



1
2
3
4
5
6
7 UNIVERSIDADE FEDERAL DO ESTADO DO PARÁ
8 INSTITUTO DE CIÊNCIAS BIOLÓGICAS
9 PROGRAMA DE PÓS-GRADUAÇÃO EM ECOLOGIA AQUÁTICA E PESCA
10
11
12
13
14
15
16
17

18 LEONARDO FERNANDES DA PAIXÃO
19
20
21
22
23
24
25
26
27
28
29
30

31 **Estudo da Biologia reprodutiva de *Crassostrea gasar* (Adanson, 1757) no nordeste**
32 **paraense.**
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48

BELÉM –PA
2012

LEONARDO FERNANDES DA PAIXÃO

49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65
66
67
68
69
70
71
72
73
74
75
76
77
78
79
80
81
82
83
84
85
86
87
88
89
90
91
92
93
94
95
96

**Estudo da Biologia reprodutiva de *Crassostrea gasar* (Adanson, 1757) no nordeste
paraense.**

Dissertação apresentada ao Programa de Ecologia Aquática e Pesca da Universidade Federal do Pará, como requisito parcial para a obtenção de grau de Mestre em ecologia Aquática e Pesca.

Orientador (a): Dr^a Rossineide Martins da Rocha – ICB/UFPA

Co-orientador (a): Maria Auxiliadora Pantoja Ferreira – ICB/UFPA

BELÉM –PA
2012

97
98
99

100 **Estudo da Biologia reprodutiva de *Crassostrea gasar* (Adanson, 1757) no nordeste**
101 **paraense.**

102 Dissertação apresentada ao Programa de Ecologia Aquática e Pesca da
103 Universidade Federal do Pará, como requisito parcial para a obtenção de grau
104 de Mestre em ecologia Aquática e Pesca, cuja banca examinadora foi
105 constituída pelos professores listados abaixo, tendo obtido o
106 conceito_____

107
108
109

110 Orientador (a):
111 Profª Drª Rossineide Martins da Rocha
112 Instituto de Ciências Biológicas – UFPA

113
114
115

116 Co-orientador (a):
117 Profª Drª Maria Auxiliadora Pantoja Ferreira
118 Instituto de Ciências Biológicas - UFPA

119

120 **Banca examinadora**

121

122

123 Profª. Drª. Laura Satiko Okada Nakaghi
124 UNESP – Jaboticabal

125
126
127

128

129 Profª. Drª. Zélia Maria Pimentel Nunes
130 UFPA – Bragança

131
132
133

134

135 Profª. Drª. Diva Anelie Guimarães
136 ICB - UFPA

137
138
139
140
141
142
143
144
145
146
147
148
149
150
151
152
153
154
155
156
157
158
159
160
161
162

Dedico aos meus pais, que me incentivaram desde o início de minha vida, minha querida irmã Luciane Melo. A Michelle Failache pela compreensão e as professoras Rossineide e Auxiliadora pela orientação.

163

164

165

166

167

168

169

170

171

172

173

174

175

176

177

178

179

180

181

182

183

184

185

186

187

188

189

“Sem a religião, a ciência é capenga; sem a ciência, a religião é cega.”

Albert Einstein

190

AGRADECIMENTOS

191 Primeiramente a Deus por ter me ungido com o seu Espírito Santo no
192 sacramento do Batismo, e por estar presente sempre em minha vida.

193 Aos meus pais Fátima e Ribamar (Zeca), por me mostrarem os verdadeiros
194 caminhos a serem seguidos, por sempre terem um ombro amigo nas horas
195 difíceis, por seu amor incondicional, por terem sempre investido e acreditado
196 em mim, Mãe e Pai amo vocês sempre.

197 A minha namorada Michelle Failache por me incentivar, acreditar, ter tido
198 paciência e sempre me mostrar que posso e que tudo se resolverá, além de
199 sempre me ouvir na hora em que eu precisava falar. E por, mesmo sem
200 conhecer muita coisa do estudo encontrava erros e pelas confirmações das
201 referências. Amor amo muito você. Muito obrigado!!!

202 A minha irmã Luciane Melo por ter sempre uma palavra de apoio, por me
203 mostra sempre o lado bom das coisas, sempre me ouvir e por me fazer
204 enxergar quando fecho meus olhos. Meu cunhado Abraão que já me teve como
205 um filho e a você meu sobrinho amado Thiago Fernandes (Peruca) pelas
206 ajudas com o computador e programas.

207 Minha tia Ray Fernandes que além de sempre me aconselhar, torcer, incentivar
208 e ouvir esteve presentes nos melhores momentos de minha vida, desde o
209 nascimento, festa do vestibular, formatura.

210 As minhas professoras e orientadoras Rossineide e Auxiliadora que desde o
211 trabalho de conclusão de curso abriram as portas do laboratório de Técnicas
212 Histológicas e hoje no Laboratório de ultraestrutura Celular, Imunohistoquímica
213 e Biologia do Desenvolvimento, que com muita paciência e dedicação
214 mostraram-me os passos de uma vida acadêmica.

215 A senhora Lia Sogab pela ajuda com os cortes histológicos perfeitos, aos meus
216 amigos do laboratório Caroline, Fabricia, Maria, Cirlene, Liziane, Yanne,
217 Melina, Ivana pela ajuda nas coletas e processamentos de rotina. Valeu
218 mesmo, amigos.

219 A Dani e Vam pela ajuda com os cálculos estatísticos e a Betânia Sena pelos
220 conselhos e ajudas com normas e referências. Muito obrigado.

221 A todos meus amigos de mestrado especialmente Paulo e Sarita pela Judá
222 com trabalhos acadêmicos e pela compreensão da minha rotina, que muitas
223 vezes era muito estafante. E Paulo muito obrigado pela força quando precisei.

224 A professora Zélia e equipe de coleta em Bragança pela imensa ajuda, a
225 Edissira que sempre trazia “meus exemplares” com cuidado e carinho.

