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PERFIL DOS HORMÔNIOS ESTERÓIDES DE MACHOS E FÊMEAS DE JUNDIÁ (*Rhamdia quelen*, QUOY &GAIMARD, 1824) DURANTE O CICLO REPRODUTIVO.

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Siglas

UFRGS	Universidade Federal do Rio Grande do Sul
UPF	Universidade de Passo Fundo
CNPq	Conselho Nacional de Pesquisa
FAPERGS	Fundação de Amparo a Pesquisa do Rio Grande do Sul
Propesq-UFRGS	Pró Reitoria de Pesquisa da UFRGS
RS	Rio Grande do Sul
GSI	Gonadossomatic Index
IGS	Índice Gonadossomático
S.E.M.	Standart Error of Mean = Desvio Padrão da Média
cpm	contagem por minuto
ANOVA	Analysis of Variance = Análise de Variância

Unidades

g	grama
mg	miligrama
ng	nanograma
pg	picograma
l	litro
ml	mililitro

µl	microlitro
m	metro
cm	centímetro
mm	milimetro
µm	micrometro
litros/minuto	litros por minuto
l / minuto	litros por minuto
g/m^2	gramas por metro quadrado
%	porcento
Kcal/EM/Kg	quilocalorias de energia metabolizável por quilo
H	horas
mg/l	miligramas por litro
° C	graus Célsius
g/l	grama por litro
ng/ml	nanogramas por ml
pg/ml	picogramas por ml

Substâncias

GtH I	gonadotrofina I
GtH II	gonadotrofina II
17,20β-P	17α,20β-dihidroxi-4-pregnen-3-ona
20β-HSD	20β-hidroxi-esteroide desidrogenase
20β-S	17α,20,21β-trihidroxi-4-pregnen-3-ona
17-P	17α-hidroxi-progesterona
11-KT	11-cetotestosterona

25OHC	25-hidroxicolesterol
T	testosterona
E_2	estradiol 17β

RESUMO

O jundiá (*Rhamdia quelen*) é uma espécie nativa do sul da América do Sul, sendo uma alternativa para aquacultura de países de clima temperados e sub-tropicais. Com o intuito de mapear os perfis hormonais reprodutivos de machos e fêmeas de jundiá, amostras de plasma e gônadas foram tomadas mensalmente entre julho de 1998 e julho de 1999 e em meses pré programados entre agosto de 1999 e abril de 2000. Nos machos os níveis plasmáticos de testosterona (T) aumentaram progressivamente durante a espermatogênese. Durante o período de espermiação a T decaiu enquanto a 11-cetotestoterona (11-KT) permaneceu alta. Os esteróides C21 mostraram-se mais elevados antes do início da espermiação. As concentrações extremamente altas de 11-KT nos primeiro ciclo em contraste as do segundo, fortemente sugere que este seja o hormônio pubertal para machos destas espécie. Nas fêmeas as concentrações plasmáticas de 17β-estradiol aumentaram progressivamente durante o desenvolvimento oocitário, simultaneamente com o aparecimento das vesículas de vitelo. A T teve sua mais alta concentração coincidentemente com o pico do Índice Gônado Somático, sugerindo uma ação na maturação dos oócitos. Dois picos distintos dos progestágenos foram detectados, correspondendo as desovas detectadas, sugerindo que um ou mais destes hormônios aja como "esteróide indutor de maturação final". Altos níveis de 11-KT foram encontrados nas fêmeas de jundiá. A identidade da 11-KT foi confirmada por cromatografia de camada delgada. A relevância fisiológica da 11-KT para as fêmeas desta espécie permanece desconhecida, apesar de sua distribuição ao longo do ciclo sugira sua participação. Referente a resposta ao estresse, os jundiás se comportaram como a maioria dos peixes teleósteos estudados até o momento. Concluindo, as concentrações dos esteróides reprodutivos indicaram ações semelhantes as propostas para os demais peixes, com peculiaridades no tocante a 11-KT.

Palavras-chave: ciclo reprodutivo, cortisol e estresse, desova, estrógenos e andrógenos, Jundiá, progestágenos, puberdade, *Rhamdia quelen*.

1. INTRODUÇÃO:

1.1 A piscicultura e o Jundiá

Com o avanço da piscicultura como modelo econômico alternativo, o conhecimento da biologia e fisiologia reprodutiva das espécies que possuem interesse econômico torna-se indispensável.

A população mundial vem crescendo em acelerada progressão. A necessidade de produção de alimentos de qualidade superior e em quantidades capazes de suprir a crescente demanda, reforça a necessidade de investimentos em pesquisa em cultivos alternativos. A aquacultura se encaixa neste contexto como atividade produtora de proteína animal de alta qualidade e em grande quantidade por área utilizada.

O cultivo de peixes vem experimentando um crescimento anual significativo da ordem de 20%. Com o contínuo decréscimo dos estoques pesqueiros, a produção de peixes cultivados é encarada como uma alternativa para suprir a crescente demanda por carne de pescado.

Ao mesmo tempo, a cultura alimentar mundial volta-se para os alimentos saudáveis e que de alguma forma possam contribuir para o estabelecimento e a preservação da saúde do ser humano. Neste contexto a carne de pescado vem sendo vista como uma excelente fonte de proteína e alimento extremamente saudável. Pesquisas demonstram que os altos teores dos ácidos graxos ômega-3 presentes nas carnes de pescado atuam como eficazes

preventivos de cardiopatias. Médicos do mundo inteiro já apontam o consumo semanal de peixes como fator de vida saudável.

O Brasil por suas características hídricas tem potencial para se tornar em pouco tempo o maior produtor mundial de pescado cultivado. Somente o lago da hidrelétrica de Itaipu poderia elevar o Brasil a esta posição se corretamente explorado. O aproveitamento deste tipo de barragem e de mananciais de águas públicas esbarra muitas vezes na legislação que proíbe o uso de espécies exóticas. Assim, é de fundamental importância a pesquisa e o domínio tecnológico sobre os peixes nativos do Brasil e, para nós em especial, os adaptados ao clima sul-brasileiro, como é o caso do jundiá.

O Estado do Rio Grande do Sul é privilegiado com uma extensa lâmina d'água. Barragens para irrigação das extensas lavouras de arroz, barragens para movimentação de usinas hidrelétricas e um sem número de açudes bebedouros para o gado, perfazem uma área de água, ainda hoje sub-utilizada.

As espécies brasileiras de peixes são extremamente aptas ao cultivo, necessitando apenas serem criteriosamente pesquisadas afim de desenvolver pacotes tecnológicos capazes de promover seus cultivos economicamente viáveis. A adaptação de tecnologia estrangeira pode ser uma alternativa de curto prazo, mas mesmo assim pesquisas são necessárias para a correta utilização deste conhecimento nos nossos peixes.

A única alternativa real e factível para a aquacultura brasileira tornar-se independente e auto-sustentável é a pesquisa voltada ao desenvolvimento de tecnologia nacional própria para nossos peixes.

Dentre as espécies nacionais, os bagres tem despertado crescente interesse por serem peixes rústicos de rápido crescimento e fornecedores de carne de excelentes qualidades e em quantidades (% de filé) acima dos outros peixes mais cultivados.

Neste contexto, escolheu-se o jundiá da espécie *Rhamdia quelen* para concentrar as pesquisas. O jundiá é uma espécie nativa do sul da América do Sul, são peixes do gênero *Rhamdia,* sendo o mais conhecido e adaptado ao clima do Rio Grande do Sul o *Rhamdia quelen.* Apesar de ser resistente ao frio, desenvolve-se melhor nas épocas mais quentes, quando apresenta boa conversão alimentar. Atinge entre 600 e 800g em seis a sete meses de cultivo. Apresenta carne firme e sem espinhos intramusculares, sendo bem aceito no mercado (SAINT-PAUL, 1986).

As pesquisas sobre esta espécie no Estado, eram até o presente momento, mais direcionadas a pesquisa aplicada, se restringindo a aspectos referentes a larvicultura, desenvolvimento, crescimento e herdabilidade de características produtivas. Até o início deste trabalho não havia nenhum estudo publicado, sobre a fisiologia reprodutiva da espécie.

Ao que se sabe não existem atualmente estudos sobre a fisiologia reprodutiva em bagres brasileiros. Somente *Rhamdia hilarii*, outra espécie de jundiá, existem algumas publicações do final da década de 70 e início da de 80, objetivando o estudo da morfo-fisiologia hipofisiária (VAL-SELLA, 1980 a,b). Alguns estudos foram desenvolvidos para caracídeos tropicais como o Pacú (*Piaractus mesopotamicus*) (ANDRADE, 1996; GAZOLA et al, 1996). Desse modo evidencia-se a necessidade de maior número de estudos quanto aos níveis hormonais relacionados ao controle da maturação gonadal em nossos peixes.

Tendo em vista a estado atual do conhecimento das alterações endócrinas durante o ciclo reprodutivo de espécies nativas é notória a necessidade do intensificar estudos para que se possa associa-los a eventos morfológicos gonadais nas varias fases do ciclo reprodutivo.

Sob o ponto de vista da fisiologia, os perfis hormonais associados às alterações morfológicas das gônadas, poderão contribuir para um melhor conhecimento da estratégia reprodutiva desse peixe, para seleção de reprodutores capazes do produzirem gametas viáveis e do boa qualidade e no desenvolvimento ou melhoria das técnicas de propagação da espécie. Sob o ponto do vista da aquacultura informações que permitam desenvolver sistemas do produção eficientes certamente impulsionarão o cultivo desse peixe em bases auto-sustentáveis firmando sua posição como peixe nativo com tecnologia nacional eficiente e, mais importante, disponibilizando-o como alimento.

1.2. Aspectos da Fisiologia Reprodutiva dos Peixes

A oogênese em peixes constitui-se de um período de crescimento primário (pré-vitelogênico), um período de incorporação de vitelo, a vitelogênese, um período de maturação final dos oócitos, seguindo-se a desova propriamente dita e uma fase de recuperação do ovário para um próximo ciclo.

O desenvolvimento oocitário dos peixes guarda certa relação com o tipo de desova: ao tipo sincrônico estão associadas espécies que desovam uma única vez morrendo a seguir; ao tipo sincrônico em grupo, associam-se espécies de desova total, a qual ocorre durante um curto intervalo de tempo, e ao tipo assincrônico relaciona-se a desova parcelada, durante períodos mais um menos prolongados (de VLAMING et al., 1984).

A assincronia no desenvolvimento dos oócitos aparentemente conduz a um tipo de desova oportunista, a qual seria particularmente útil em ambientes onde as condições de desova são favoráveis por períodos prolongados, apesar da ocorrência de flutuações ou instabilidade que podem interromper o período de desova. Assim sendo, o animal estaria preparado para tirar vantagem dessas flutuações de curto prazo, desovando um pequeno

lote de oócitos sempre que as condições permitissem, prolongando seu período de desova distribuindo adequadamente o esforço energético necessário ao processo reprodutivo.

Desse modo, a caracterização precisa dos estágios do desenvolvimento gonadal, através do estudo dos fenômenos que ocorrem no ovário dessas espécies, em especial nos indivíduos mantidos em cativeiro, levarão a uma melhoria dos métodos de intervenção exógena, adaptáveis aos diferentes estágios de maturação ovariana.

Alterações anuais na esteroidogênese e na gemetogênese tem sido descritas para muitas espécies de peixes teleósteos (SCOTT et al., 1983 *Oncorhynchus mykiis*; KIME et al., 1991 *Sparidentex hasta*; RINCHARD et al., 1993 *Gobio gobio*; BLYTHE et al. 1994, *Morone sexatilis* e ESTAY et al., 1998 *Oncorhynchus kisutch*). As espécies mais pesquisadas apresentam desenvolvimento ovariano síncrone, com apenas uma desova durante a vida, como por exemplo os salmonídeos, ou diversas desovas durante a vida, mas apenas uma por ano. Muito poucas pesquisas foram realizadas com peixes de desovas múltiplas.

Existe um pequeno número de pesquisas sobre esteroidogênese e ciclo hormonal reprodutivo em peixes brasileiros, sempre com peixes da família *Characidae* como o Pacu (ANDRADE, 1996 e GAZOLA, et al. 1996). Trabalhos objetivando bagres brasileiros não foram encontrados na literatura.

Nos últimos anos o interesse no estudo da esteroidogênese gonadal em peixes tem crescido, com o objetivo de desenvolver métodos para controle da reprodução das espécies com interesse comercial (VENKATESH et al., 1992).

Gonadotrofinas e hormônios esteróides estão presentes no plasma de peixes durante o processo de espermatogênese. Como ocorre em mamíferos, uma gonadotrofina estimula a produção de andrógenos pelos testículos, enquanto que uma segunda, estimula o crescimento testicular sem causar produção de andrógenos (COCHRAN, 1992).

Em trutas arco-íris (*Oncorhynchus mikyss*) já foi comprovada a existência de duas gonadotrofinas (GtH I e GtH II) (SUSUKI et al., 1988 e Swanson et al., 1990 apud KOIDE et al., 1992).

Em bagres africanos (*Clarias gariepinus*) a primeira purificação de gonadotrofinas ocorreu em 1986 (Goss et al., 1986 apud KOIDE et al., 1992), apenas em uma forma. Reforçando, KOIDE et al. (1992) detectaram apenas uma gonadotrofina nestes bagres, sendo homóloga fisicoquimicamente a GtH II dos salmonídeos.

Ainda não foi comprovada a existência de duas gonadotrofinas em jundiás, e, tampouco, até o presente estudo, se sabia quais e em que quantidade eram os esteróides envolvidos na fisiologia reprodutiva dos machos e fêmeas desta espécie.

