maximal when energy availability is lowest. Among the many adaptations that have evolved in response to this winter energy "bottleneck" (Bronson and Heideman 1994; Goldman and Nelson 1993; Nicholls et al. 1988), cessation of breeding is probably most important. Other winter adaptations include elevated basal metabolic rate, increased capacity for nonshivering thermogenesis, increased nest building, increased pelage density, decreased locomotor activities, increased incidence of torpor and communal huddling, increased gut absorption, and altered body mass (Lynch et al. 1973; Lynch 1973; Heldmaier et al. 1981; Dark et al. 1983; Dark and Zucker 1986; Wunder 1984; Ruby and Zucker 1992; Moffatt et al. 1993). The timing of many of these seasonal traits is regulated by photoperiod (reviewed in Bronson and Heideman 1994; Moffatt et al. 1993); the onset of autumnal day lengths induces the constellation of winter adaptations in rodents. In every population

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examined, some individuals appear to ignore photoperiodic information and maintain "summer-like" reproductive systems during short-day exposure (Nelson 1987). The extent to which other, nonreproductive winter adaptations are linked to the reproductive response to day length remains to be determined completely (Moffatt et al. 1993).

Seasonal changes in adrenal size and function have also been documented for many cricedid rodents (Lee and McDonald 1985), especially arvicoline species (Tähkä 1980; Dobrowolska and Gromadzka-Ostrowska 1983). However, the functional consequences of these seasonal changes in adrenal activities have been a matter of dispute (Lee and McDonald 1985). These changes may reflect seasonal variation in stress responses to annual energetic challenges (e.g., Christian 1980; Lochmiller et al. 1994) or reproductive activity (Lee and McDonald 1985; Ferin 1993). In either case, photoperiod appears to be the environmental cue for these seasonal changes in adrenal function (e.g., Tähkä 1980).

Basal corticosterone levels reported for voles range from 3 to 10 times those reported throughout the day in laboratory strains of mice and rats (Olsen and Seabloom 1973; Seabloom 1985). Prairie voles (*Microtus ochrogaster*) exhibit the highest corticosterone levels among arvicoline rodents. Basal concentrations in this species during the circadian peak average approximately 1000 ng/mL (Carter et al. 1995; Taymans et al. 1995). Average peak blood corticosterone concentrations in mice have been reported to range between 100 and 300 ng/mL (Persengiev et al. 1991). The extent to which prairie voles are buffered by the relatively high levels of corticosterone remains unspecified (but see Taymans et al. 1995).

Chronically elevated levels of adrenocortical hormones, especially the glucocorticoids, suppress immune function in both humans and nonhuman animals (Ader and Cohen 1993; Brown-Borg et al. 1993; Hauger et al. 1988; Jefferies 1991; Stein and Miller 1993). Adrenalectomy reverses the effects of sustained glucocorticoid exposure on immune function (del Rey et al. 1984). The precise mechanisms by which the immune system interacts with the hypothalamic–pituitary–adrenocortical axis are unknown, but this interaction probably involves cytokine release rates from activated immunological cells (e.g., Betancur et al. 1994; Khansari et al. 1990; Payne et al. 1994; Zhou et al. 1993). Regardless of the mechanism, substantial evidence links elevated glucocorticoid levels with suppressed immune function.

The goal of this study was to evaluate the effects of day length on adrenal and spleen masses, as well as on basal serum corticosterone and immunoglobulin G (IgG) values, in male prairie voles. In exp. 1 the effects of day length on paired adrenal and splenic masses and serum corticosterone and IgG levels were assessed in males that did not regress their reproductive systems in response to chronic short-day exposure. In exp. 2, the effects of day length on these same parameters were examined in males that inhibited reproductive function in response to long-term maintenance on short days. If adrenal, splenic, and IgG responses to photoperiod are linked to reproductive function, then photoperiodic differences in these parameters should be observed in exp. 2, but not in exp. 1. If adrenal, splenic, and IgG responses to photoperiod are independent of reproductive responsiveness to day length, then in both experiments males should display photoperiod-mediated differences in these parameters.