226 A CAPES pela concessão de bolsa.

227 A professora Simone Damasceno com Apoio estatístico e risadas em
228 momentos importantes.

229 Aos professores que compõe a banca examinadora

230 À Pós-Graduação em Ecologia Aquática e Pesca – UFPA

231 Aos professores, que contribuíram para o meu crescimento na vida acadêmica

232 E a todos que de alguma forma me ajudaram nessa conquista.

233

234 Muito obrigado!!!

235

236

237

238

239

1. Introdução.....	11
2. Objetivos.....	14
2.1 Objetivo Geral	14
2.2 Objetivos Específicos	14
3. Referências.....	15
4. Artigo	
4.1 Effects of salinity and rainfall on the reproductive biology of the mangrove oyster (<i>Crassostrea gasar</i>): implications for farming practices.	19
4.1.2 Resumo.....	19
4.1.2 Abstract.....	20
4.1.2 Introdução.....	21
4.1.3 Material e Métodos.....	22
4.1.4 Resultados.....	25
4.1.5 Discussão.....	31
4.1.7 Referências.....	34

241
 242
 243
 244
 245
 246
 247
 248
 249
 250
 251
 252
 253
 254
 255
 256
 257
 258
 259
 260
 261
 262
 263
 264
 265
 266
 267
 268
 269

270 **Índice de figuras**

271 **Figura 1** – Espécime de *Crassostrea gasar*, coletado no município de Augusto Corrêa -
272 PA. A- valvas fechadas; B- Valvas abertas e região da gônada.
27312.

274 **Figura 2:** The study area in Augusto Corrêa in the Brazilian state of Pará (modified
275 from NOAA, National Oceanic Atmospheric Administration, 2010).
27623.

277
278 **Figura 3:** Seasonal variation in (a) salinity and rainfall and (b) temperature (°C) and pH
279 in Augusto Corrêa PA during the study period. A – rainy-dry transition period; B – dry
280 season; C - dry-rainy transition period and D - rainy season.
281 25.

282
283 **Figura 4:** Gonadal stages of female and male *C. gasar*: A – Immature female; B-
284 Immature male; C – Maturing female; D – Maturing male; E – Mature female; F – Mature
285 male; G – Spawned female; H – Spawned male. M – mantle; F – Follicles; T –
286 spermatic tubules; L – lumen; O – oocytes; Spt – sperm.
287 27.

288
289 **Figura 5:** Hermaphroditic gonad of *C. gasar*. A – Spermatic tubules containing mature
290 oocytes (O) and B – ovarian follicles with spermatozoids in the lumen and oocytes in
291 vitellogenesis..... 28.

292
293 **Figura 6:** Seasonal variation in salinity during the 2009 and 2010 reproductive cycles
294 of *C. gasar* females. A – dry-rainy transition period; B – dry season; C - rainy-dry
295 transition period and D - rainy season.
296 29.

297
298 **Figura 7:** Seasonal variation in rainfall during the 2009 and 2010 reproductive cycles of
299 *C. gasar* females. A – dry-rainy transition period; B – dry season; C – rainy-dry
300 transition period and D - rainy season.
301 29.

302
303 **Figura 8:** Seasonal variation in salinity during the 2009 and 2010 reproductive cycles
304 of *C. gasar* males. A – dry-rainy transition period; B – dry season; C – rainy-dry
305 transition period and D - rainy season.
306 30.

307
308 **Figura 9.** Seasonal variation in rainfall during the 2009 and 2010 reproductive cycles of
309 *C. gasar* males. A – dry-rainy transition period; B – dry season; C – rainy-dry transition
310 period and D - rainy season.
311 30.

312

313

314

315

316 **Índice de tabelas**

317 Tabela 1: Relative frequency of gametogenic stages in male and female *C. gasar* during
318 the different seasonal periods.
319 31.
320

321

322

323

324

325

326

327

328

329

330

331

332

333

334

335

336

337

338

339

340

341

342

343 **Introdução geral**

344 O litoral brasileiro apresenta diferentes tipos de ecossistema, dentre eles os
345 manguezais que são encontrados desde o Estado do Amapá até o de Santa Catarina
346 (Souza-Filho, 2005). Este ecossistema possui espécies típicas e adaptadas ao substrato
347 que sofre diariamente variações de maré o que acarreta oscilações dos fatores
348 ambientais, como por exemplo a salinidade (Tomlinson, 1986); sendo um ambiente
349 favorável para o desenvolvimento, crescimento e sobrevivência dos estágios iniciais do
350 ciclo de vida de animais marinhos (Schuler *et al.* 2000). Peixes, moluscos (ostras e
351 mexilhões) e crustáceos (caranguejos e camarões), são os componentes faunísticos dos
352 manguezais que mais se destacam, pois são constituídos por expressiva biomassa,
353 proporcionando excelentes fontes de proteína animal de alto valor nutricional (Kjerfve
354 *et al.* 1997).

355 O cultivo de ostras constitui um importante recurso econômico por apresentar
356 um excelente potencial de comercialização (Manzoni, 2001). É uma atividade que pode
357 gerar recursos e oportunidades de negócios para empresas, comunidades e associações,
358 criando empregos diretos e indiretos. Essa atividade apresenta as vantagens: do ponto de
359 vista social promovendo a inclusão social, devido ao aumento da renda familiar,
360 proporcionando igualdade dos gêneros e fortalecendo a dieta alimentar da família; do
361 ponto de vista econômico, promovendo a abertura de novos mercados gerando
362 empregos e rendas; e do ponto de vista ambiental, reduzindo a pressão extrativista sobre
363 os estoques de populações naturais (Gomes *et al.* 2008).

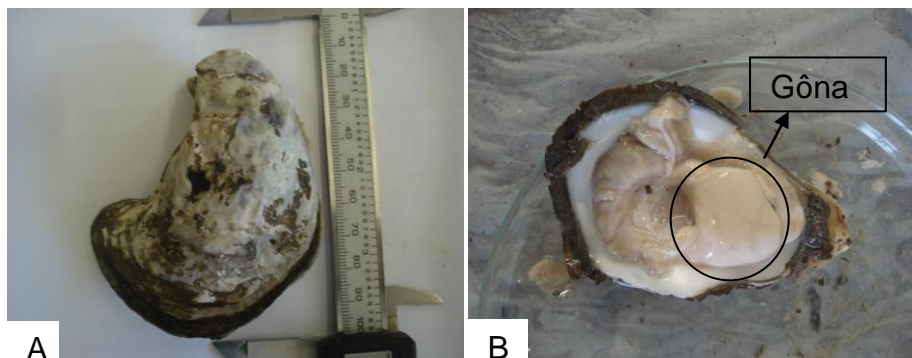
364 O cultivo de ostras no Brasil é recente, iniciou nas décadas de 70 e 80, nos
365 estados da Bahia, Rio de Janeiro, São Paulo e somente no início dos anos 90 se
366 consolidou no Estado de Santa Catarina (Rupp *et al.* 2008). Dentre os dezessete Estados
367 brasileiros localizados na região litorânea, apenas cinco constam como produtores de

368 moluscos com cultivo em nível comercial. No Pará os experimentos pioneiros com
369 cultivo de ostras com finalidades comerciais iniciaram nos anos de 2001 a 2003, na
370 comunidade de Nova Olinda, município de Augusto Corrêa (Sampaio, 2007).