Em linhas gerais, o controle endócrino da maturação gonadal em fêmeas de teleósteos adultas inicia-se com a estimulação que fatores ambientais (temperatura, fotoperíodo, etc.) exercem sobre o hipotálamo, conduzindo à secreção de GnRH (Hormônio Liberador de Gonadotrofinas), e sobre a hipófise, resultando na produção e liberação de GtHs (Gonadotrofinas) na corrente sangüínea. Estas, por sua vez, chegam aos ovários, promovendo a síntese de esteróides, como estrógenos (principalmente 17β-estradiol) nas fases pre-vitelogênica e vitelogênica e progestágenos (principalmente 17α-hidroxi-progesterona e 17α,20β-dihidroxi-progesterona) nas fases finais de maturação e ovulação.

O 17β-estradiol é sintetizado nas camadas foliculares dos oócitos. A camada teca, sob influência de GtH, converte colesterol em andrógenos, principalmente testosterona. Esta é transportada para a camada granulosa, onde é aromatizada a 17β-estradiol pela enzima aromatase, também sob influencia de GtHs. A habilidade das células da granulosa em secretar 17β-estradiol depende, assim, da atividade da enzima aromatase, o que varia de acordo com o estágio de maturação dos folículos ovarianos (YOUNG et al., 1983). O

17β-estradiol por sua vez, atua sobre o fígado, estimulando a síntese de vitelogenina, que, no oócito, promove a incorporação de vitelo e crescimento, (processe conhecido como vitelogênese), incorporação esta realizada por micropinocitose, sob ação de GtH.

Na grande maioria dos peixes investigados até o presente, durante a vitelogênese, os níveis de 17β-estradiol e testosterona são elevados, o que promove um feed-back negativo na secreção de GtHs hipofisarias. No final da vitelogênese, ocorre a diminuição da secreção de 17β-estradiol e testosterona, e, consequentemente, os níveis plasmaticos desses hormônios diminuem. Desse modo, o feed-back negativo e removido e o nível de GtH plasmática aumenta. Essa GtH, então, promove a síntese e a liberação de 17,20β-dihidroxi-progesterona.

De acordo com NAGAHAMA (1990) a 17α,20β-dihidroxi-4-pregnen-3-ona (17,20β-P) é o principal hormônio indutor de maturação final dos oócitos nos peixes estudados até aquele momento, e sua produção cresce com o aumento dos níveis das gonadotrofinas que estimulam a enzima 20β-hidroxi-esteroide desidrogenase (20β-HSD) que acelera a transformação biosintética de 17α-hidroxi-progesterona em 17,20β-P. É interessante perceber que sem a provisão do precursor 17-P, não há produção de 17,20β-P (NAGAHAMA 1990). Assim, o nível plasmático de 17-P pode ser um indicativo da concentração de 17,20β-P.

Até a ovulação, a 17α,20β-dihidroxi-progesterona exerce um feedback negativo sobre a GtH hipofisaria, o qual é removido após esse processo, quando a GtH volta a aumentar.

Em fêmeas jovens, há um feedback positivo exercido pelos esteróides gonadais sobre a hipófise. Assim, existe uma produção de gonadotrofina que, embora baixa, estimula o desenvolvimento das gônadas. (Revisões em GOETZ, 1983; FOSTIER et al, 1983 e SCOTT & CANARIO, 1987).

Outros esteróides, como os corticosteróides de origem interrenal, principalmente o cortisol, tem sido relatados como importantes na maturação final dos oócitos de alguns peixes através de um efeito sinergistico da GtH (GOETZ, 1983), sugerindo um envolvimento da interrenal na maturação do oócito. Entretanto, existem ainda controvérsias (CANARIO & SCOTT, 1988).

Diversas funções tem sido propostas para a testosterona nas fêmeas (FOSTIER et al., 1983). A testosterona pode ter uma ação vitelogênica direta, ou indireta, por ser substrato para produção de estradiol pela ação da aromatase nas células da granulosa. Em muitos peixes, um pico de testosterona antes da ovulação, sugere que este hormônio tenha uma função na maturação final do oócitos (SCOTT et al., 1984).

Em salmonídeos machos, a testosterona é, quantitativamente o principal andrógeno, e a 11-cetotestosterona, o mais ativo (CAMPBELL et al., 1980 e Scott et al., 1980 apud LEATHERLAND et al., 1982). Em outros trabalhos (SCOTT & BAYNES, 1982 e Fostier et al., 1982 apud SCHULZ, 1984) a 11-hidroxitestosterona é que é reportada como o principal andrógeno.

A 11-cetotestosterona é responsável pelos caracteres sexuais secundários em machos de salmão, sendo tão potente a ponto de induzir estes caracteres em machos imaturos injetados com este hormônio (LEATHERLAND et al., 1982).

Durante o ciclo reprodutivo de machos da maioria das espécies já pesquisadas, observou-se um decréscimo nas concentrações plasmáticas de testosterona e 11-cetotestosterona no início da espermiação em trutas arco-íris. No mesmo estudo detectou um pico de 17,20β-P no início da estação de desova (Colombo et al., 1987 apud BARRY et al., 1990).

Da mesma forma, SCOTT et al. (1984) detectaram diminuição da testosterona e 11-cetotestosterona e aumento da 17,20β-P e gonadotrofina, no início da estação de desova.

De acordo com FOSTIER et al. (1983), nos machos a GtH I e a GtH II estimulam a síntese testicular de vários esteróides e a espermatogênese. Em muitas espécies examinadas, os testículos produzem testosterona e 11-cetotestosterona. BILLARD (1976) mostrou que doses de testosterona exógena, mantém a espermatogênese em peixes dourados (*Carassius auratus, L.*) hipofisectomizados, sugerindo que este andrógeno controle este processo. Por outro lado, FOSTIER et al. (1983) afirma que é a 17,20β-P, outro hormônio produzido pelos testículos, que exerce o maior controle sobre a espermiação em teleósteos.

Além da possível ação sinérgica entre os esteróides sexuais e os corticosteróides interrenais, principalmente o cortisol, o estudo dos perfis de cortisolemia durante o ciclo reprodutivo e após situações estressantes é justificado pelo fato do estresse ser muito associado a situações de manejo intensivo em cativeiro e de ter conseqüências deletérias na reprodução dos peixes.

Segundo Brett (1958) *apud* IWAMA (1993), "estresse é o estado produzido por fatores ambientais ou não, que provocam resposta adaptativa no animal frente a uma alteração da função normal". Existem uma série de outras definições para a resposta ao estresse, mas há um consenso quanto ao fato desta, representar reação a um estímulo, e poder alterar o estado de homeostase do peixe (BARTON & IWAMA, 1991).

A resposta ao estresse compreende uma série de alterações fisiológicas. Os efeitos são divididos em primários, secundários e terciários. Entre os efeitos primários encontram-se os aumentos de catecolaminas, adrenalina e noradrenalina, e corticosteróides no plasma. Entre os secundários, existem efeitos metabólicos, como as alterações na glicemia, no ácido láctico e no glicogênio hepático e muscular. Como efeitos hematológicos, alterações no hematócrito e no número de linfócitos. Ainda relatam os efeitos hidrominerais, como alterações nas concentrações plasmáticas de cloro, sódio, potássio, proteínas e na

osmolaridade do plasma. Os efeitos terciários principais são a queda de desempenho produtivo e reprodutivo e a diminuição da resistência às doenças (WENDELAAR-BONGA, 1997).

Nos peixes teleósteos, a elevação plasmática do cortisol, é reconhecida como a principal resposta hormonal ao estresse, e utilizada como indicadora da presença desta resposta (PATIÑO et al. 1987 e BALM et al. 1989).

A resposta crônica ao estresse, muito associada ao cativeiro, tem como principal característica a manutenção de altas concentrações plasmáticas de cortisol por longo período. Esta exposição prolongada ao cortisol pode ocasionar uma série de efeitos terciários, como queda de desempenho produtivo e reprodutivo (PICKERING et al. 1987; CARRAGHER et al. 1989; PICKERING & POTTINGER, 1989).

Em peixes, a influência do cortisol na depressão da atividade reprodutiva ainda permanece incerta. Segundo CARRAGHER et al. (1989), o cortisol liberado em situações de estresse, tem efeito deletério na reprodução, evidenciado pela redução em vários parâmetros reprodutivos de trutas arco-íris (*Oncorhynchus mykiss*) e trutas marrons (*Salmo trutta*), tratadas com cortisol exógeno. Os autores não descartam a possibilidade de que muitos destes efeitos sejam decorrentes da queda de condição geral do peixe, quando exposto a elevação crônica de corticosteróides, entretanto, afirmam que o cortisol tem efeitos diretos na liberação de hormônios reprodutivos para os tecidos alvo.

POTTINGER & PICKERING (1990) demostraram em trutas arco-íris (*Oncorhynchus mykiss*) que o cortisol diminui o número de sítios de ligação para o estradiol no fígado, diminuindo assim a vitelogênese.

Por outro lado, PANKHURST et al. (1995), levantam uma dúvida sobre o efeito do cortisol na reprodução. Numa série de experimentos *in vitro*, onde folículos ovarianos de três espécies, carpa comum (*Cyprinus carpio*), "goldfish" (*Carassius auratus*)

e de "snappers" (*Pagrus auratus*), foram incubados na presença ou não de cortisol, com GtH, testosterona ou 25-hidroxicolesterol (25OHC), não houve evidência de que o cortisol inibe diretamente a esteroidogênese ovariana. Os autores porém, não descartam o envolvimento do cortisol na inibição da reprodução e especulam que outros hormônios, que são secretados em resposta ao estresse, possam estar envolvidos nesta depressão da reprodução.

2 – OBJETIVOS GERAIS

Devido as características de produção do jundiá, a sua potencialidade no sul do Brasil e demais países de clima subtropical ou temperado, justifica-se o estudo de sua fisiologia reprodutiva, sendo uma alternativa à espécies tropicais,. Além disso, existem poucos grupos de pesquisa se dedicando à espécie, limitando-se a trabalhos nas áreas de pesquisa aplicada. Sendo assim, os objetivos principais deste trabalho são os seguintes:

- caracterizar o ciclo maturacional de jundiás (*Rhamdia quelen*) nascidos e criados em cativeiro;
- Determinar e quantificar a presença de 17β estradiol, testosterona, 11-cetotestosterona, 17α-hidroxi-pregnen-tri-ona, 17α,20β-dihidroxi-pregnen-tri-ona e 17α,20,21β-trihidroxi-pregnen-tri-ona em fêmeas e de testosterona, 11-cetotestosterona, 17α-hidroxi-pregnen-tri-ona, 17α,20β-dihidroxi-pregnen-tri-ona em machos durante o ciclo reprodutivo;
- Determinar a produção de testosterona e 11-cetotestosterona por testículos incubados *In vitro*.
- Investigar a resposta o estresse de transferência de tanques em jundiás criados em cativeiro, estabelecendo possíveis interrelações entre o cortisol com a maturação gonadal.

3.1. Animais

No presente estudo, foi utilizada uma população de cerca de 600 jundiás (*Rhamdia quelen*, Quoy & Gaimard, 1824) machos e fêmeas (1:1), imaturos no início do experimento, seguindo-se o acompanhamento de dois ciclos reprodutivos (1998-1999 e 1999-2000), nas instalações do Laboratório de Piscicultura da Universidade de Passo Fundo, localizado em Passo Fundo (RS) (28°15'S / 52°24''W, 687 m acima do nível do mar).

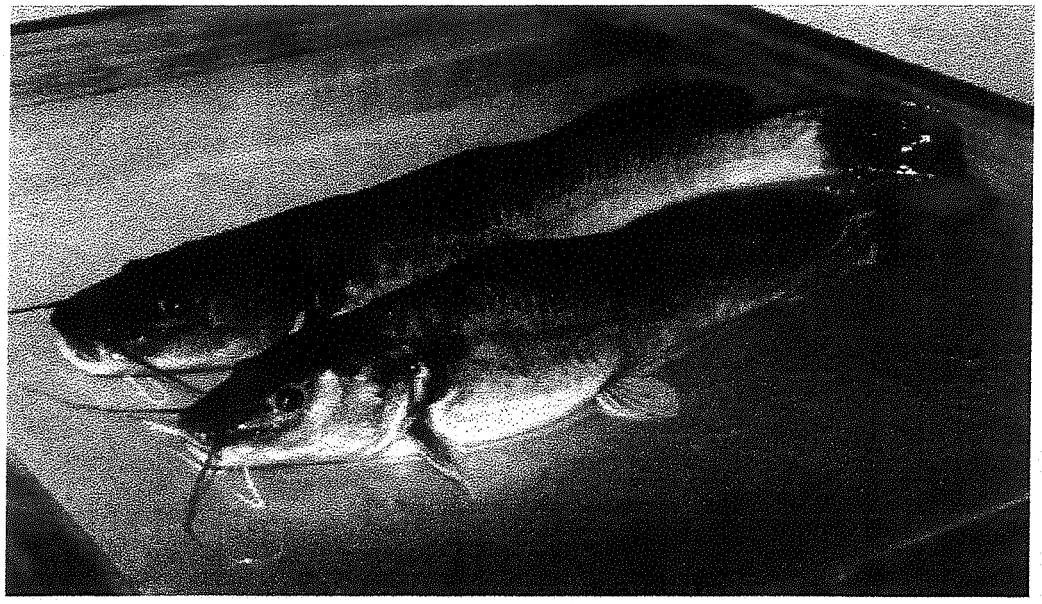

Figura 1. Exemplares adultos de jundiá *Rhamdia quelen.*

Foram efetuadas coletas mensais entre julho de 1998 e junho de 1999, seguindo-se coletas em meses previamente programados de acordo com os estádios maturacionais

desejados no ciclo 1999-2000. Em cada mês foram amostrados machos e fêmeas, sendo os resultados publicados em dois artigos independentes.

Os animais foram mantidos em viveiros escavados em terreno natural, de 20,0m x 5,0 m x 1,0 m, com volume de água de aproximadamente 100 m^3. Os viveiros foram abastecidos individualmente por gravidade, com água oriunda de represa, com fluxo de água constante de 6 litros/minuto. Foram criados em monocultivo, estocados a uma carga inicial de 1800 g/m^2.