Material and methods

Animals and housing conditions

Adult (>60 days of age) male prairie voles (*Microtus ochrogaster ochrogaster*) were born from breeding pairs in our laboratory colony (16 h light (L) : 8 h dark (D) photoperiod (lights on between 07:00 and 23:00 EST) at 21 ± 2°C with 50 ± 5% relative humidity) established from descendants of animals trapped near Urbana, Illinois, and last outbred 3 years previously. Voles were weaned at 21 days of age and housed with same-sex siblings. Three weeks prior to the initiation of the experiment, siblings were separated and housed individually in polypropylene cages (27.8 × 7.5 × 13 cm) in colony rooms with a 16 h L : 8 h D cycle (lights on at 07:00 EST). Food (Agway Prolab 1000, Syracuse, N.Y.) and tap water were available at all times before and during the study. Breeding pairs produced males that are predominantly nonresponsive to the inhibitory effects of short day lengths on reproductive parameters (exp. 1) or males that regress the reproductive systems in response to short days (exp. 2). Variation in reproductive response to short days was observed within experiments because the responsive and nonresponsive phenotype is not yet fixed in our breeding colony.

Experimental protocol

At the start of the study, voles either remained in 16 h L : 8 h D photoperiod or were moved to another colony room with a 8 h L : 16 h D photoperiod. After 8 weeks, animals were lightly anesthetized under methoxyflurane vapors (Metofane: Pitman Moore, Mundelein, Ill.), weighed, and bled from the retro-orbital sinus of the eye within 2 min of anesthesia (Riley 1960). All samples were collected between 12:00 and 14:30 EST, i.e., during the lowest part of the circadian cycle of corticosterone secretion (Taymans et al. 1995). Blood samples were stored for 1 h at room temperature to allow clot formation prior to centrifugation at 4°C for 1 h at 3500 rpm; serum was stored at −80°C.

Animals were then killed by cervical dislocation. Spleens and adrenal glands were removed and weighed at autopsy. The masses of paired testes, epididymides, and seminal vesicles, as well as the epididymal fat pads were recorded. Both absolute and relative (corrected for body mass) organ masses were recorded. Two iterations of the study were conducted. In exp. 1, a high proportion (26/28) of short-day reproductively nonresponsive animals was assigned to the short-day experimental condition. In exp. 2, a high proportion (20/28) of short-day reproductively responsive animals was assigned to the short-day experimental condition.