371 As populações de ostras estão adaptadas as condições específicas de seu habitat,
372 pois cada estuário tem suas particularidades, os fatores ambientais alteram o
373 metabolismo do animal, influenciando no crescimento, desenvolvimento e reprodução
374 das espécies (Wakamatsu 1973; Vilanova & Fonteles-Filho 1989).

375 As ostras de maior importância econômica pertencem ao gênero *Crassostrea*, o
376 qual é dióico, não apresenta dimorfismo sexual, a sua reprodução é sexuada com
377 fecundação externa. As gônadas têm um aspecto esbranquiçado e difuso e quando estão
378 sexualmente maduras envolvem totalmente a glândula digestiva, sendo necessário o uso
379 das técnicas histológicas para diferenciação entre machos e fêmeas (Christo & Absher,
380 2008).

381
382
383
384



385
386
387
388
389

Figura 1 – Espécime de *Crassostrea gasar*, coletado no município de Augusto Corrêa - PA. A- valvas fechadas; B- Valvas abertas e região da gônada.

390 Na região amazônica dentre as espécies utilizadas no cultivo destaca-se
391 *Crassostrea gasar* (Adanson, 1757) encontrada, principalmente, fixada as raízes aéreas
392 do mangue vermelho (*Rhizophora mangle*) ou sobre zonas intertidais e costões rochosos
393 (Nascimento, 1983). Podem ocorrer tanto na zona infralitoral, onde permanecem
394 continuamente submersas, quanto na zona entre-marés, onde ficam alternadamente

395 emersas pelas variações semi-diurnas da maré (Fernandes, 1979; Pereira *et al.* 1988;
396 Rios, 1994).

397 Em qualquer organismo de interesse comercial o conhecimento da estrutura
398 gonadal é fundamental permitem melhorar a qualidade produtiva desses animais. Em se
399 tratando do gênero *Crassostrea* estudos sobre a estrutura gonadal estão limitados a
400 algumas espécies como: *C. gigas* (Komaru *et al.* 1994; Dong *et al.* 2005; Franco *et al.*
401 2008; Yurchenko *et al.* 2009) e *C. rhizophorae* (Matos *et al.* 1999). Com relação a
402 espécie *C. brasiliiana* não há estudos sobre estrutura gonadal e dinâmica celular da
403 linhagem germinativa. Afim de gerar informações a respeito da biologia reprodutiva do
404 animal é necessário a definição dos estádios gonadais, do período reprodutivo e da
405 relação entre a produção de gametas e os fatores ambientais para a espécie.

406 Nesse contexto, o presente trabalho teve como objetivo estudar a biologia
407 reprodutiva e analisar a morfologia do gametogênese de *Crassostrea gasar* (Adanson,
408 1757) no estuário amazônico e influência dos fatores abióticos sobre a reprodução da
409 espécie no município de Augusto Corrêa no Estado do Pará, onde foi implantado a
410 atividade de cultivo em condições ainda inadequadas para o estabelecimento da
411 produção em escala comercial.

412

413

414

415

416

417

418

419

420 **OBJETIVO GERAL**

421 - Estudar a biologia reprodutiva e analisar a morfologia da gametogênese de
422 *Crassostrea gasar* (Adanson, 1757) no estuário amazônico.

423

424 **OBJETIVOS ESPECÍFICOS**

425 - Descrever os aspectos morfológicos e estruturais das gônadas de *Crassostrea gasar*.

426 - Caracterizar os estádios gonadais.

427 - Determinar a época da reprodução da espécie.

428 - Verificar a influência das variáveis físicas e químicas da água na reprodução da
429 espécie.

430 - Caracterizar ultraestruturalmente a espermatogênese de *Crassostrea gasar*.

431 - Descrever a participação das proteínas do citoesqueleto na maturação gonadal de
432 machos.

433

434

435

436

437

438

439

440

441

442

443

444

445 **Referências bibliográficas**

446 CHRISTO, S. W.; ABSHER, T. M. Crescimento da prodissoconch de ostras do gênero
447 *Crassostrea* Sacco, 1897 (Bivalvia, Ostreidae). **Boletim do Instituto de Pesca**, v. 34, p.
448 71-77, 2008.

449

450 DONG, Q.; EUDELIN, B.; HUANGA, C.; STANDISH, K.; ALLEN, Jr.;
451 TERRENCE, R. T. Commercial-scale sperm cryopreservation of diploid and tetraploid
452 Pacific oysters, *Crassostrea gigas*. **Cryobiology**, v. 50, p. 1–16, 2005.

453

454 FRANCO, A.; BERTHELIN, C. H.; GOUX, D.; SOURDAINE, P.; MATHIEU, M. Fine
455 structure of the early stages of spermatogenesis in the Pacific oyster, *Crassostrea*
456 *gigas* (Mollusca, Bivalvia). **Tissue and Cell**, v. 40, p. 251-260, 2008.

457

458 FERNANDES, L. M. B. Ecologia e cultivo da ostra do nordeste brasileiro. 1979. 70 pp.
459 Dissertação de Mestrado. Universidade Federal Rural de Recife, Pernambuco, 1979.

460

461 KJERFVE, B.; DE LACERDA L. D.; DIOP, E. H. S. Mangroves of Brazil. **Mangrove**
462 **Ecosystems Studies in Latin America and African**, United Nations Educational,
463 Scientific and Cultural Organization, Paris-France, 349 p., 1997.

464

465 KOMARU, A.; KONISHI, K.; WADA K. T. Ultrastructure of spermatozoa from induced
466 triploid pacific oyster, *Crassostrea gigas*. **Aquaculture**, v. 123, p. 217-222, 1994.