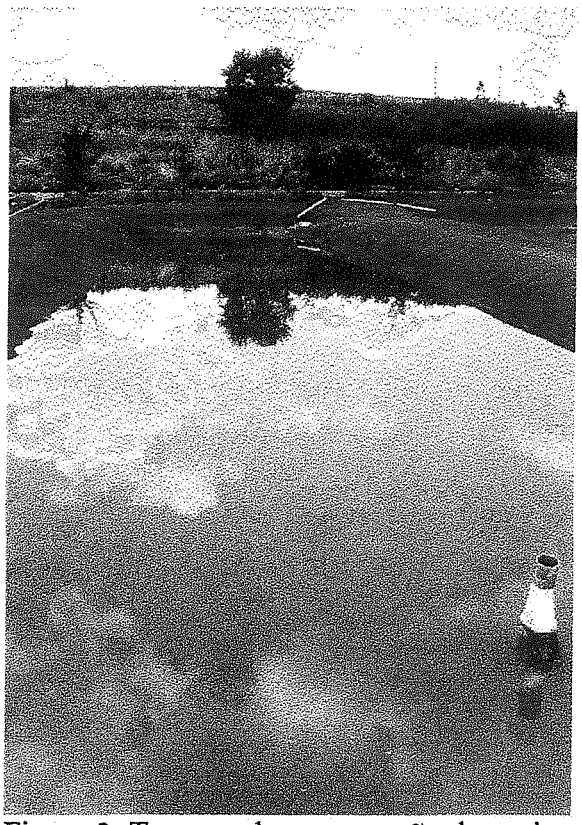

Figura 2. Tanques de manutenção dos peixes.

Os peixes foram alimentados com dieta seca industrializada e extrusada com 30% de proteína bruta e 3000 Kcal/EM/Kg. O alimento foi fornecido a lanço em duas porções, as 10:00 e as16:00 h, à proporção de 5,0 % da biomassa inicialmente, passando a 3% no final do período experimental. Nos meses de junho a agosto a alimentação ocorria em dias

alternados à taxa de 1,0 % da biomassa. O ajuste das quantidades ofertadas foi feito mensalmente com base nos pesos obtidos nas amostragens.

A qualidade de água dos viveiros foi monitorada durante todo o período experimental, sendo que o oxigênio dissolvido esteve entre 5,0 a 7,0 e a amônia abaixo de 0,2 mg/l em todo período. O pH oscilou entre 6,8 a 7,2.

A implicação de fatores ambientais no processo de maturação das gônadas tem sido relatada na literatura, portanto, dados de temperatura do ar, fotoperíodo e pluviosidade foram obtidos mensalmente junto ao Departamento de Climatologia da Embrapa Trigo, localizada em Passo Fundo. Dados de temperatura da água foram tomados duas vezes ao dia, as 8:00h e as 15:00 h.

3.2 – Captura dos Peixes e Obtenção das Amostras

Visando minimizar ao máximo alguns fatores causadores de estresse, os peixes foram capturados com rede de arrasto, conduzida lentamente em toda a largura do tanque, procurando obter em um único arrasto, todos os exemplares necessários para a coleta, sempre as 8:00h da manhã. A seguir, foram transportados para o laboratório e acondicionados em tanques de concreto (capacidade 4.000 l), com água corrente.

3.3 – Experimentos Adicionais

3.3.1. Produção de testosterona por testículos incubados *In Vitro*

Para os machos, além das determinações de esteróides no plasma, foi determinada a produção de testosterona por fragmentos de testículo em meio de cultivo celular. Após o sacrifício, um pequeno pedaço da porção média do testículo foi pesada e colocada em meio 199 modificado e gelado (Meio 199 com sais de Hank e L-glutamina, Gibco BRL, Life Technologies $_{TM}$). O pedaço foi desintegrado com auxílio de tesoura de ponta fina e

incubado no meio 199 modificado por duas horas no agitador metabólico Dubnoff a 25°C, com pH 7.4 e em atmosfera de O_2:CO_2 (95:5; v/v).

Após a incubação os pedaços de testículos foram removidos por centrifugação e o meio congelado a –25° C até a análise.

Para análise, os esteróides foram extraídos das alíquotas de meio através do solvente orgânico acetato de etila. O solvente foi adicionado ao meio na dose de 5ml de solvente para 2 ml de meio, agitado, centrifugado e separado o sobrenadante, repetindo-se duas vezes esta operação, resultado em cerca de 10ml de solvente com os esteróides. Este solvente foi então evaporado em atmosfera de nitrogênio e ressuspendido em metanol para estocagem.

No extrato foram quantificadas a testosterona e a 11-cetotestosterona por radioimunoenssaio e a quantidade relacionada com o peso de testículo que foi incubado, obtendo-se o resultado em ng de hormônio / mg de tecido.

3.3.2. Resposta aguda ao estresse após transferência de tanques

O estressor "transferência de tanques" foi escolhido por se tratar de um manejo muito comum nas práticas de reprodução da espécie, onde peixes são geralmente transferidos para tanques de reprodução.

Para este experimento foram utilizados machos e fêmeas adultos de jundiá. O estoque foi mantido em dois tanques separados por sexo de 100m^3 de volume, a densidade de 0,8 g/l. A renovação de água foi de 6 l / minuto e o nível de oxigênio dissolvido 0mantido entre 5,0 e 7,0 mg/l, com o pH variando entre 6.8 e 7.2. Os peixes foram alimentados com dieta seca industrializada contendo 30% de proteína bruta, à percentagem de 2% da biomassa.

3.4. Determinação dos níveis plasmáticos hormonais

3.4.1. Radioimunoenssaios

Os radioimunoenssaios de 17β-estradiol, testosterona, 17α-hidroxi-progosterona e cortisol foram realizados utilizando kits comerciais (Coat-A-Count®, DPC Los Angeles, CA). Esses kits envolvem o uso de tubos de polipropileno cobertos na superfície interna com anticorpos específicos. Kits comerciais para diagnóstico de doenças humanas são rápidos, convenientes e simples de usar, e têm sido usados para ensaiar hormônios de peixes (GAZOLA et al., 1996) desde que adequadamente validados para a espécie em questão.

O plasma preservado a –25° C em tubos Eppendorf foi descongelado a temperatura ambiente, e foram retiradas alíquotas para realização dos ensaios. Após incubação do plasma por três horas a 37° C (100 µl para estradiol, 50µl para 17α-hidroxi-progesterona e testosterona e 25 µl para cortisol) inclusive padrões e hormônios radiomarcados. O hormônio marcado não ligado foi removido e os tubos invertidos e deixados sobre papel absorvente. Todas as amostras foram ensaiadas em duplicata. A radioatividade que permaneceu ligada aos tubos foi quantificada em contador Gama.

A precisão e a reprodutibilidade dos kits forarn ensaiadas como descrito em GAZOLA et al. (1996). Em resumo, alíquotas da mesma amostra foram ensaiadas em diferentes dias e dentro da mesma determinação. As variações intra-ensaios e inter-ensaio ficaram abaixo dos 8 e 12 % respectivamente. As curvas dos padrões dos ensaios e aquelas obtidas por diluições sucessivas de amostras de plasma de jundiá, foram comparadas, observando-se paralelismo entre elas. A sensibilidade dos kits foram avaliadas pelo menor valor detectável dentro dos limites de confiança de 0 a 90%, sendo 8 pg/ml para estradiol, 40 pg/ml para testosterona, 70 pg/ml para 17-P e 150 pg/ml para cortisol. O método não sofre interferência de contaminantes comuns (hiperlipemia, bilirrubina, hemoglobina), nem

da presença de heparina.

Os ensaios visando mensurar as concentrações de 11-cetotestosterona, 17,20β-dihidroxi-progesterona e 17,20,21β-trihidroxi-progesterona foram realizados na Inglaterra no CEFAS Fisheries Laboratory em cooperação com o Dr. Alexander P. Scoot. Devido as dificuldade de enviar amostras congeladas entre os países, foi decidido adaptar o procedimento usado por TVEITEN *et al.* (2000) para o wolffish (*Anarhichas lupus* L.).

As características do anti-soro contra 17,20β-P, 20β-S e 11KT são descritas em SCOTT *et al.* (1982) e TVEITEN *et al.* (2000).

3.4.2. Identificação da 11-cetotestosterona

Para identificação da 11-KT em cromatografia de camada delgada, como descrito em INBARAJ et al. (1997).

3.5. Tratamento dos dados

Para análise dos resultados, foram computados os dados de todos os animais coletados, agrupados por sexo e pelo mês de coleta. As diferenças estatísticas foram determinadas utilizando-se análise da variância (ANOVA). Foi utilizado ainda, o teste de comparações múltiplas de Tukey para determinar quais os dados que variaram significativamente ($P<0,05$, $P<0,01$ e $P<0,001$).

4 – ARTIGOS

4.1 Artigos Publicados

4.1.1.

Título: Plasma levels of cortisol and glucose in response to capture and tank transference in *Rhamdia quelen* (Quoy & Gaimard), a South American catfish.

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Plasma levels of cortisol and glucose in response to capture and tank transference in *Rhamdia quelen* (Quoy & Gaimard), a South American catfish

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Abstract

Males and females of *Rhamdia quelen* (Quoy & Gaimard) were subjected to capture and tank transference in order to evaluate the stress response. This process provoked a characteristic stress response in both sexes, with cortisol values reaching a peak in one hour after stress. High levels of cortisol found in females were explained by the increase of energy demand and the mobilization in the vitellogenic phase. Several previous studies have shown that cortisol is strongly correlated to these periods in another catfish.

Keywords: cortisol, glycaemia, jundiá, *Rhamdia quelen* (Quoy & Gaimard), stress response

Introduction

his paper provides first data about physiological responses to stress in jundiá (*Rhamdia quelen*, Quoy & Gaimard). This fish species is endemic to South America and is relatively resistant to cold environments. In culture conditions, it reaches 500–600 g of body weight in 6–8 months, and is a good alternative for fish production in southern Brazil where, due to the climate conditions, the fish are subjected to several partial harvests and to tank transference. These activities are able to provoke a characteristic stress response, which is generally assessed by measuring plasma cortisol levels in stressed fish (Wendelaar Bonga 1997). The aim of the present study was to evaluate cortisol and glucose levels after net capture and tank transference.

The study was conducted at the facilities of the University of Passo Fundo (28°15'S/52°24"W, 687 m above sea level). One-year-old adult male and female *R. quelen* (400 ± 50 g SEM) were kept in 100 m^3 tanks (separated by sex) with a stocking density of 0.8 g L^{-1}. The level of dissolved oxygen was 5.0–7.0 mg L^{-1} and the pH ranged from 7.0 to 7.2. The fish were fed once a day with commercially available food (30% crude protein). The basal cortisol level was obtained by sampling eight males and eight females from the pond 1 week before the experiment.

To conduct the experiment, all the fish were captured with a net and transferred to 10 concrete tanks (4 m^3, stocking density of 2.0 g L^{-1}, five tanks per sex). The transference was in 100-L containers, and the time between capture and release did not exceed 2 min. Males and females were sampled immediately, 1, 4, 12 and 24 h after the transference. For each sampling, one tank for each sex was used. In order to avoid cumulative stress effects caused by repeated captures (Pottinger & Mosuwe 1994 and Barcellos, Nicolaiewsky, Souza & Lulhier 1999), the fish were maintained for 1, 4, 12 and 24 h without being disturbed.

Except for basal levels, where the fish were captured and immediately sampled underwater, the fish were anaesthetized with buffered MS222 (Finquel®, 300 mg L^{-1}), and blood samples (1–2 mL) were taken from the caudal vein into heparinized syringes, centrifuged for 10 min at 10 000 *g*, and stored at −25 °C. Eight males and eight females were sacrificed to verify the stage of gonadal devolpment.

Cortisol was measured in unextracted duplicate plasma samples with the commercially available [^{125}I] DPC-cortisol RIA test (Coat-A-Count®, DPC Los Angeles, CA). The results obtained were validated with the standard curve of the kit. The intrassay coefficient of variation was 6% and the sensitivity was 50 of pg mL^{-1}. Plasma glucose was analysed by a colorimetric test based on the oxidase/peroxidase reaction (EnzColor Bio Diagnóstica, SP, Brazil).

Ideally, this type of experiment should be analysed by analysis of variance. However, because data in each sample time were obtained from fish of the same tank, a simple comparison of means by the Student's *t*-test was employed, with a significance level of $P < 0.05$. Comparisons between each sample time against the basal level of respective sex were made. In order to compare the results of both sex, comparisons between each sample time of males and females were also made. All data were expressed as the mean ± SEM. This type of statistical analysis was used by Pottinger & Pickering (1992).

The males and females (Fig. 1) showed similar curve patterns of plasma cortisol, with peaks 1 h after the transference. Afterwards the values decreased progressively, but had not declined to basal levels after 24 h. The basal values for plasma cortisol were 15.8 ± 3.12 ng mL^{-1} for males and 29.6 ± 5.45 ng mL^{-1} for females. Males and females (Fig. 2) showed similar curve patterns of plasma glucose, with peaks 1 h after the transference. The basal values of glycaemia were 61.3 ± 5.8 mg dL^{-1} for males and 68.7 ± 9.3 mg dL^{-1} for females. After 4 h, the male glycaemia decreased until levels approximated the pre-stress levels, but the females maintained a high level of glycaemia until 4 h after the transference. In all sampling points, females had higher cortisol concentrations than males, and a higher level of glycaemia at 4 and 12 h sampling. The GSI calculated among the sacrificed animals was 11.89% ± 1.06% for females and 8.36% ± 0.9% for males. Microscopic staging showed that females were in final vitellogenesis and the males in final maturation of spermatozoa.