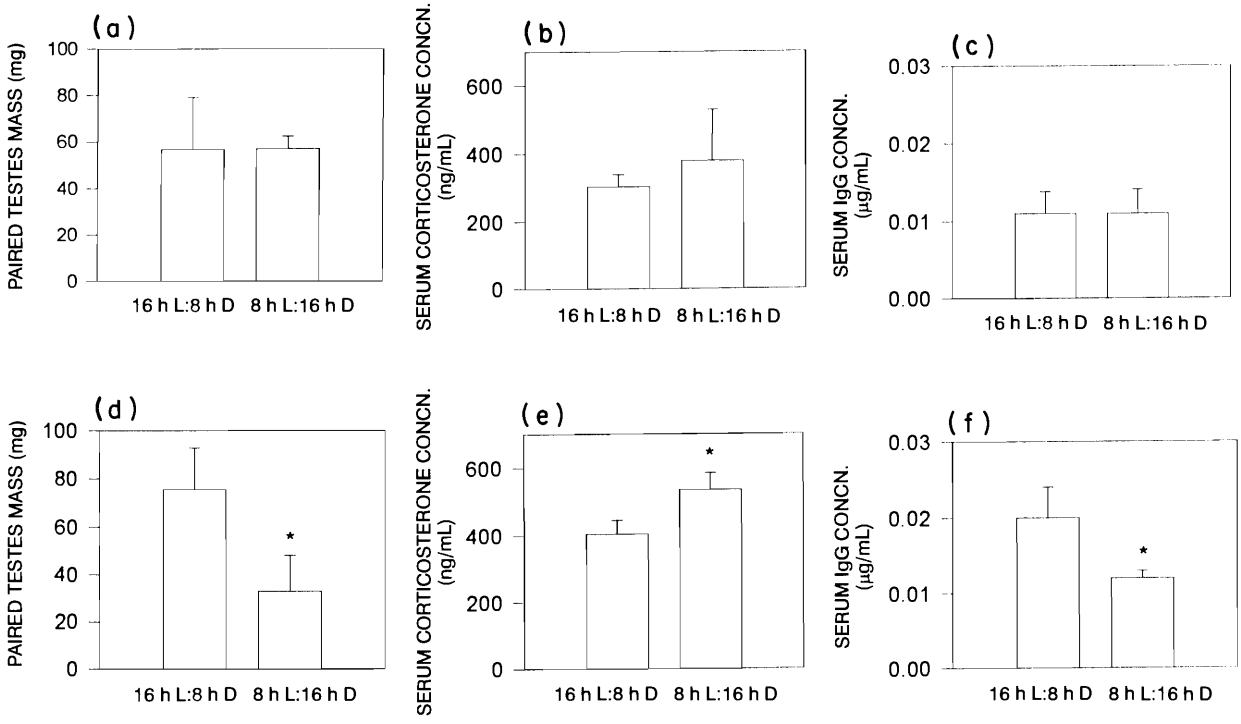
Serum corticosterone assay

Total corticosterone was assayed by radioimmunoassay (RIA) using the ICN Biomedicals, Inc. (Carson, Calif.) ^{125}I kit. The vole serum was diluted 1:2000; all other instructions furnished by ICN were followed. This RIA has been completely validated for use in prairie voles (Taymans et al. 1995). The corticosterone assay was highly specific; ICN reports that this RIA cross-reacts less than 0.3% with other steroid hormones. All blood samples were assayed for corticosterone in a single assay. The intra-assay coefficient of variation was <5%.

IgG antibody assay

IgG levels in the blood samples were determined using a sandwich ELISA that was validated for use in prairie voles. Serial dilutions yielded values in parallel to the standard curves. Immunoplates (96-well, Nunc®, MaxiSorp, Naperville, Ill.) were incubated overnight at 4°C with 100 μL per well of a goat polyclonal antibody

Fig. 1. (*a*) Mean (±SEM) mass (mg) of paired testes of male prairie voles on long (16 h L : 8 h D) or short (8 L : 16 D) days (experiment 1). The males in this study were predominantly nonresponsive to the typical inhibitory effects of short days on the size of the reproductive system. (*b*) Mean (±SEM) serum corticosterone levels of these nonresponsive male voles on long or short days. (*c*) Mean (±SEM) serum IgG concentrations of these nonresponsive male voles on long or short days. (*d*) Mean (±SEM) mass (mg) paired testes of male prairie voles on long or short days (experiment 2). These males inhibited the size of their reproductive systems in short days. (*e*) Mean (±SEM) serum corticosterone levels of these responsive male voles on long or short days. (*f*) Mean (±SEM) serum IgG concentrations of these responsive male voles on long or short days.



raised against mouse IgG (Cappel, Durham, N.C.) diluted 1:3000 in a carbonate–bicarbonate buffer (0.1 M, pH 9.6). The following day the plates were washed 4 times with phosphate-buffered saline (PBS; 0.05 M, pH 7.4) containing 0.05% Tween-20 and 0.001% NaN_3, using an automatic microplate washer (Model 1550, Bio-Rad Laboratories, Richmond, Calif.). A standard curve (upper limit 10 µg/mL; lower limit 0.001 µg/mL) was prepared using purified mouse IgG (Sigma Chemical Corporation, St. Louis, Mo.) diluted in standard diluent (PBS (0.05 M, pH 7.4) containing 0.05% Tween-20). The standards (100 µL per well in triplicate) and samples of vole serum (100 µL per well in duplicate) diluted 1:100 with standard diluent were placed in wells on the plates. The plates were incubated overnight at 4°C. The following day the plates were washed and 100 µL of alkaline phosphatase conjugated sheep – anti-mouse IgG (Cappel) diluted 1:2500 in standard diluent was added to each well. The plates were incubated overnight at 4°C. The following day the plates were washed and 100 µL of substrate buffer (0.1 mM *p*-nitrophenyl phosphate in diethanolamine buffer (0.1 M, pH 9.5) containing 5 mM $MgCl_2$) was added to each well. The plates were incubated for 30 min and the optical density of the resulting colored product in each well was measured at 405 nm using a microplate reader (Bio-Rad Laboratories, Model 450). The absolute concentrations of IgG in the samples were determined relative to the standard curve.

Statistical analysis

All data were analyzed using independent Student's *t* tests (SYSTAT, Inc., Evanston, Ill.). Differences between group means were considered statistically significant if $p \leq 0.05$. Some of the data in exp. 1 violated the assumption of homogeneity of variance; consequently, these data were analyzed by Mann–Whitney rank sum tests.

Results

Experiment 1

The body mass of male prairie voles was unaffected by photoperiod. Similarly, photoperiod did not significantly affect the mass of paired testes, paired epididymides, seminal vesicles, paired adrenals, or spleens ($p > 0.05$ in all cases) (Table 1). Voles maintained in long-day conditions had significantly heavier epididymal fat pad masses ($t_{52} = 2.39$, $p < 0.05$) than short-day animals. Serum corticosterone concentrations did not differ significantly between groups ($p > 0.05$) (Fig. 1). Neither serum corticosterone nor IgG concentrations were affected by photoperiod ($p > 0.05$ in both cases) (Fig. 1). Mann–Whitney rank sum tests conducted for both testis mass and blood serum corticosterone concentrations revealed no significant differences between means ($p > 0.05$ in both cases).