467

468 GOMES, R. S.; ARAÚJO, R. C.; NETO-DANTAS, M. P. Contribuição da ostreicultura
469 para a formação da renda familiar: estudo de caso do projeto de ostreicultura
470 comunitária da Fundação Alphaville, Eusébio, Ceará. *Ciências do mar*, v. 42, p. 72-84,
471 2008.

472 MATOS, E.; CORRAL, L.; MATOS, P.; AZEVEDO, C. Spermatogenesis and sperm
473 structure os *Crassostrea rhyzophorea* (Mollusca, Bilvania) from estuarine region of the
474 amazon river. **Brazil Journal of Morphology Science**, v. 16, n. 1, 1999.

475

476 MANZONI, G. C. **Ostras**: Aspectos bioecológicos e técnicas de cultivo. 1ª. ed. Itajaí:
477 Univali, 2001. 30 p.

478 NASCIMENTO, I. A. Cultivo de ostras no Brasil: problemas e perspectivas. **Ciência e**
479 **Cultura**, v. 35, p. 871-876, 1983.

480

481 PEREIRA, O. M.; AKABOSHI, S.; CHAGAS-SOARES, F. Cultivo experimental de
482 *Crassostrea brasiliana* (Lamarck, 1819) no Canal da Bertioiga, São Paulo. **Boletim do**
483 **Instituto de Pesca**, v. 1, p. 55-65, 1988.

484

485 DONG, Q.; EUDELIN, B.; HUANGA, C.; STANDISH, K.; ALLEN, Jr.;
486 TERRENCE, R. T. Commercial-scale sperm cryopreservation of diploid and tetraploid
487 Pacific oysters, *Crassostrea gigas*. **Cryobiology**, v. 50, p. 1–16, 2005.

488

489 RIOS, E.C. **Seashells of Brazil**. Rio Grande: Fundação Universidade do Rio Grande.
490 1994. 330p.

491

492 RUPP, G.S.; DE OLIVEIRA NETO, F. M.; GUZENSKI, J. Estado actual del cultivo de
493 moluscos bivalvos en la región sudeste-sur de Brasil. En A. Lovatelli, A. Farías e I.
494 Uriarte (eds). Estado actual del cultivo y manejo de moluscos bivalvos y su proyección
495 futura: factores que afectan su sustentabilidad en América Latina. **Actas de Pesca y**
496 **Acuicultura**, v. 12, p. 77–89, 2008.

497

498 SAMPAIO, S. S. **Energia que vem da ostra: do extrativismo para o cultivo**. Histórias
499 de sucesso, Agronegócios, Aqüicultura e Pesca. SEBRAE, 2007.

500

501 SCHULER, C. A. B.; ANDRADE, V. C.; SANTOS, D. S. O manguezal: composição e
502 estrutura. In: BARROS, H. M.; ESQUINAZI-LEÇA, S. J.; MACEDO, T. L. (edis).
503 Gerenciamento participativo de estuários e manguezais. Recife, Cap. 3, p. 89-102, 2000.

504

505 SOUZA-FILHO, P. W. M. Costa de manguezais de macromaré da Amazônia: cenários
506 morfológicos, mapeamento e quantificação de áreas usando dados de sensores remotos.
507 **Revista Brasileira de Geofísica**, v. 23, p. 427-435, 2005.

508

509 TOMLINSON, P. B. **The botany of mangroves**. London: Cambridge University Press,
510 New York, 413p., 1986.

511 VILANOVA, M. F. V.; FONTELES-FILHO, A. A. Análise da biometria e do fator de
512 condição da ostra-do-mangue, *Crassostrea rhizophorae* (Guilding, 1828) (Mollusca,
513 Bivalvia) no estuário do rio Ceará, Ceará, Brasil. **Ciência e Cultura**, v. 41, p. 1117-
514 1124, 1989.

515

516 YURCHENKO, O. V.; RADASHEVSKY, V. I.; HWEY-LIAN, H.; REUNOV, A. A.
517 Ultrastructural comparison of the spermatozoa of the Pacific oyster *Crassostrea gigas*
518 inhabiting polluted and relatively clean areas in Taiwan. **Aquatic Ecology**, v. 43, n. 2,
519 p. 513-519, 2009.

520

521 WAKAMATSU, T. **A ostra de Cananéia e seu cultivo**. In: Superintendência do
522 Desenvolvimento do Litoral Paulista e Instituto Oceanográfico – USP, 141 p., 1973.

523

524

525

526

527

528

529

530

531

532

533

534

535

536

537

538

539

CAPÍTULO I

540

541

542

543

544

545

546

547

548

549

550

551

552

553

554

555

556

557

558

559

560

561

562

563

564

565 A publicação está sob as normas da Revista: Aquaculture Research.

566 **Effects of salinity and rainfall on the reproductive biology of the mangrove oyster**
567 **(*Crassostrea gasar*): implications for farming practices.**

568

569 Leonardo Paixão^{ab}, Maria Auxiliadora Ferreira^b, Zélia Nunes^c, Fabrícia Fonseca-Sizo^{ab},
570 Rossineide Rocha^{a*}

571

572 ^aLaboratory of Cellular Ultrastructure, Institute of Biological Sciences, Federal
573 University of Pará, Brazil

574 ^bLaboratory of Developmental Biology and Immunohistochemistry, Institute of
575 Biological Sciences, Federal University of Pará, Brazil

576 ^cInstitute for Coastal Studies, Federal University of Pará, Bragança, Pará, Brazil

577

578 *Corresponding author:

579 Av. Augusto Corrêa n° 01, CEP 66075-900. Tel: 055 91 3201 7876, Brazil

580 E-mail: rmrocha@ufpa.br

581

582 **Resumo**

583 O presente estudo analisou o ciclo reprodutivo e estabeleceu a relação dos
584 fatores abióticos com os estádios de desenvolvimento gonadal de *Crassostrea gasar*
585 criadas no estuário amazônico. Foram coletados mensalmente cerca de 20 ostras no
586 período de agosto de 2009 a dezembro de 2010. Os meses de coleta foram agrupados
587 em quatro períodos sazonais (seco, transicional seco/chuvoso, chuvoso e transicional
588 chuvoso/seco). No local da coleta foram mensurados *in situ* salinidade, pH, temperatura
589 e oxigênio dissolvido. A gônada foi dissecada e submetida ao procedimento histológico.
590 Um total de 351 animais foram coletados, sendo 190 fêmeas, 161 machos e 2