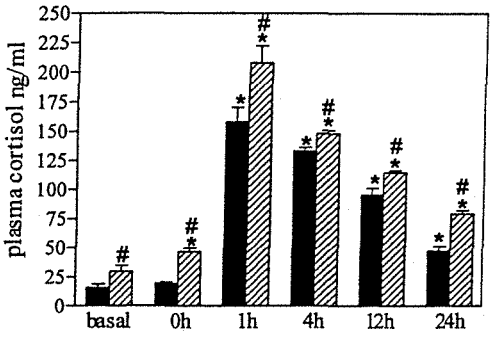

Figure 1 Plasma cortisol levels of male (solid bar) and female (traced bar) *Rhamdia quelen* (Quoy & Gimard), sampled immediately, 1, 4, 12 and 24 h after tank transfer. *denotes statistical differences between sampling times and basal level of respective sex; #denotes statistical differences between sexes in each sampling time. Each point is mean ± SEM (n = 8–12).

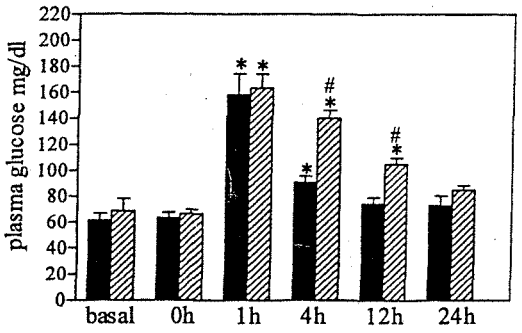

Figure 2 Plasma glucose levels of male (solid bar) and female (traced bar) *R. quelen* sampled immediately, 1, 4, 12 and 24 h after tank transfer. *denotes statistical differences between sampling times and basal level of respective sex; #denotes statistical differences between sexes in each sampling time. Each point is mean ± SEM (n = 8–12).

The resting plasma cortisol levels of male and female *R. quelen* (15.8 ± 3.12 and 29.6 ± 5.45 ng mL respectively) were similar to those obtained in other teleost fish. According to Pickering & Pottinger (1989), resting or unstressed levels of circulating corticosteroids in fish are less than 30–40 ng mL^{-1}.

One hour after transfer, the net capture and tank transfer provoked a characteristic acute stress response with the plasma cortisol peak reaching 158.12 ng mL^{-1} for males and 207.95 ng mL^{-1} for females. According to Pickering & Pottinger (1989),

peak post-stress elevations in plasma cortisol of salmonids are typically 40–200 ng mL^{-1}.

In a review by Barton & Iwama (1991), few papers about fish of the family Siluridae were cited, and those cited were all of *Ictalurus puntactus* (Rafinesque) with pre-stress cortisol levels varying from 5 to 51 ng mL^{-1} and post-stress varying from 30 to 309 ng mL^{-1}. The values we measured in the *R. quelen* are approximated by those previously obtained in *I. puntactus*.

Our results show that 24 h was not enough time for fish to recover to pre-stress cortisol levels in both male and female *R. quelen*. In other teleosts, such as *Salmo trutta* L. (Pickering, Pottinger, Carragher & Sumpter 1987) and *Oreochromis niloticus* (L.) (Barcellos *et al.* 1999) this time is sufficient.

Plasma glucose showed a peak 1 h after the transference and decreased to pre-stress initial values after 4 h in males, and after 24 h in females. These results are similar to those found by Kebus, Collins, Brownfield, Amundson, Kayes & Malison (1992) in rainbow trout [*Oncorhynchus mykiss*, (Walbaum)] when the plasma glucose and cortisol reach a peak over the same time period. The hyperglycaemia observed in these experiments probably occurs through the effects of catecholamines, which determines hepatic glycogenolysis. The massive liberation of catecholamines after stress is well documented in teleosts (Wendelaar Bonga 1997).

One possible explanation for the higher cortisol levels found in females and its high glycaemia for 12 h is the fact that these females were in exogenous vitellogenesis (accumulation of yolk vesicles in vitellogenic oocytes). The energy demand in this phase is extremely augmented, and several previous studies have shown that cortisol is strongly correlated to these periods in another catfish. In a paper about the Indian catfish *Heteropneustes fossilis* (Bloch), Lamba, Goswami & Sundararaj (1983) reported high levels of cortisol coinciding with vitellogenesis and the spawning period. To confirm this, Ding, Lim & Lam (1994) found a potentiation by cortisol administration of the induction of vitellogenin gene transcription by estradiol in tilapia (*Oreochromis aureus*, Steindachner).

In conclusion, the physiological response of *R. quelen* to capture and tank transfer was similar to that reported in other teleost fish. However, our data are the first on the physiological responses to stress in this fish and thus provides a basis for more detailed studies aiming to evaluate the impact of stress response in broodstock and in culture of *R. quelen*.

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4.1.2

Título: Steroid profiles in cultured female jundiá, the Siluridae *Rhamdia quelen* (Quoy and Gaimard, Pisces Teleostei), during the first reproductive cycle.

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Steroid Profiles in Cultured Female Jundiá, the Siluridae *Rhamdia quelen* (Quoy and Gaimard, Pisces Teleostei), during the First Reproductive Cycle

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The jundiá *Rhamdia quelen* (Quoy and Gaimard) is a teleost species from the Siluridae family and is an important species for aquaculture in temperate and subtropical climates. Gonad and blood tissue samples were taken from cultured jundiá females between 1998 and 1999. Plasma concentrations of 17β-estradiol (E_2), testosterone (T), 11-ketotestosterone (11-KT), 17-hydroxy-4-pregnene-3,20-dione (17-P), 17,20β-dihydroxy-4-pregnen-3-one (17,20β-P), and 17,20β,21-trihydroxy-4-pregnen-3-one (20β-S) were measured by radioimmunoassay and potential correlations with the stage of oogenesis and sexual maturation examined. During the experimental period two spawning episodes were observed. Plasma concentrations of E_2 increased progressively during oocyte development, simultaneously with the appearance of yolk vesicles and increasing amounts of deposited yolk. In female jundiá, the T peak occurred in October and was coincident with the peak in gonadosomatic index. Two distinct peaks of progestogens were detected, corresponding to the two spawning episodes, suggesting that one or more of these steroids might act as the "maturational-inducing steroid" in jundiá. Unusually large amounts of 11-KT were also measured in the plasma of mature jundiá females. The identity of 11-KT was confirmed by thin-layer chromatography. Although the profiles of the other steroids are compatible with the roles proposed for the action of these hormones in other teleosts, the role of 11-KT, normally found only in males, is unknown. © 2001 Academic Press

Key Words: 17β-estradiol; C21 steroids; *Rhamdia quelen*; testosterone; 11-ketotestosterone.

INTRODUCTION

The jundiá (*Rhamdia quelen*, Quoy and Gaimard) is an endemic species in South America. It can withstand cold winters and can grow fast in the summer. In aquaculture systems, at a density of two to four fish per square meter, it will reach 600–800 g body weight in 8 months. It is a suitable species for fish production

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in the southern part South America, which makes it ideal for any region with a temperate or subtropical climate. Some research has been carried out on nutrition and stocking density of jundiá, but data are absent on its reproduction. An understanding of reproduction is particularly necessary in aquaculture species, as gamete production is often adversely affected by the conditions of captivity. Jundiá has an asynchronous mode of oocyte development. The other species with asynchronous oocyte development that have been studied in South America belong to the tropical Characidae family (Andrade, 1996; Gazola *et al.*, 1996).

In teleost females, several different ovarian steroids play different roles at different stages of oocyte development. The best known is 17β-estradiol (E_2), which is produced by the follicular layers and which induces hepatic synthesis and secretion of the yolk precursor protein vitellogenin (Wallace, 1985).

Two other steroids, 17α,20β-dihydroxy-4-pregnen-3-one (17,20β-diOHP) and 17α,20β,21-trihydroxy-4-pregnen-3-one (20β-S), have established roles in the induction of oocyte final maturation (Nagahama, 1990; Trant *et al.*, 1986; Trant and Thomas, 1989; King *et al.*, 1994).

Testosterone (T) has been reported in the plasma of a number of female teleosts, but its precise role has not been determined (Rinchard *et al.*, 1993). Also, 11-ketotestosterone (11-KT), a steroid commonly found in males, has been reported in the plasma of some female teleosts (Slater *et al.*, 1994).

This study characterizes the plasma profile of E_2, T, 11-KT, 17,20β-diOHP, 20β-S, and 17-hydroxy-4-pregnen-3-one (a putative precursor to 17,20β-diOHP) during the first reproductive cycle of female jundiá.

METHODS

The study was conducted between July 1998 and July 1999 at the facilities of the University of Passo Fundo, in Rio Grande do Sul, Brazil (28°15'S/52°24'W, 687 m above sea level).

Fish. The animals used in the present study were 6-month-old juvenile female jundiá weighing between 300 and 600 g. They were kept in 100-m^2 tanks with 1 m of maximum depth. The water flow rate was 6 liters/min and the level of dissolved oxygen varied between 5.0 and 7.0 mg/liter and the pH between 7.0 and 7.2. The fish were fed once a day *ad libitum* with commercial fish pellets (30% of crude protein).

The experimental tank was observed twice a day to detect group spawning and the presence of fry and larvae at the surface. The spawning behavior of jundiá was associated with mild agitation in the tank and some of the fish swimming near the water surface (unpublished observations).

Sampling schedule and procedures. Six females were sampled monthly. In each month the sampling was programmed to occur until the 12th day. The fish were captured with a pen net in the early morning (0800 h) and immediately anesthetized with buffered MS222 (Finquel, Sandoz, 300 mg/liter). After the fish were immobilized by the anesthetic, total body weight (±0.1 g) and length (±0.1 cm) were measured. Blood samples (1–2 ml) were taken from the caudal vessel with a heparinized syringe and centrifuged for 10 min at 3000*g*, and the plasma was stored at −25° until required for radioimmunoassay. The fish were sacrificed by spinal section and decapitation and both ovaries removed, weighed (±0.001 g), and fixed in Bouin's solution for 24 h for histological examination. Tissues were embedded in Paraplast, cut into 5-μm sections, and stained with hematoxylin–eosin.

The gonado-somatic index (GSI) was calculated as the weight of gonads divided by total body weight times 100.

Radioimmunoassay of steroids. Plasma E_2, T, and 17-P were measured in unextracted plasma samples, in duplicate, with commercially available RIA kits ([^{125}I]DPC-estradiol RIA test, [^{125}I] DPC-total testosterone RIA test, [^{125}I] DPC-17-OHP RIA test; DPC Med Lab Produtos Hospitalares LTDA., SP, Brazil). Parallelism of the dilution curves of the plasma samples with the standard curve was demonstrated in all assays, with correlation coefficients ranging from 0.959 to 0.999. The inter- and intraassay coefficients of variation varied between 9 and 12% and between 6 and 9%, respectively.

The assays for 17,20β-P, 20β-S, and 11-KT were carried out in the United Kingdom at the CEFAS Fisheries Laboratory. Because of the difficulties in shipping frozen plasma between countries, it was decided to adapt a procedure that was used by Tveiten *et al.* (2000) to prepare sulfated steroids from the plasma

of common wolffish (*Anarhichas lupus* L.). Aliquots of plasma (100 μl) were mixed with 1 ml ethanol (to precipitate the proteins) and centrifuged at 3000*g*. The supernatants were placed in sealed vials and sent by airmail to the United Kingdom. On arrival, the supernatants were transferred to glass tubes, evaporated under a stream of nitrogen at 45°, reconstituted in 200 μl of distilled water, and extracted with 4 ml diethyl ether. After evaporation of the ether at 45°, the residues were redissolved in 1 ml RIA buffer. The validity of the extraction method was tested by comparing the concentration of testosterone in the 50 ethanol-treated plasma samples (37.35 ± 2.84 ng/ml) with that measured by direct assay (42.64 ± 4.32 ng/ml). The coefficient of correlation between the concentrations measured by the two methods was 0.8728 ($P < 0.05$).

The characteristics of the antisera for 17,20β-P, 20β-S, and 11-KT are described in Scott *et al.* (1982) and Tveiten *et al.* (2000).

For identification of 11-KT on thin-layer chromatography (TLC), a 1-ml pool was made from 10 extracts that had been prepared for RIA and that contained large amounts of 11-KT. A 1-ml pool was also made from 10 male plasma extracts. The steroids were reextracted with diethyl ether, dried down, mixed with ca. 2000 dpm 11-[^{3}H]KT as a marker, and run in separate lanes on TLC as described by Inbaraj *et al.* (1997). The lanes were scraped off in 4-mm bands, eluted with 1 ml assay buffer, and assayed for 11-KT.

Climatic conditions. Maximum and minimum water and air temperatures were recorded daily. The natural day length was calculated from sunrise to sunset in Passo Fundo, and the rainfall levels were obtained at the meteorological station of Passo Fundo.

Data analysis. All data are expressed as means ± SEM. Data were statistically analysed by an analysis of variance (ANOVA) followed by the comparison of means using Tukey's multiple range test (Zaar, 1996), at a significance level of 0.05. A Hartley test was carried out to verify the homogeneity of variance, and a log transformation was performed when necessary.

RESULTS

Climatic conditions. Changes in water and air temperatures, photoperiod, and rainfall during the experimental period are shown in Fig. 1. Water temperature varied from 8° during winter (July) to 31° in summer (January). Day length was longest in December (13 h 45 min) and shortest in July (10 h 14 min).

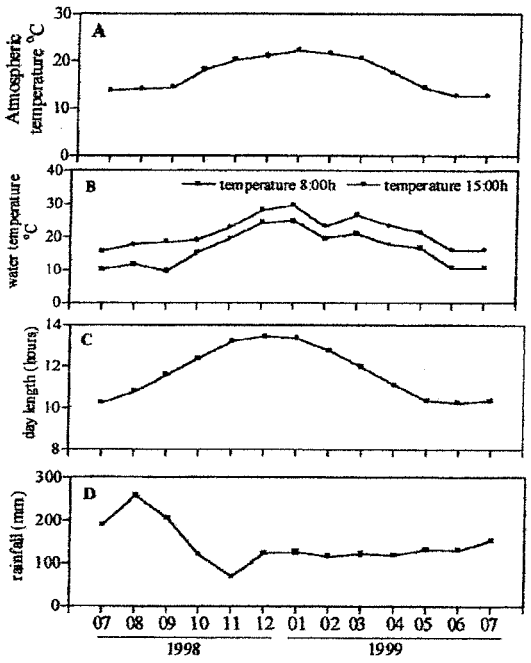

FIG. 1. Climatic conditions in Passo Fundo during the experimental period. (A) Atmospheric temperature. (B) Water temperature: (squares) temperature at 1500 h, (circles) temperature at 0800 h. (C) Photoperiod (hours of light). (D) Rainfall (mm).