Experiment 2

In contrast to exp. 1, short-day prairie voles weighed significantly more than their long-day counterparts ($t_{50} = 3.04$, $p < 0.01$). Despite the elevated body mass, animals maintained on short days had reduced absolute values of all reproductive parameters examined (Table 1); paired testicular mass ($t_{49} = 6.65$, $p < 0.001$), paired epididymal mass ($t_{49} = 6.47$, $p < 0.001$), seminal vesicle mass ($t_{48} = 5.00$, $p < 0.001$), and epididymal fat pad mass ($t_{49} = -2.88$, $p < 0.001$) were also reduced in short-day voles compared with long-day voles after correction for body mass. Animals

Table 1. Body and organ masses of males that maintained (experiment 1) or regressed (experiment 2) reproductive organ size on short days.

Photoperiod treatment	Body mass (g)	Epididymal mass (mg)	Seminal vesicle mass (mg)	Epididymal fat pad mass (mg)	Splenic mass (mg)	Adrenal mass (mg)
			Experiment 1			
16 h L : 8 h D (n = 27)	38.9±1.2	112.2±7.0	106.3±8.0	72.4±4.9	38.2±2.4	14.0±2.1
8 h L : 16 h D (n = 27)	40.9±1.8	112.4±1.0	116.0±2.0	62.0±12.1	38.5±2.0	13.2±3.0
			Experiment 2			
16 h L : 8 h D (n = 29)	37.5±1.9	167.4±16.0	105.1±11.0	116.0±16.0	42.2±2.3	15.2±1.1
8 h L : 16 h D (n = 26)	43.2−1.3*	75.3±8.0*	47.1±8.0*	75.2±8.0*	42.4±3.2*	10.4±0.5*

Note: Values are given as means ± SEM.
*Statistically significant difference between means within an experiment.

maintained on short days possessed significantly reduced adrenal mass ($t_{49} = 3.19, p < 0.001$), but splenic mass was unaffected ($p > 0.05$); the same pattern of results was obtained when these organ masses were corrected for body mass ($p < 0.05$ in all cases).

Serum corticosterone concentrations were significantly elevated in prairie voles on short days compared with those on long days ($t_{46} = -2.03, p < 0.05$) (Fig. 1). In contrast, serum IgG concentrations were significantly depressed in short-day voles compared with long-day animals ($t_{46} = -2.11, p < 0.05$) (Fig. 1).

Discussion

Prairie voles that maintained reproductive organ size on short days failed to display any photoperiod-mediated differences in body, splenic, or adrenal masses. These males also failed to show any effect of photoperiod upon serum corticosterone or IgG concentrations. In contrast, males that displayed reduced reproductive system size on short days also exhibited elevated body mass and decreased absolute adrenal mass in comparison with long-day animals. Short-day males that inhibited their reproductive systems on short days also displayed increased serum corticosterone concentrations and decreased IgG concentrations compared with their long-day counterparts.

The interaction between the adrenal glands and reproductive function is complex. In some species, castration has no effect on corticosterone levels (e.g., *Microtus townsendii*; McDonald and Taitt 1982), whereas in other species castration blocks the androgen-mediated inhibition of corticosterone-binding globulin (CBG) levels (e.g., *Antechinus stuartii*; McDonald et al. 1981; Lee and McDonald 1985). In the absence of androgens, blood CBG levels may increase, thereby decreasing the free corticosterone and limiting the pool of biologically active steroid hormone. Exposure to short days, and subsequent reproductive regression, coincided with adrenal gland atrophy and reduced steroidogenesis in bank voles (*Clethrionomys glareous*) (Tähkä 1979). In the present study, reproductive regression on short days (functional castration) was associated with a decrease in paired adrenal mass, but an increase in serum corticosterone concentration. Although reduced organ size corresponding to increased secretion may seem counterintuitive, several previous studies have suggested that adrenal mass may not accurately reflect adrenal function (e.g., Lee and McDonald 1985; Morton and Lewis 1980; Tähkä 1980). Such factors as reproductive activities can affect adrenal masses and secretory function. In the present study, males that maintained the size of their reproductive systems on short days also maintained splenic and adrenal masses, as well as serum corticosterone and IgG concentrations.

Basal IgG levels were reduced in voles displaying reproductive inhibition in short days. High circulating IgG levels can be interpreted either as increased antibody production in preparation for coping with antigens or as a reaction to a recent infection. Six "sentry" voles were maintained in each experimental room and were examined by the veterinarian staff of the Department of Comparative Medicine at Johns Hopkins University. No evidence of any viral or bacterial infections was noted. Further studies are required to determine the effects of an immunological insult on immune function in long- and short-day animals in varying states of reproductive activities (Lochmiller et al. 1994).