591 hermafroditas. Histologicamente machos e fêmeas foram classificados em quatro
592 estádios gonadais: I- imaturo, II - em maturação, III - maturo e IV - desovado (fêmeas) e
593 espermiado (machos). Dentre os fatores abióticos analisados apenas a salinidade e a
594 precipitação pluviométrica apresentaram diferenças estatisticamente significantes
595 durante o estudo. Houve correlação entre esses dois fatores e a maturação gonadal,
596 sugerindo que esses fatores estejam influenciando na reprodução, visto que foram
597 encontrados predominância de indivíduos maturos (III) no período chuvoso e
598 transicional chuvoso/seco (baixa salinidade e alta precipitação pluviométrica). Nos
599 períodos seco e transicional seco/chuvoso (alta salinidade e baixa precipitação) foram
600 encontrados indivíduos nos estágios imaturo (I), em maturação (II) e
601 desovado/espermiado (IV). Por conseguinte, para o cultivo é indicado que a coleta de
602 sementes seja feita nos períodos seco e transicional seco/chuvoso.

603 **Palavras-chaves:** reprodução, histologia, desenvolvimento gonadal, fatores abióticos e
604 ostras

605

606 **Abstract**

607 The present study analyzed gonadal development in *Crassostrea gasar* from an
608 Amazonian estuary and characterized the relationship between the reproductive cycle
609 and the variation in abiotic factors. Samples of approximately 20 oysters were collected
610 each month between August, 2009, and December, 2010. The study period was divided
611 into four seasonal periods (dry, dry-rainy transition, rainy, and rainy-dry transition). The
612 salinity, pH, temperature, and dissolved oxygen concentration of the water were
613 measured *in situ* during the collection of specimens. The gonads were dissected and
614 analyzed histologically. A total of 353 specimens were collected, of which 190 were
615 female, 161 were male, and two were hermaphrodite. Histologically, the specimens

616 were classified in four gonadal stages: I – immature; II – maturing; III – mature, and IV
617 – spawned (females) and spermiated (males). Of the abiotic factors analyzed, only
618 salinity and rainfall varied significantly over the study period, with correlations being
619 found between these factors and gonadal maturation, suggesting that they influence the
620 reproductive cycle of the species. A predominance of mature (stage III) and maturing
621 individuals were recorded during the rainy and rainy-dry periods (low salinity and high
622 rainfall), whereas immature (I) and spawned/spermiated (IV) specimens were more
623 common during the dry and dry-rainy periods (high salinity and low rainfall). Given
624 this, the collection of seeds for farming would be recommended during the latter period,
625 when spawned/spermiated oysters were more common.

626

627 **Keywords:** Histology, gonadal development, abiotic factors, oysters, Amazonian
628 estuary

629

630 **1. Introduction**

631 Mangroves are among the most important ecosystems of tropical coastal zones
632 in both biological and economic terms (Cintrón-Molero and Schaeffer-Novelli, 1999).
633 They are considered to be essential nursery areas for many groups of animals, in
634 particular fishes, crustaceans (crabs and shrimps) and mollusks (oysters and mussels).
635 These animals are able to tolerate considerable variation in tidal conditions and salinity
636 (Pereira et al., 2003).

637 The oysters of the genus *Crassostrea* are dioic, and present sexual reproduction
638 with external fertilization. The gonads have a diffuse whitish aspect, but no sexual
639 dimorphism, requiring histological analysis in order to differentiate males from females
640 (Christo and Absher, 2008). In addition to the two native species of *Crassostrea* oyster

641 – *Crassostrea rhizophorae* (Guilding, 1828) and *Crassostrea gasar* (Adanson, 1757) –
642 an exotic species, *Crassostrea gigas* (Thunberg, 1795), can be found on the Brazilian
643 coast. The exotic *C. gigas* is farmed commercially in the south of the country (Varela et
644 al., 2007). Molecular studies (partial sequencing of the COI gene) have revealed that the
645 genus *Crassostrea* is present in the Amazon region, where it is represented by
646 *Crassostrea gasar*, *Crassostrea rhizophorae*, and *Crassostrea* sp. (Melo et al., 2010). *C.*
647 *gasar* is prevalent in the northeast of the Brazilian state of Pará, where it is now being
648 farmed on a small scale.

649 As exogenous factors may affect the duration of gonadal stages, and thus the
650 production of gametes, a good working knowledge of the relationship between
651 environmental variables and gonadal development is an important prerequisite for the
652 development of programs for the commercial exploitation of shellfish (Gosling, 2003).
653 Given this, the present study analyzed the reproductive cycle of *C. gasar* to determine
654 whether and to what extent variation in the physical and chemical of the water influence
655 the reproduction of the species in an estuary in Pará. These results provide an important
656 database for the development of effective farming and management techniques in the
657 region.

658

659 **2. Material and methods**

660

661 **2.1. Study area and specimen collection**

662 Oysters, *C. gasar*, were farmed in rectangular oyster bags (1 m x 0.5 m) on the
663 Emboraí River in the town of Nova Olinda (0°52'54" S, 46°26'54" W) located in the
664 municipality of Augusto Corrêa in the Brazilian state of Pará (Fig. 2). An average of 20
665 oyster specimens were collected each month between September, 2009, and December,

666 2010. The study period was divided into four periods – the dry season (September-
667 December, 2009, and August-December, 2010), dry-rainy transition period (January-
668 March, 2010), rainy season (April-June, 2010), and the rainy-dry transition period
669 (August, 2009, and July, 2010).