During the experimental period (from July 1998 to July 1999) two spawning episodes were observed. These occurred in November (1 day before sampling) and in January (about 7 days after sampling).

Gonadosomatic index. GSI values remained low until the beginning of vitellogenesis (July 1998) and increased rapidly to a peak of 12.28 ± 0.76% in spring (October) (Table 1). They decreased in November and then rose to a second peak in summer (December, 9.1 ± 1.22%), remained high during January (7.48 ± 0.61%), decreased in February and March, peaked for a third time in April (8.63 ± 2.12%), and then dropped suddenly (without the fish spawning) in May (2.13 ± 0.55%).

Maturational stages. The stages, based on the histological and macroscopic appearance of ovary and on gonadosomatic index (GSI), are summarized in Table

TABLE 1
Stages Based on Oocyte Histological and Macroscopic Characteristics and on Gonadosomatic Index (GSI)

Stage	Type of oocytes present[a]	Predominant[b]	Month and GSI (%)
1. Immature	Perinucleolus primary and secondary stages	Stages are distributed similarly	Jul 1998, 1.05 ± 0.13 Aug 1988, 1.14 ± 0.24
2. Early vitellogenesis	Perinucleolus primary and secondary stages, cortical alveoli stage	Cortical alveoli	Sep 1998, 2.11 ± 0.7
Late vitellogenesis and mature	Perinucleolus secondary stage, cortical alveoli stage, vitellogenic oocytes	Vitellogenic oocytes	Oct 1998, 12.28 ± 0.76
3. Postspawn	Perinucleolus primary and secondary stage, cortical alveoli, vitellogenic oocytes	Stages are distributed similarly	Nov 1998 4.03 ± 0.48
First/second spawn			Jan 1999, 7.48 ± 0.61
4. Second mature	Perinucleolus secondary stage, cortical alveoli, vitellogenic oocytes	Vitellogenic oocytes	Dec 1999, 9.1 ± 1.22 Mar 1999, 7.27 ± 0.77 Apr 1999, 8.63 ± 2.12
5. Regressed	Perinucleolus primary and secondary stage and vitellogenic atretic oocytes	Atretic oocytes	May 1999, 2.13 ± 0.55 Jun 1999, 2.27 ± 0.77

[a] The oogonial stage is present in all stages of maturation.
[b] The predominance of oocytes was defined by the type frequency.

1. The predominance of oocytes in each stage was defined by the type frequency.

Plasma sex steroid concentrations. Profiles of plasma concentrations of E_2, T, 17-P, 17,20β-P, and 20β-S are shown in Fig. 2. All numerical data were expressed as means ± SEM.

Plasma E_2 concentrations were low (0.15 ± 0.056 ng/ml) in July 1998, and then progressively increased, to reach a peak in summer (November, 9.1 ± 1.21 ng/ml). In December, the E_2 concentration decreased and remained low from January to July 1999.

Plasma T concentrations were below 15 ng/ml between July and September 1998 but rose to a peak of 53.5 ± 0.8 ng/ml in October (spring).

Plasma 17-P concentrations increased progressively between July and September 1998. Two peaks were observed in October (0.97 ± 0.13 ng/ml) and January (0.94 ± 0.22 ng/ml).

Plasma 17,20β-P increased from undetectable concentrations (<0.2 ng/ml) in August 1998 to 0.73 ± 0.2 ng/ml in September. The highest value was reached in October, preceding the first spawning (1.29 ± 0.18 ng/ml). In January 1999 (second spawning), a moderate elevation was detected.

Plasma 20β-S concentrations showed a pattern similar to those of 17,20β-P with the highest values being found in October 1998 (first spawning) and January 1999 (second spawning).

Plasma 11-KT concentrations (Figs. 3A, 3B) were undetectable in August 1998 and then increased to 91.2 ± 5.61 ng/ml in September. A second elevation (94.37 ± 22.9 ng/ml) was observed in January 1999. In February, the 11-KT concentrations decreased and remained low (Fig. 3A). The distribution of 11-KT in the three major phases of the reproductive cycle is shown in Fig. 3B. The concentrations before the beginning of the active reproductive phase (i.e., September to February) were undetectable. They showed an abrupt increase ($P < 0.01$) during the active reproductive phase. After this phase, concentrations decreased significantly ($P < 0.01$).

Fractionation on TLC (Fig. 4) showed that the bulk of the 11-KT immunoactivity in female and male plasma eluted in exactly the same position as synthetic tritiated 11-KT. A small amount of activity from the females ran ahead of the main peak.

DISCUSSION

During the experimental period, two spawning episodes were observed. In our practical observations with tagged females, some of these fish spawned two to four times during the spawning season (October to March). In our experiment two spawning episodes were observed, but we cannot discard that other spawning had occurred since our observations were made in early morning (6:00 AM) and the jundiá can

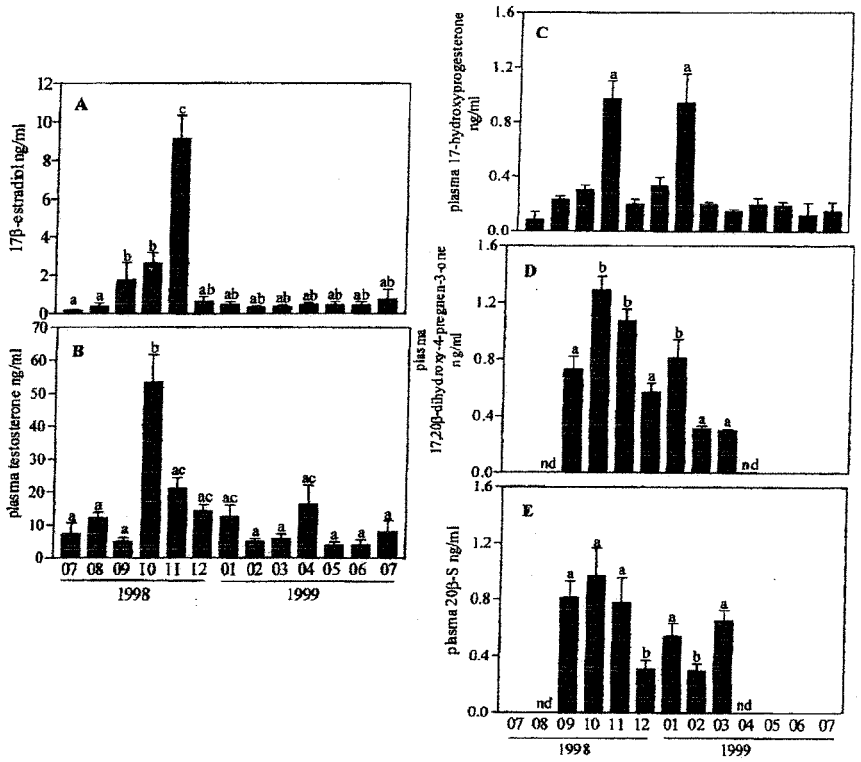

FIG. 2. Changes in sex steroid concentrations during the experimental period. Results expressed as means ± SEM for four to eight fish. (A) 17β-Estradiol. (B) Testosterone. (C) 17-Hydroxy-4-pregnen-3-one. (D) 17,20β-Dihydroxy-4-pregnen-3-one. (E) 17,20β,21-Trihydroxy-4-pregnen-3-one. Different letters above histograms indicate statistically significant differences. Tukey's multiple range test ($P < 0.05$). nd, under the detection limit.

also spawn before that time (3:00 to 7:00 AM). We cannot exactly confirm if the females sampled after the second spawn had spawned in the first spawn; thus the term "second spawn" refers to population.

The GSI has been used as a useful index for monitoring the progression of gametogenesis in female teleost fish. In female *R. quelen* the GSI reaches 12.28 ± 0.76% of body weight. As in other teleosts, the GSI of female jundiá rapidly increases during the months before the spawning season. The morphological features of female jundiá oocytes do not differ significantly from those of other teleosts with asynchronous oocyte development.

The present work revealed a progressive increase in plasma concentrations of E_2 during the months before first spawning, reaching 2.6 ± 0.6 ng/ml in October, 10-fold higher than the values for July and August. Interestingly, one day after the first spawning, in November, E_2 was detected at its highest concentration of 9.1 ± 1.2 ng/ml. This was probably due to the surge in gonadotropin concentration that occurs concomitantly with oocyte final maturation and spawning (Nagahama, 1990). On the other hand, this high estrogen level after the first spawning may be responsible for the initiation of vitellogenesis in the second batch of oocytes for spawning in January, and, since the gonads developed very rapidly from September to October, a major E_2 peak may have occurred between our sampling days. A more frequent sampling during the critical period could certainly help clarify this point.

In female jundiá, the T peak occurred in October and was coincident with the peak in GSI. The positive correlation (data not shown) between T and GSI suggests that T is a strong indicator of the stage of oocyte development as in *Chanos chanos* Forsskal (Marte and Lam, 1992).

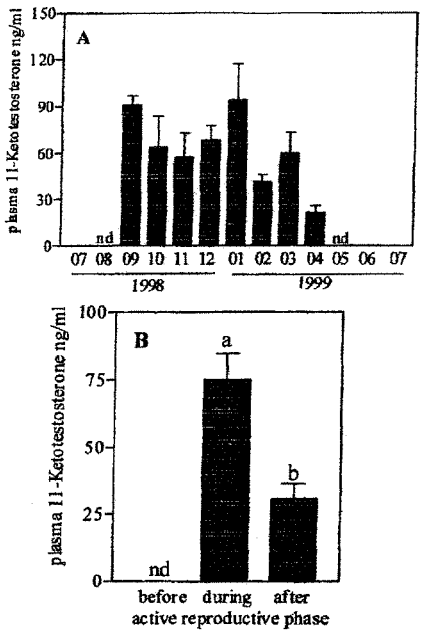

FIG. 3. Changes in 11-ketotestosterone during the experimental period. Results expressed as means ± SEM. (A) Monthly values. (B) Distribution across the major reproductive phases. The "active reproductive phase" was considered to be from September 1998 to February 1999. Before (N = 18), during (N = 30), and after (N = 30) active reproductive phase. Different letters above histograms indicate statistically significant differences. Tukey's multiple range test ($P < 0.05$). nd, under the detection limit.

The present study showed two distinct peaks of 17-P, both occurring 1 month before spawning. This steroid has been reported in plasma of numerous teleost females like the bitterling, *Acheilognathus rhombea* Temminck and Schlegel (Shimizu *et al.*, 1985), the gray mullet *Mugil cephalus* Linnaeus (Chang *et al.*, 1995), and the white sucker *Catostomus comersoni* Lacepède (Scott *et al.*, 1984).

The C21 steroid 17,20β-P had its highest concentrations in October, coincident with the first peak of 17-P. A second elevation in 17,20β-P was observed in January, coincident with the second peak of 17-P. This secretory pattern supports the possibility that the 17,20β-P might act as the MIS (Maturational Inducing Substance). Shimizu *et al.* (1985) in the bitterling (*A. rhombea*) measured both 17-P and 17,20β-P and found very similar profiles, concluding that both hormones have important actions in final oocyte maturation in this fish. The relatively low concentrations of 17,20β-P in the blood plasma of jundiá (up to 1.5 ng/ml), as opposed to salmonids (up to 500 ng/ml), are typical of multiple spawning species (see Discussion in Scott *et al.*, 1998).

The C21 steroid 20β-S was also in evidence in the plasma of mature jundiá. This steroid, as has been proposed for Atlantic croaker *Micropogonias undulatus* (Trant *et al.*, 1986; Trant and Thomas 1989) and striped bass *Morone saxatilis* L. (King *et al.*, 1994), can have a MIS function in female jundiá.

Although 11-KT is the male androgen in teleosts, it has been detected in the plasma of a number of sexually mature female fish; for example, Morrison *et al.* (1985) found 11-KT concentrations of ca. 30 ng/ml in female coho salmon, *Oncorhyncus kisutch*. In the same species, Fitzpatrick *et al.* (1986) found 11-KT concentrations varied throughout the spawning run, between 0.6 and 21.7 ng/ml, but without a readily discernible pattern. In the chinook salmon *Oncorhynchus*

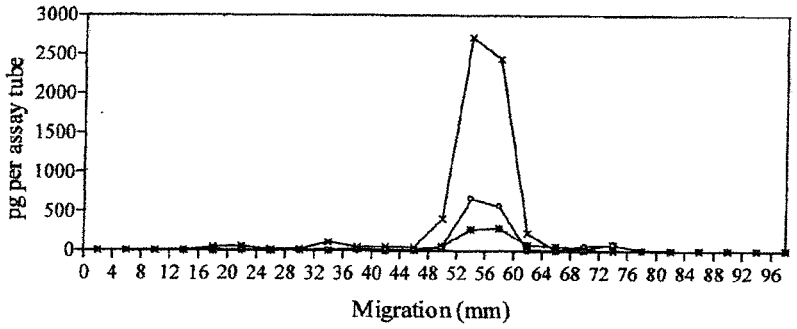

FIG. 4. Migration on TLC of 11-KT immunoactivity extracted from male (x) and female (o) jundiá plasma. The elution position of 11-[^{3}H]KT is also shown (in dpm × 10).

tshawytscha, Slater *et al.* (1994) found concentrations of 11-KT varying from 0.1 to 20 ng/ml during the reproductive cycle. In migrating females of two Australian *Anguilla* spp., Lokman *et al.* (1998) measured 11-KT concentrations between 3 and 20 ng/ml. The origin of 11-KT in females is unknown. Cuisset *et al.* (1995) detected 11-KT in Siberian sturgeon (*Acipenser baeri* Brandt) females. They investigated its possible biosynthesis by various tissues and observed a small amount of production of 11-KT and other 11-oxygenated androgens by the ovary, interrenal, and blood cells. The mammalian adrenal has also been show to produce 11-oxygenated androgens, especially when stimulated by adrenocorticotropin (Wassermann *et al.*, 1973).