In the present study, reproductive regression was associated with elevated glucocorticoid levels and reduced basal IgG concentrations. Thus, on the basis of these results, it would seem that voles on short days that maintained reproductive functioning might possess an adaptive advantage over males that inhibited breeding during the winter, i.e., they could sire offspring throughout the short days of winter, and they may maintain relatively low corticosterone and high antibody levels. However, previous studies have indicated that significant energy costs are associated with maintenance of winter breeding (Moffatt et al. 1993); for example, males with functional reproductive systems remain too aggressively disruptive to benefit from energy-saving communal huddling. Further studies are required to assess the effects of social system and social interactions on immune function.

Because both androgens and glucocorticoids can suppress immune function (Grossman 1985), further studies are required to understand the mechanisms underlying the apparent elevation of IgG levels in short-day voles that underwent gonadal regression and presumably underwent functional castration in terms of blood levels of androgens. Although blood testosterone levels were not assayed, seminal vesicle mass, a reliable index of circulating androgens, was reduced in short-day voles in exp. 2. One trait that could possibly vary in the field and affect breeding and survival is responsiveness to day length (Lochmiller et al. 1994; Moffatt et al. 1993; Nelson 1987). Additional studies are necessary to assess the effects of photoperiod on immune function in ener-

getically stressed animals. Again, males that maintained the size of their reproductive systems on short days also maintained splenic and adrenal masses as well as serum corticosterone and IgG concentrations. These results suggest that nonreproductive adaptations may be linked to reproductive responsiveness to day length.

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A new species of the congrogadin genus *Rusichthys* from southern Oman (Perciformes; Pseudochromidae), with notes on its osteology

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Abstract: Three specimens of a distinctive new species of congrogadin belonging to the basal lineage (*Rusichthys*) of the subfamily are reported here. The new species differs from its congener in having more dorsal-fin and anal-fin rays and more gill rakers on the outer surface of the first gill arch, in colour pattern, and in various osteological details (especially of the caudal skeleton). ***Rusichthys explicitus*** n.sp. is presently known from a single collection taken from 27 m off southwest Oman, whereas *R. plesiomorphus* is presently known from two specimens trawled in 140 m on the Lamu Banks off northern Kenya.

Résumé : Trois spécimens d'une espèce nouvelle très distincte de congrogadiné appartenant à la lignée de base (*Rusichthys*) de la sous-famille ont été capturés. La nouvelle espèce se distingue par le nombre plus grand de rayons à ses nageoires dorsale et anale, par le nombre plus grand de branchiocténies à la surface externe de son premier arc branchial, par sa coloration et par plusieurs aspects de son ostéologie (particulièrement sur le squelette caudal). Les spécimens de ***Rusichthys explicitus*** n.sp., ont été capturés au cours d'une seule récolte à 27 m de profondeur au large de la côte sud-ouest d'Oman, alors que *R. plesiomorphus* n'est représenté que par deux spécimens connus, récoltés au chalut dans 140 m d'eau sur les bancs de Lamu, au large de la côte nord du Kenya.
[Traduit par la Rédaction]

Introduction

Rusichthys was erected by Winterbottom (1979) to describe a morphologically generalized congrogadin from off the coast of Kenya. In 1985, Godkin and Winterbottom demonstrated that *Rusichthys* was the sister-group to all other congrogadins, and provided the morphological "missing link" between congrogadins and anisochromin pseudochromids. The specimen used for the osteological study of *Rusichthys* was in poor condition, and the bones of the posterodorsal region of the suspensorium and the anteroventral region of the cranium were lost. These details are reported here from the cleared and stained specimen of a new species of *Rusichthys* described herein recently collected in southern Oman, northern Indian Ocean. *Rusichthys* spp. differ from all other congrogadins in having only four pores in the suborbital series and in lacking pored lateral-line scales (both apomorphic) and in retaining a I 4 pelvic fin and 14–15 pectoral-fin rays (both plesiomorphic). The new species is 1 of 22 recently discovered along the south and central coasts of Oman (Randall and Hoover 1995).

Methods

Methods are as outlined in Winterbottom (1986). Values for the holotype are underlined where pertinent. Abbreviations for repositories of material follow Leviton et al. (1985).

Key to the species of *Rusichthys*

1*a*. Dorsal fin II 32–33; anal fin 26–27; gill rakers on first arch 2 + 5 ..... *Rusichthys plesiomorphus*
1*b*. Dorsal fin II 46–48; anal fin 36–38; gill rakers on first arch 4 + 10 ..... *Rusichthys explicitus* n.sp.

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