670

671

672

673

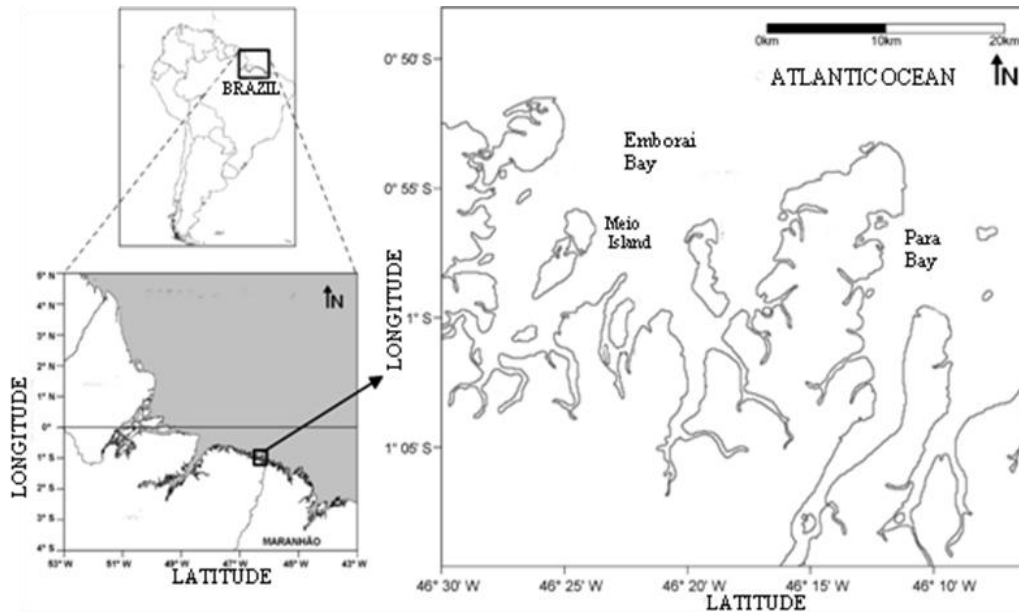
674

675

676

677

678



679 **Fig. 2:** The study area in Augusto Corrêa in the Brazilian state of Pará (modified from
680 NOAA, National Oceanic Atmospheric Administration, 2010).

681

682 2.2. Environmental variables

683 During specimen collection, the salinity, pH, temperature (°C), and dissolved
684 oxygen concentration (mg/L) of the water were measured *in situ* using a multi-
685 parameter probe (Horiba U10). These measurements were taken during ebb spring tides.
686 Rainfall data were obtained from the National Institute of Meteorology's automatic
687 meteorological station at Bragança (INMET, 2010).

688 2.3. Processing of the samples

689 Following collection, the oysters were packed in isothermal boxes for
690 transportation to the laboratory, where they were opened for the removal of the gonads
691 and processing for electronic and light microscopy.

692

693 **2.4. Light microscopy**

694 The gonad samples were fixed in Bouin's solution for 24 hours and then
695 embedded in paraffin using standard histological techniques. Sections of 5 μm were
696 obtained and stained with hematoxylin and eosin (HE) for analysis. Photomicrographs
697 were taken with a Carl Zeiss optical microscope (Axiostar Plus 1169-151). Following
698 this analysis, four gonadal stages were established, adapted from the classification of
699 Rodríguez-Jaramillo et al. (2008).

700

701 **2.5. Transmission electron microscopy (TEM)**

702 Fragments of the gonads were fixed in Karnovsky's solution at pH 7.3 and 4°C
703 for 24 hours and post-fixed in 1% osmium tetroxide. The samples were contrasted with
704 uranyl acetate (1%), followed by dehydration in growing solutions of acetone and
705 included in Epon. The ultra-thin cuts were contrasted with uranyl acetate and lead
706 citrate and analyzed by transmission electron microscopy (LEO 906 E).

707

708 **2.6. Statistical analysis**

709 The physical and chemical variables were analyzed using ANOVA and the
710 Kruskal-Wallis (K-W) nonparametric analysis of variance, depending on the normality
711 of the data. A univariate analysis was then employed to test the possible relationship
712 between environmental factors and the stages of gonadal development in male and
713 female *C. gasar*. The SIMPER analysis was used to verify the contribution of each
714 gonadal stage within each seasonal period. The Chi-square test with Yates' correction
715 (Snedecor & Cochran, 1980) was used to determine the significance ($p < 0.05$) of

716 deviations in the sex ratio. The analyses were run in the Statistica V 6.0 and Primer 6.0
 717 programs.

718

719 **3. Results**

720 Monthly rainfall ranged from 0 to 360 mm, with significantly higher values
 721 being recorded in the rainy season (K-W, $p < 0.05$). Salinity varied from 19 to 39 and
 722 was significantly higher during the dry season (ANOVA, $p < 0.05$). Temperature, pH,
 723 and dissolved oxygen concentrations all fluctuated slightly during the course of the
 724 study period, but did not vary significantly between seasons. Water temperature ranged
 725 between 28.4 and 30.1°C, pH from 7.3 to 8.35, and dissolved oxygen concentrations
 726 from 3.62 to 5.95 mg/L (Fig. 3).