The possibility that the 11-KT in female jundiá is synthesized by ovaries or interrenal needs to be explored. However, the possibility that it derives from fish-to-fish transfer of 11-KT from males to females, as has been shown for testosterone in a closed circulation tank system (Budworth *et al.*, 1994), cannot be discarded.

Studies of other teleosts have indicated that temperature and photoperiod are primary environmental cues regulating gonadal recrudescence and spawning (MacKenzie *et al.*, 1989; Peter and Yu, 1997). These two climatic factors probably also affected the reproduction of jundiá, since T and E_2 concentrations increased in synchrony with the elevation of temperature and increasing photoperiod.

In conclusion, the profiles of E_2, T, 17-P, 17,20β-P, and 20β-S in females suggest their participation in different phases of the reproductive cycle, and in a manner similar to that of other teleosts that have been studied.

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4.2. Artigo Submetido

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Steroid profiles in cultured male jundiá, the Siluridae *Rhamdia quelen* (Quoy and Gaimard, Pisces Teleostei).

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Running headline: Steroid profiles in female R. quelen

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Abstract

The jundiá *Rhamdia quelen* (Quoy and Gaimard) is a teleost species from the *Siluridae* family and is an important species for aquaculture in temperate and subtropical climates. Gonad and blood samples were taken from cultured *R. quelen* males between 1998 and 2000, and plasma levels of testosterone (T), 11-ketotestosterone (11-KT), 17-hydroxy-4-pregnene-3,20-dione (17-P), 17,20β-dihydroxy-4-pregnen-3-one (17,20β-P) by radioimmunoassay measured and potential correlations with the stage of spermatogenesis and sexual maturation examined. The *In vitro* production of T and 11-KT by incubated testis was also analysed. The plasmatic levels of T increased progressively during spermatogenesis and reached its peak at the beginning of the spontaneous spermiation. The higher production of T by the testis occurred simultaneously. During the milt-producing period, both plasma and testis T decrease, when the 11-KT concentrations are still high. The extremely high concentrations of 11-KT during the first cycle in contrast to second cycle suggests its role as pubertal steroid in male jundiá. The 17-P and 17,20β-P showed higher levels during the period preceding the spermiation and decrease afterwards. Concluding, the data suggests that the 11-KT plays a important role during puberty in male jundiá and that the pattern of plasma and testis T and 11KT changes are consistent with the general roles of these hormones in male fish reproduction.

Key words – jundiá, Rhamdia quelen, testosterone, 11-ketotestosterone, puberty.

Introduction

In the southern part of South American, and in other regions with a temperate or subtropical climates, there is a crescent interest in species that can support cold winters and that can grow fast in the warm months of summer. The jundiá (*Rhamdia quelen,* Quoy and Gaimard) is one of these species. It will reach 600-800g of body weight in eight months at stocking densities of two to four fish/m2. The study of diverse aspects of reproductive physiology in aquaculture species is particularly necessary, as gamete production is often adversely affected by the conditions of captivity.

In the last years, the interest in the study of gonadal steroidogenesis in teleost has increased, in order to develop methods for reproduction control in species with commercial value (Venkatesh et al., 1992). According to Fostier et al. (1983), in male fish, GtH I and GtH II stimulate the testicular synthesis of some steroids and spermatogenesis. In most species of fish examined, testes produce both testosterone and 11-ketotestosterone.

Billard (1976) postulate that high doses of exogenous T maintain spermatogenesis in hypophysectomized goldfish (*Carassius auratus*, L.), suggesting an androgen control of this process. On the other hand, the 17α,20β-dihydroxy-progesterone (17,20β-P), another hormone produced by the testis, seems to control the spermiation in male teleosts (Fostier et al., 1983).

In the Siluridae family, some works has been shown the 11-KT as a possible pubertal steroid, wich have a direct stimulatory effect on first spermatogenesis events (Miura et al., 1991; Borg, 1994) and in mature males of teleosts in general, its function is related to spermiation (Fostier et al., 1987).

Until now, few researches have been dedicated to jundiá, always focusing in the management aspects (e.g. nutrition and stocking density). No data about its reproductive physiology were found in the literature. This study characterizes the plasma profile of T,

11KT, 17,20β-P, and 17-P (a putative precursor for 17,20β-P) during the first reproductive cycle of male jundiá. The study also characterizes the *in vitro* production of T and 11-KT by testis.

Material and Methods

The study was conducted between July 1998 and February 2000, at the facilities of the University of Passo Fundo, Rio Grande do Sul (28°15'S / 52°24''W, 687 m up of sea level).

Fish

The animals used in the present study were first maturing eight-month-old juveniles male jundiá (*Rhamdia quelen*) weighing between 300 and 600g. The females stocked together were tagged and not considered in this study. Sample broodstock were kept in $100m^2$ tanks with 1m of maximum depth. The water renovation was 6 l/m and the level of dissolved oxygen was maintained from 5.0 to 7.0 mg/l and the pH ranged between 7.0 to 7.2. The fish were fed once a day with commercial available food (30% of raw protein).

The experimental tank was observed twice a day to detect group spawning and the presence of fry and larvae at the surface. The spawning behaviour of jundiá was associated with mild agitation in the tank and some of the fish swimming near the water surface (Barcellos et al., 2001).

Sampling schedule and procedures

Six males were sampled monthly in the first cycle. In second cycle the sampling were made in pre determined months (July, September to November 1999 and February 2000). In each month the sampling was programmed to occur until the 12 day. The fish were captured with a pen net in the early morning (0800h) and immediately anaesthetized with buffered MS222 (Finquel® Sandoz, 300 mg/l). After the fish were immobilized by the anaesthetic, total body weight (±0.1g) and length (± 0.1 cm) were measured. Blood

samples (1-2 ml) were taken from the caudal vessel with a heparinized syringe, centrifuged for 10 minutes at 3,000g, and the plasma stored at –25°C until required for radioimmunoassay. The fish were sacrificed by spinal section and decapitation and both testis removed, weighed (± 0.001g), and fixed in Bouin's solution for 24 h for histological examination. Tissues were embedded in paraplast®, cut into 5μm sections and stained with hematoxylin-eosin. A small piece of the median portion of one testis were removed, weighed and put in ice-cold 199 modified medium (Medium 199 with Hank's salts and L-glutamine, Gibco BRL, Life Technologies TM).

The gonado-somatic index (GSI) was calculated as the weight of gonads divided by total body weight times 100.

Incubation procedures

The piece of testis was submerged in ice-cold 199 medium and minced with scissors. The minced testis was incubated in 199 modified medium (Sigma Chemical Co., St. Louis, USA) for two hours in Dubnoff metabolic shaker at 25°C, pH 7.4 and gassed with O_2:CO_2 (95:5; v/v).

After incubation, the testes were removed by centrifugation and the medium stored at -25°C until analysis.

Extraction

Two ml of medium sample were extracted with 5 ml of ethyl acetate (2X). The ethyl acetate evaporated to dryness under nitrogen.

Radioimmunoassay of steroids

The concentrations of T and 17-P were measured in unextracted plasma and medium (T) samples, in duplicate, with commercially available RIA kits ([^{125}I] DPC-total testosterone RIA test, [^{125}I] DPC- 17-P RIA test; DPC Med Lab Produtos Hospitalares LTDA., SP, Brazil). Parallelism of the dilution curves of the plasma samples with the

standard curve was demonstrated in all assays, with correlation coefficients ranging from 0,959 to 0,999. The inter- and intra-assay coefficients of variation varied between 9 to 12% and 6 to 9%, respectively.

The assays for 17,20β-P and 11-KT were carried out in the UK at the CEFAS Fisheries Laboratory. Because of the difficulties in shipping frozen samples between countries, it was decided to adapt a procedure that was used by Tveiten *et al.* (2000) to prepare sulfated steroids from the plasma of common wolffish (*Anarhichas lupus* L.). Aliquots of plasma or incubation medium (100 µl) were mixed with 1 ml ethanol (to precipitate the proteins) and centrifuged at 3000 g. The supernatants were placed in sealed vials and sent by airmail to the UK. On arrival, the supernatants were transferred to glass tubes, evaporated under a stream of nitrogen at 45°C, reconstituted in 200 µl of distilled water, and extracted with 4 ml diethyl ether. After evaporation of the ether at 45 °C, the residues were redissolved in 1 ml RIA buffer. The validity of the extraction method was tested by comparing the concentrations of testosterone in the ethanol-treated 50 plasma samples (59.3 ± 6.8 ng/ml) with those measured by direct assay (55.7 ± 7.0 ng/ml). The coefficient of correlation between the concentrations measured by the two methods was 0.9988 ($P<0.05$).

The characteristics of the antisera for 17,20β-P and 11KT are described in Scott *et al.* (1982) and Tveiten *et al.* (2000).

For identification of 11KT on thin-layer chromatography (TLC), a 1 ml pool was made from ten extracts which had been prepared for RIA and which contained high amounts of 11KT. The steroids were re-extracted with diethyl ether, dried down, mixed with ca. 2000 dpm [^{3}H]-11KT as a marker and run in separate lanes on TLC as described by Inbaraj *et al.* (1997). The lanes were scraped off in 4 mm bands, eluted with 1 ml assay buffer and assayed for 11KT.

Climatic conditions

Maximum and minimum water and air temperatures were recorded daily. The natural day length was calculated from sunrise to sunset in Passo Fundo, and the rainfall levels were obtained at the meteorological station of Passo Fundo.

Data Analysis

All data are expressed as means ± S.E.M. Data were statistically analysed by an analysis of variance (ANOVA) followed by the comparison of means using Tukey's multiple range test (Zaar, 1996), at a significance level of 0.05. A Hartley test was carried out to verify the homogeneity of variance, and a log-transformation was performed when necessary.

Results

Changes in water and air temperatures, in photoperiod and in the rainfall, during the study period are shown in figure 1. Pond temperature varied from 8°C during winter (July) to a maximum value (31°C) in summer (January). Day length was longer in December (1345h) and shorter (1014h) in July.

Gonadossomatic Index (GSI, table I)

In winter (July and August) the GSI remained low. In early spring (September) the GSI increased ($P<0.05$), and reached a first peak in the middle of spring (October, 8.03 ± 0.64%). After the decrease ($P<0.05$) occurred in November, the GSI reached a second peak in summer (January, 8.25 ± 0.82%) decreasing afterwards (February to April). The GSI severely dropped in autumn (may, 0.81 ± 0.34%). In June and July, the increase slowly begin, 1.18 ± 0.34 and 1.6 ± 0.25 %, respectively. In September 1999 the GSI increased to a peak of 7.38 ± 0.68 % and after presents a progressive decrease to 4.42 ± 0.93 % in november. In February 2000 the GSI was 2.92 ± 0.98 %.

Maturational Stages

The stages, based on the histological and macroscopic appearance of testis and on Gonadosomatic Index (GSI) are shown in Table 1.

Plasma T and 11-KT concentrations

Profiles of plasma T and 11-KT concentrations are shown in figure 2.

Plasma T concentrations (Fig. 2A) begun to increase in late winter (August), reaching a peak in late spring (November) (55.7 ± 7.0 ng/ml, $P<0.01$). Later on, T levels decreased progressively until minimum levels in autumn. In the second cycle, the plasma T concentrations are high in September and reaches a peak in October (58.8 ± 5.7 ng/ml, Spring, $P<0,01$) In November the values decreases and in February 2000 presents a moderate non-significative elevation.

Plasma 11-KT concentrations (Fig. 2B) were low in July, August and September 1998, and begun to increase in october, reaching a extremely high concentration of 1243.42 ± 337.32 ng/ml in December 1998. After this elevated levels the 11-KT concentrations declines to approximately 100-250 ng/ml in all other measurements including the second cycle.

Testis productions of T and 11KT

Profiles of testis production of T and 11KT are shown in figure 3.

The concentrations of T (Fig. 3A) measured in incubation medium showed the higher level in November, the same month of the plasmatic peak. This value is higher than preceding values ($P<0.001$). In December the values were still high and in the posterior months they decreased progressively.

Concentrations of 11-KT (Fig. 3B) measured in incubation medium show a peak of 0.48 ± 0.19 ng/mg of tissue in December (P<0,01), coinciding with the 11-KT plasmatic peak. Before and after this peak the values are low.

Fractionation on TLC (Fig. 4) showed that the bulk of the 11-KT immunoactivity in male and female plasma eluted in exactly the same position as synthetic tritiated 11-KT. A big amount of activity from the males ran ahead of the main peak.

Plasma C21 steroids concentrations

The plasmatic levels of 17-P and 17,20 β-P were show in figure 5.

The plasma 17-P levels are higher in months preceding the beginning of spermiation, when the spermiation period begun it was declined. In the second cycle the values obtained in September, October and November 1999 are similar to the first cycle. After the ending of the spermiation period, the levels dropped down to minimal levels.

Plasma 17,20β-P (Fig. 5B) increase from undetectable concentrations (<0.2 ng/ml) in August 1998 to 0.31 ± 0.07 ng/ml in September. The highest value of 0.81 ± 0.14 ng/ml, was reached in October 1998 (first spawning episode, puberty). In the second cycle the values were low in contrast with the 1st cycle, but presents highest concentrations in the same months.

Discussion

The GSI has been used as a useful index for monitoring the progression of the gametogenesis in male teleost fish, because the testis mass during maturation makes a good percentage of their body mass. In male *Rhamdia quelen* the testis reach 8.25 ± 0.82% of body weight, when the testis are completely mature. Like in other teleost fish, the GSI of male jundiá rapidly increases during the months before the spermiation and presents relative high levels afterwards, reaching another peak in January. During the regression stage, the GSI drops until initial levels (≈ 1%).