727

728

729

730

731

732

733

734

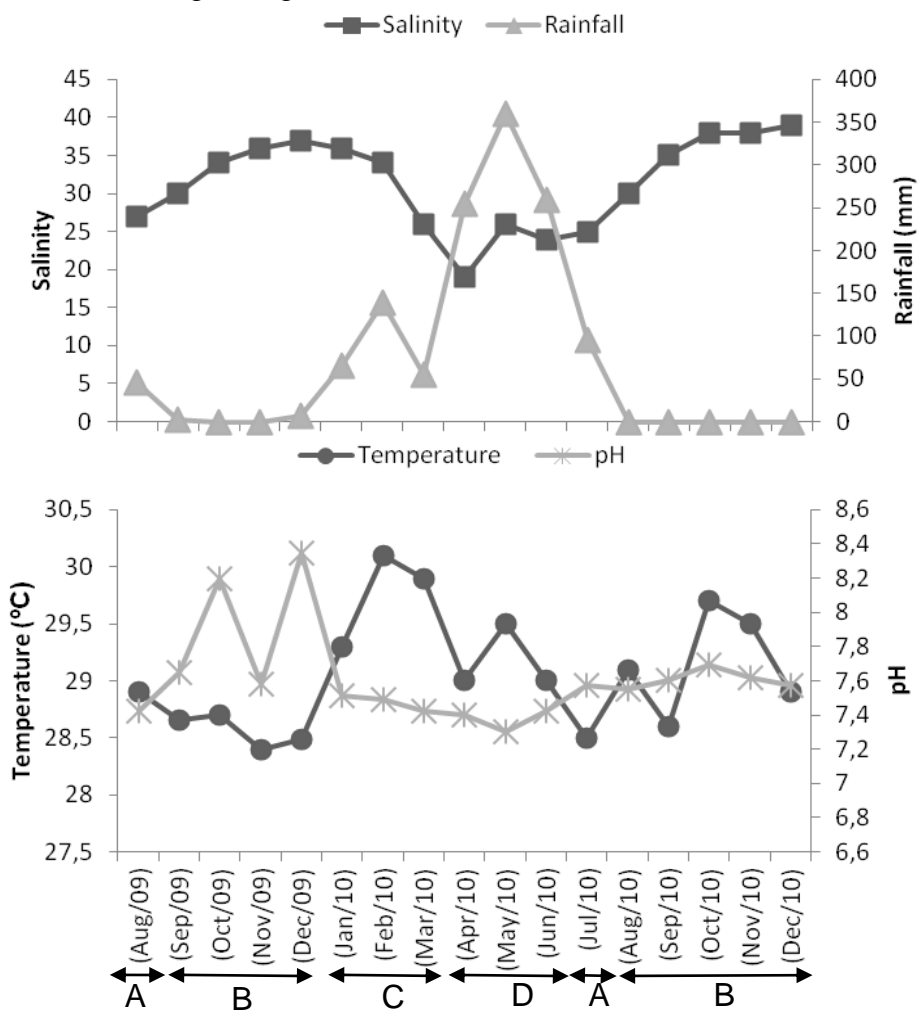
735

736

737

738

739



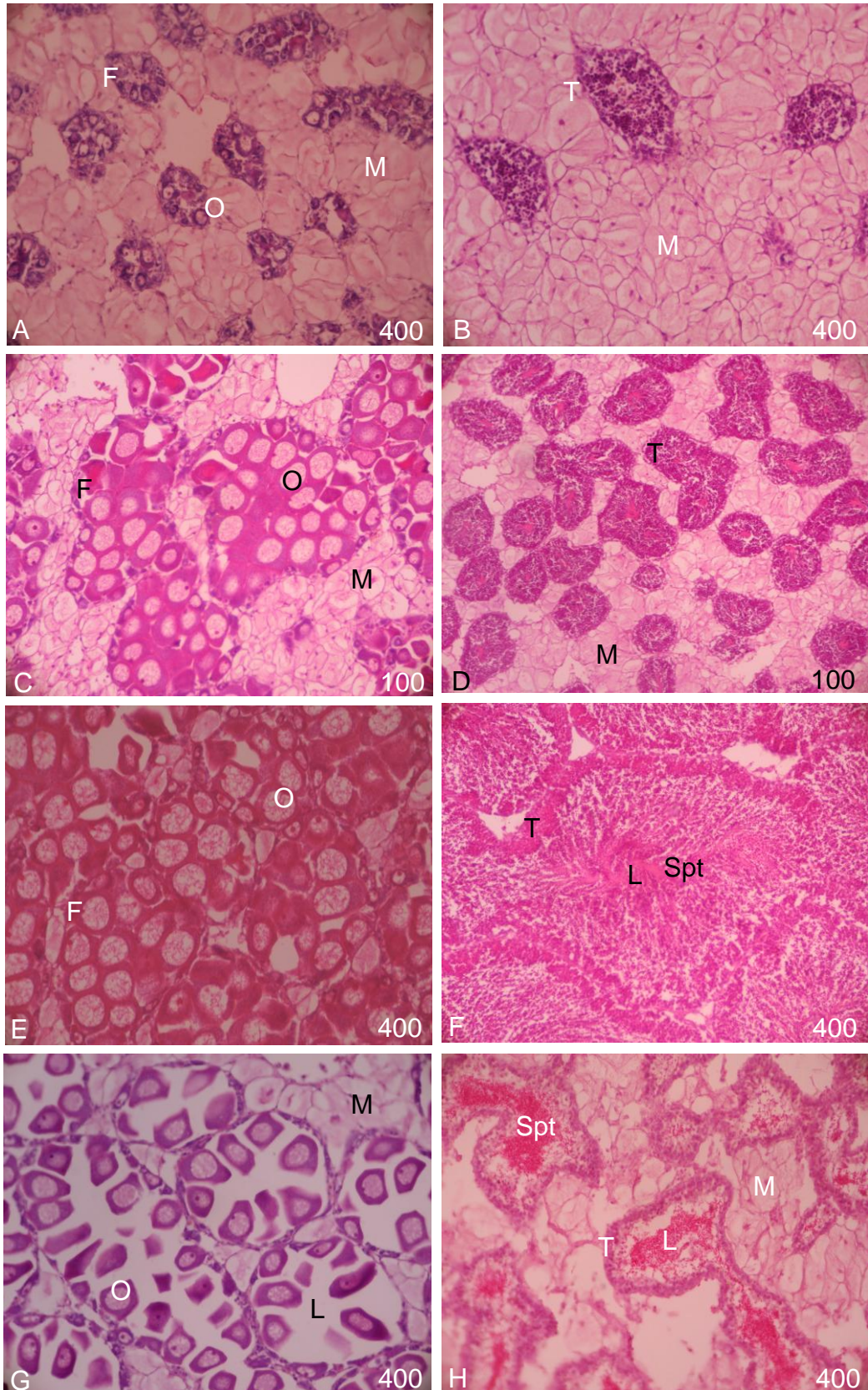
740 **Fig. 3:** Seasonal variation in (a) salinity and rainfall and (b) temperature (°C) and pH in
741 Augusto Corrêa PA during the study period. A – rainy-dry transition period; B – dry
742 season; C - dry-rainy transition period and D - rainy season.
743

744 A total of 353 individuals were analyzed. No sexual dimorphism was observed
745 macroscopically. Histological examination of the specimens identified 190 (55%)
746 females, 161 (45%) males, and two (0.5%) hermaphrodites. While the sex ratio favored
747 the females – 1.1:1 – the difference was not significant (Chi-square: $p > 0.05$).