The morphological features of male jundiá spermatogenesis do not differ significantly from other teleosts with the same cellular types.

The plasmatic levels of T in male jundiá increases progressively until the beginning of spermiation and decreases afterwards while sperm production is still rising. In trout (*Oncorhynchus mykiss*, Walbaum) Pavlidis et al. (1994) found similar results. In their work, T peak occurred as spermiation begun and after, it declined, but sperm production continued.

In the *Chanos chanos* (Forsskal) (Marte and Lam, 1992) the males were divided in four stages: immature, early mature, late mature and spent or regressed males. The T levels increased from immature to early mature males and dropped in late mature males. The abrupt rise in early mature males indicates that T may play a role in the initiation of spermatogenesis in this fish.

In the pacific herring (*Clupea harengus Palassi*) Carolsfeld et al. (1996), it was found elevated levels of T and 11-KT in males during the beginning of spermatogenesis. These levels were reduced in ripe fish during the milt producing period.

Our results showed that the T production by the testis presented the higher value simultaneous to the plasma T peak at the beginning of the spermiation period, decreasing afterwards. In rainbow trout (*Oncorhynchus mikyss*), Schulz and Blüm (1990) also found a reduction of T production by the testis after the beginning of the spermiation period.

In the king-fish (*Odonthestes perugiae*, Evermann & Kendall), other native specie from southern South America, the peak of *in vitro* T production by the testis occurred in September at the beginning of the spermiation period (Porawski, 1999).

Other androgens are reported as spermatogenesis regulatory hormones in fish like 11-oxygenated androgens (Fostier et al.,1983). In male jundiá the 11-KT is quantitatively predominant to T during all reproductive cycle.

A direct stimulatory effect on spermatogenesis by 11-KT, the predominant circulating androgen in males of several fish species (Borg, 1994), was observed by Miura et al. (1991). The extremely high concentrations of 11-KT at the initiation of the 1st reproductive cycle in relation to relative low levels found in 2nd cycle strongly suggests that the 11-KT can act as a "pubertal steroid" in male *Rhamdia quelen.*

This is in line with the concept that 11-KT has a direct stimulatory effect on testis and stimulates the spermatogenesis toward and beyond the first meiotic division, as has been show in Japanese Eel by Miura et al., 1991.

In addition, Cavaco et al. (1995) show that the 11-oxygenated androgens significantly stimulate testicular growth and induce precocious differentiation of seminal vesicles in African catfish (*Clarias gariepinus*).

There is a species variability in the concentrations of 11-KT measured in teleost fish, with the peak concentrations varying from 3 ng/ml in the *Pagrus auratus* (Carragher and Pankhurst, 1993) to 115 ng/ml in *Oncorhynchus kisutch* (Morrison et al., 1985). The extremely high concentrations found in pubertal male jundiá (up to 1,0 μg/ml) may indicate a specie peculiarity in the biosynthesis of this hormone. This peculiarity maybe justify the presence of relatively high levels of 11-KT in females of this specie (Barcellos et al., 2001).

According to Baynes and Scott (1985), T may be important during an early phase of the testicular growth, whereas 11-KT may be the principal hormone during the late spermatogenesis and spermiogenesis. Since the T levels in jundiá were high until initiating of spermiation and 11KT remain predominant in all spermiation period, our results seem to confirm this assertion.

The levels of 17-P were higher in months preceding the beginning of the spermiation. In the white sucker (Scott et al.,1984), the levels of both 17-P and 17,20βP

increased during spawning, and in rainbow trout only 17α,20βP increased during the spermiation (Scott and Baynes, 1982). In male jundiá, after the beginning of spermiation, the 17-P and T levels decreased and low levels were maintained during this period. The abrupt reduction in T concentration may be also explained by its conversion in downstream metabolites as the 11-KT (Fostier et al., 1983).

The 17,20β-P show the highest values in the initiation of spermiation period, suggesting that its play a role in the final spermatozoid maturation and hydration, like postulated to other teleost males (Fostier et al., 1987).

Taken together, the concentrations found in the sex steroids analysed, may indicate that the 11-KT can be the pubertal steroid in male jundiá, and that this hormone is related to spermiation also in posterior reproductive cycles, acting in the same period that C21 steroids, probably responsible for final spermatozoid maturation and milt formation.

Studies in other teleost have indicated that temperature is a primary environmental cue in regulating the gonadal recrudescence (*Ictalurus puntactus*, MacKenzie et al.,1989) In our study, we detected the T levels begin to increase with the increase of the temperature. The photoperiod, that modulates sexual development and spawning in many fish (Peter & Yu, 1997), would probably affect the reproduction of jundiá, since the T levels increased progressively in period of crescent photoperiod. Thus, our results provide a basis for more detailed studies aiming to elucidate the relative contribution of environmental conditions in regulating hormone dynamics in male jundiá.

In conclusion, the profiles of T, 11-KT, 17-P and 17,20β-P in males suggest their participation in different phases of the reproductive life (specially 11-KT during puberty) cycles, and in similar manner to other teleosts that have been studied

Figure captions

Figure 1. Climatic conditions in Passo Fundo during the experimental period. (A) Atmospheric Temperature. (B) Water temperature: (squares) temperature at 1500h, (circles) temperature at 0800h. (C) Photoperiod (hours of light) and (D) rainfall (mm).

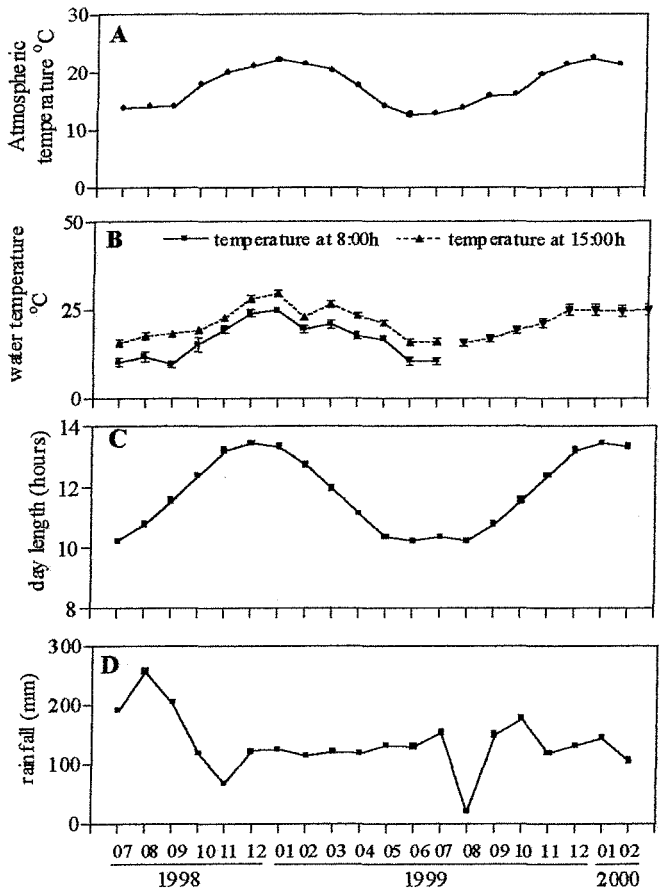
Figure 2: Changes of plasma testosterone (A) and 11-ketotestosterone (B) during the experimental period. Results expressed as mean ± S.E.M. for 4-8 fish. Different letters above histograms indicate statistically significant differences. Tukey's multiple range test ($P<0.05$). *nm* = not measured.

Figure 3. Changes of testis production of testosterone (A) and 11-ketotestosterone (B) during the experimental period. Results expressed as mean ± S.E.M. Different letters above histograms indicate statistically significant differences. Tukey's multiple range test ($P<0.05$). *nm* = not measured.

Figure 4: Migration on TLC of 11KT immunoactivity extracted from male (x) and female (o) jundiá plasma. The elution position of [^{3}H]-11KT is also shown (in dpm x 10).

Figure 5: Changes of plasma 17-hydroxy-progesterone (A) and 17,20β-dihydroxy-progesterone (B) during the experimental period. Results expressed as mean ± S.E.M. for 4-8 fish. Different letters above histograms indicate statistically significant differences. Tukey's multiple range test ($P<0.05$). *nm* = not measured.

Figure 1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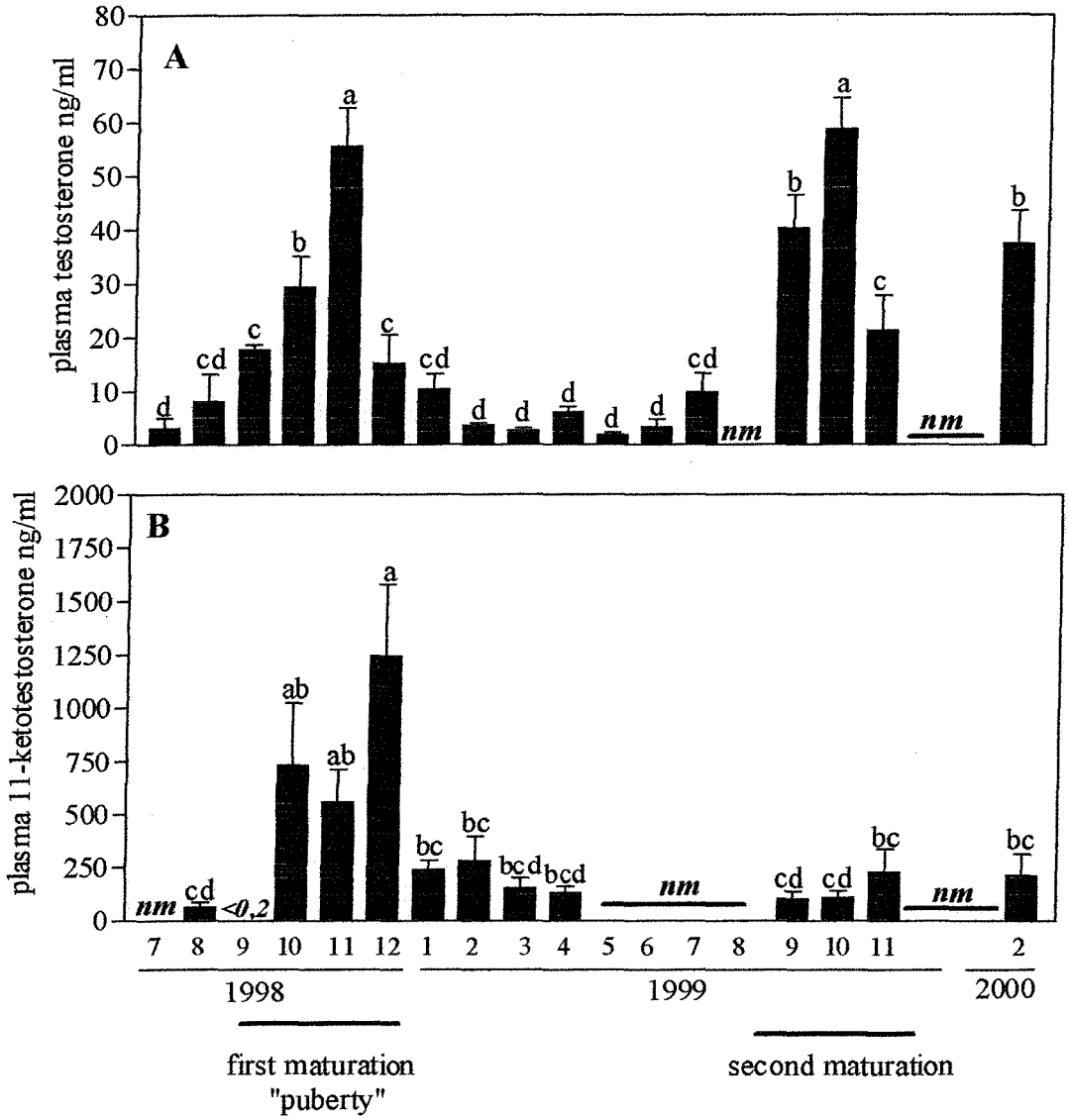
Atmospheric temperature °C
30
20
10
0
B
water temperature °C
temperature at 8:00h
temperature at 15:00h
50
25
0
C
day length (hours)
14
12
10
8
D
rainfall (mm)
300
200
100
0
07 08 09 10 11 12 01 02 03 04 05 06 07 08 09 10 11 12 01 02
1998
1999
2000

Figure 2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70
60
50
40
30
20
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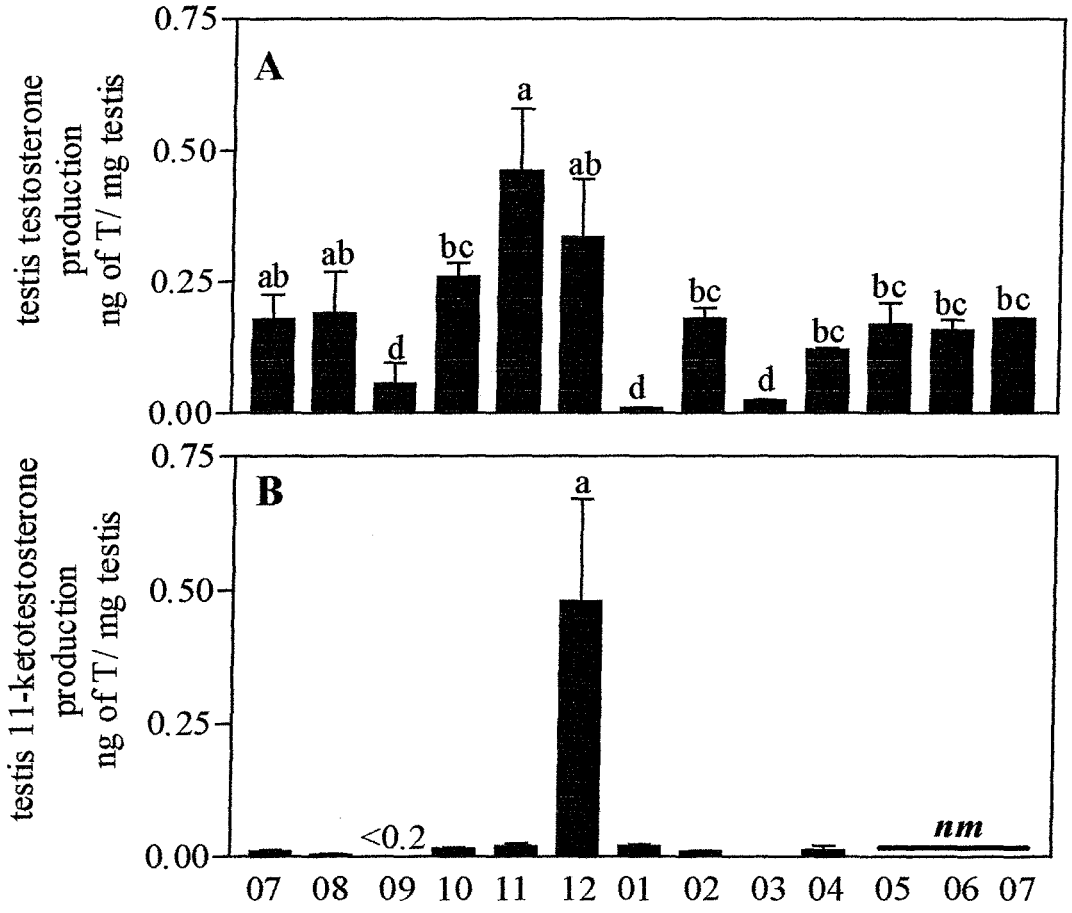
0
d
cd
c
b
a
c
cd
d
d
d
d
d
cd
nm
b
a
c
nm
b
B
plasma 11-ketotestosterone ng/ml
2000
1750
1500
1250
1000
750
500
250
0
nm
cd
<0,2
ab
ab
a
bc
bc
bcd
bcd
nm
cd
cd
bc
nm
bc
7 8 9 10 11 12 1 2 3 4 5 6 7 8 9 10 11 2
1998
1999
2000
first maturation
"puberty"
second maturation

Figure 3


A
testis testosterone production ng of T/ mg testis
0.75
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0.00
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ab
d
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ab
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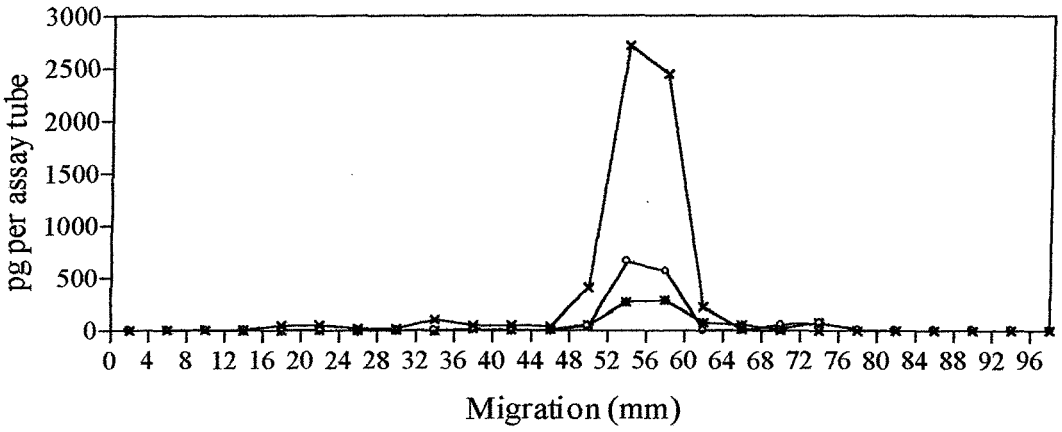
bc
d
bc
bc
bc
bc
B
testis 11-ketotestosterone production ng of T/ mg testis
0.75
0.50
0.25
0.00
<0.2
a
nm
07 08 09 10 11 12 01 02 03 04 05 06 07

Figure 4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2000
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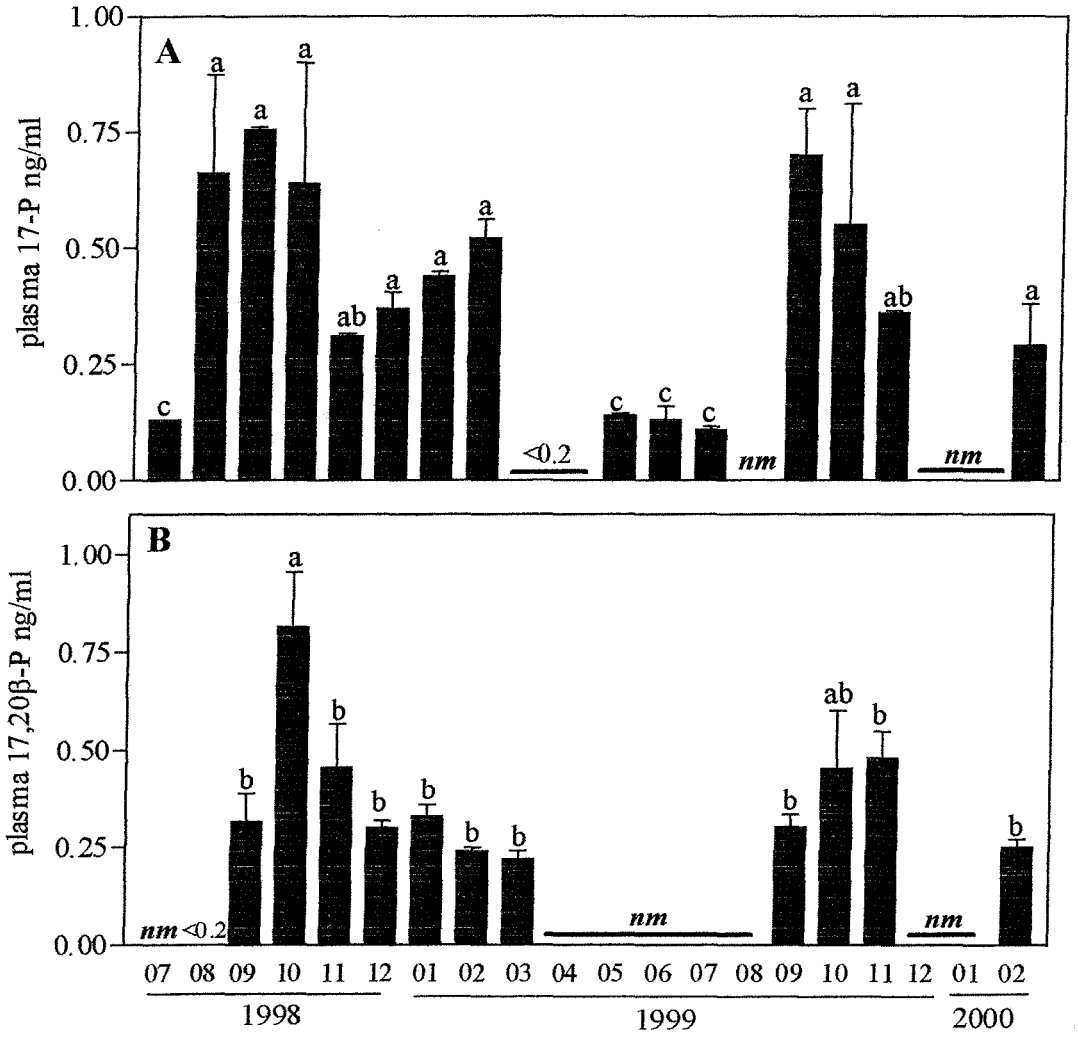
Migration (mm)

Figure 5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0.75
0.50
0.25
0.00
a a a a ab a a a c <0.2 c c c nm a a ab a nm
B
plasma 17,20β-P ng/ml
1.00
0.75
0.50
0.25
0.00
nm <0.2 b a b b b b b b nm b ab b nm b
07 08 09 10 11 12 01 02 03 04 05 06 07 08 09 10 11 12 01 02
1998
1999
2000

Table 1

Table I. Stages based on histological and macroscopic characteristics, and on Gonadosomatic Index (GSI).

Stage	Month and GSI (%)	Cell population and degree of predominance [a]
1. Immature	Jul 1998 0.41 ± 0.03	small luminal space, spermatogonia (++++)
2. Early maturing	Ago 1998 0.85 ± 0.24	Spermatocyt (++)
	Sep 1998 6.47 ± 0.28	Spermatid (++)
	Sep 1999 7.36 ± 0.68	
3. Maturing	Oct 1998 8.03 ± 0.64	Luminal space filled with spermatozoa
		In cyst, spermatogonia (+), spermatocyt
		(++), spermatid (+++)
4. Mature – Spent	Nov 1998 4.5 ± 0.66	Dilated luminal space
	Dec 1998 5.95 ±1.08	Small number of germinative cysts
	Jan 1999 8.25 ± 0.82	Spermatogonia (+)
	Feb 1999 4.48 ± 0.77	Spermatozoa (++++)
	Oct 1999 5.64 ± 1.24	
	Nov 1999 4.42 ± 0.93	
	Feb 2000 2.92 ± 0.98	
5. Regressed	May 1999 0.81 ± 0.34	Residual spermatozoa
	Jun 1999 1.18 ± 0.34	Peripheral spermatogonia (+)

[a] the predominance of the cell types was defined by the type frequency

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5 – CONCLUSÕES E PERPECTIVAS FUTURAS

Os resultados obtidos e demonstrados nos três trabalhos incluídos neste documento, fornecem uma ampla visão dos processos fisiológicos reprodutivos de machos e fêmeas de jundiás da espécie *Rhamdia quelen*, bem como um breve perfil de sua resposta ao estresse.

Alguns resultados verificados em jundiás foram de grande interesse para o estudo da fisiologia reprodutiva dos peixes teleósteos em geral, como o fato de a fêmea de jundiá produzir em grandes quantidades o hormônio denominado 11-cetotestosterona, reportado por vários autores como sendo um esteróide estritamente dos machos (LEATHERLAND et al., 1982).

A distribuição das concentrações deste hormônio nas principais fases do ciclo reprodutivo da fêmea do jundiá, fortemente sugerem uma ação deste na sua fisiologia reprodutiva, ação esta ainda desconhecida.

Durante os trâmites para a publicação do trabalho referente as fêmeas, a fonte da 11-cetotestosterona foi questionada, e uma interessante e promissora linha de pesquisa foi iniciada. Os resultados preliminares obtido até o momento, claramente indicam que o tecido interrenal, localizado na porção anterior do rim, chamada de rim cefálico, é o responsável pela conversão da testosterona circulante em 11-cetotestosterona. Estes resultados abrem uma perspectiva de continuidade das pesquisas na linha de biosíntese hormonal no jundiá.

Outro corpo de resultados que foi considerado relevante é o referente as concentrações de três diferentes progestágenos, a 17-hidroxi-4-pregnen-3,20-diona (17-P) a 17,20β-dihidroxi-4-pregnen-3-ona (17,20β-P) e a 17,20β,21-trihidroxi-4-pregnen-3-ona (20β-S).

Dos três hormônios o que apresentou uma correlação mais positiva com os meses de desova foi a 17-P, entretanto a liberação destes hormônio é curta circundando brevemente os dias de maturação final e desova e devido ao regime mensal de coletas, picos dos demais progestágenos medidos podem ter acontecido mais claramente. Estes resultados também oferecem uma clara perspectiva de continuidade nas pesquisas, afim de elucidar o exato papel destes hormônios na fisiologia reprodutiva da fêmea do jundiá.

Quanto aos machos, o resultado mais interessante foi a clara evidencia de que a 11-cetotestosterona é o esteróide indutor de puberdade. Valores extremamente altos, nunca achados para nenhuma espécie de peixes teleósteos já pesquisada, foram encontrados durante o primeiro ciclo reprodutivo dos machos de jundiá, caindo para valores considerados normais (e também próximos aos encontrados nas fêmeas) no segundo ciclo. Este dado pode abrir uma perspectiva de pesquisa no manejo reprodutivo da espécie como forma de manipular a puberdade do animais. Já nas fêmeas não foi detectado nenhum dos hormônios com distribuição indicativa de ser um indutor de puberdade.

As concentrações de testosterona medidas nos machos também foram consideradas altas, e a produção testicular deste hormônio, quando expressa em "testículo inteiro" foi extremamente alta. Estes dados quando tomados em conjunto mostram a extrema importância da testosterona e seus metabólitos na espermatogênese e espermiação de machos jundiá.

Os progestágenos medidos nos machos (17-P e 17,20-βP) apresentaram concentrações mais altas próximos a época de espermiação, sugerindo um papel importante

na maturação final dos espermatozóides, como reportado para outras espécies de peixes teleósteos mais estudadas.

O perfil da resposta ao estresse traçado para ambos os sexos, teve como resultado mais interessante a significativamente superior resposta encontradas na fêmeas. Este experimento foi realizado em somente uma fase do ciclo reprodutivo, a de maturação final, período este onde a mobilização de energia para formação do vitelo se torna indispensável.

O cortisol tem sido reportado como um hormônio chave nesta fase do ciclo reprodutivo de outros bagres como o peixe-gato indiano e africano. O perfil encontrado no jundiá, fortemente sugere que este papel também pode se dar de forma relevante nas fêmeas da espécie. Mais uma vez, vislumbra-se uma perspectiva futura de pesquisa, determinando o papel exato do cortisol na reprodução do jundiá.

Concluindo, o presente trabalho permitiu traçar um perfil hormonal reprodutivo do jundiá, correlacionando as concentrações dos diversos hormônios esteróides medidos com as diferentes épocas do ano e fases do ciclo reprodutivo e seus principais eventos fisiológicos. Os dados obtidos abrem um grande campo de pesquisa básica, biossíntese e ações hormonais, e aplicada como a manipulação da reprodução desta importante espécie para a aquacultura de exploração comercial do sul do país.

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