748 The males and females were classified in four gonadal stages. Females: 1)
749 Immature: follicles containing pre-vitellogenic oocytes (I) characterized by scarce
750 cytoplasm and well-defined nucleus; some oocytes in vitellogenesis, identified as
751 basophilic cells with prominent cytoplasm, and evident nucleoli and nuclei (Fig. 4A); 2)
752 Maturation stage: increase in follicle diameter and three cell types: pre-vitellogenic
753 oocytes (I), predominance of oocytes in vitellogenesis (II), and some vitellogenic
754 oocytes, characterized by very evident acidophile cytoplasm and nucleus (III) (Fig. 4C);
755 3) Mature stage: ovarian follicles filled by vitellogenic or mature oocytes (IV). At this
756 stage, oocytes in vitellogenesis were also found (II) (Fig. 4E); and 4) Spawned stage:
757 follicles with highly visible lumen containing pre-vitellogenic oocytes (I) and oocytes in
758 vitellogenesis (II) (Fig. 4G).

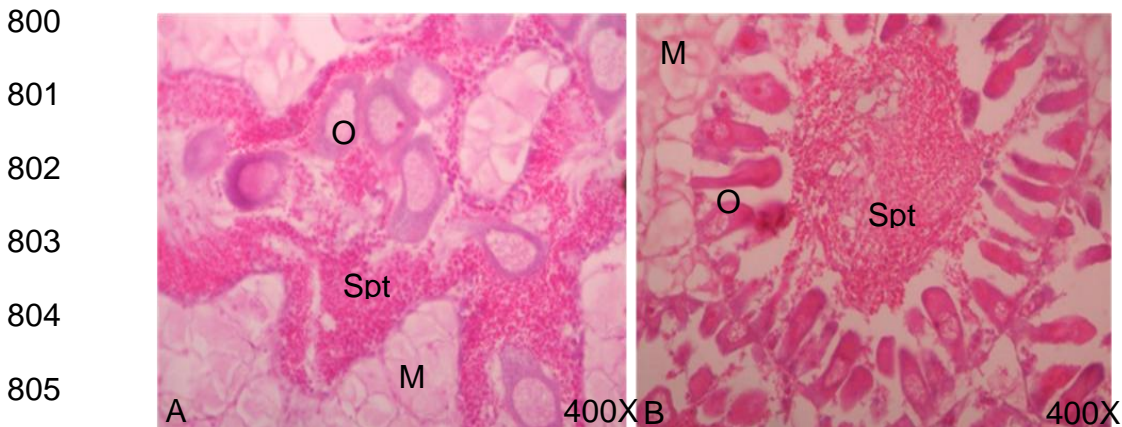
759 Males: 1) Immature stage: testicular parenchyma characterized by spermatocytic
760 tubules irregular in form and size. Spermatogonia adjacent to the tubule wall (Fig. 4B);
761 2) Maturation stage: increase in the diameter of the tubule, which contains
762 spermatogonia, spermatocytes, spermatids, and spermatozooids (Fig. 4D); 3) Mature
763 stage: spermatocytic tubules wider in diameter with a predominance of spermatozooids, but
764 some spermatogonia still anchored to the tubule wall (Fig. 4F); and 4) Spermiated stage:
765 characterized by a reduction of the tubule diameter and the presence of few
766 spermatozooids in the lumen (Fig. 4H).

767
768
769
770
771
772
773
774
775
776
777
778
779
780
781
782
783
784
785
786
787
788
789



790 **Fig. 4:** Gonadal stages of female and male *C. gasar*: A – Immature female; B- Imature
791 male; C – Maturing female; D – Maturing male; E – Mature female; F – Mature male; G
792 – Spawned female; H – Spawned male. M – mantle; F – Follicles; T – spermatic
793 tubules; L – lumen; O – oocytes; Spt – sperm.

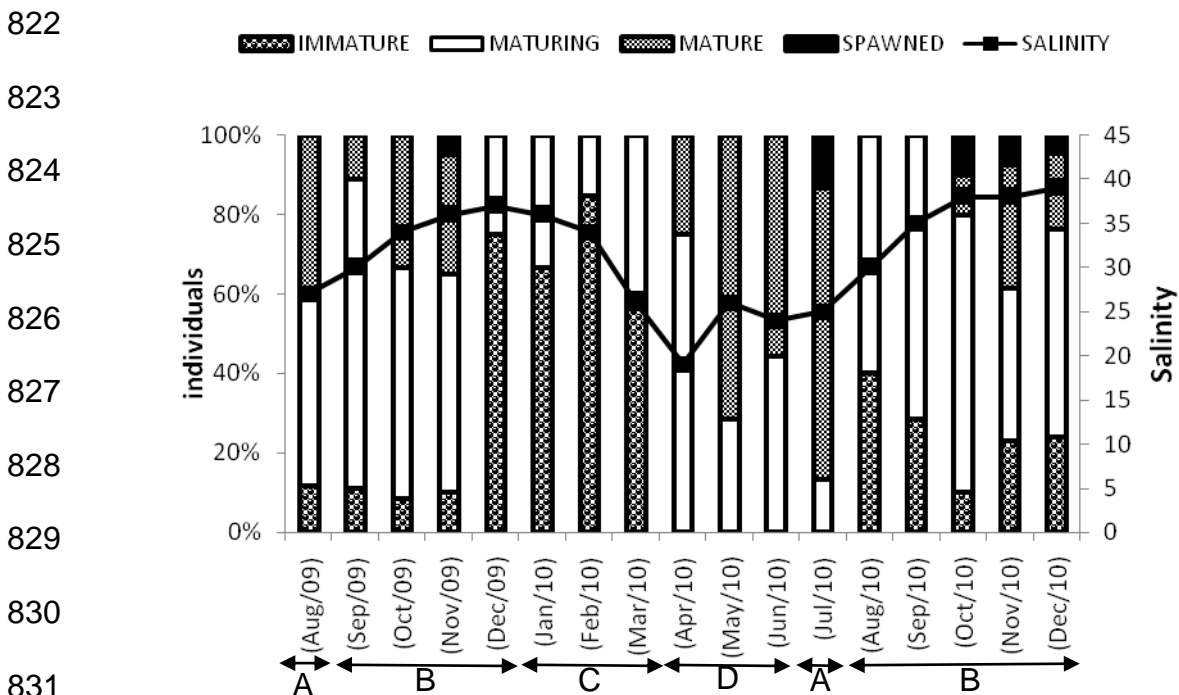
794 Two different hermaphrodite forms were observed: 1) spermatic tubules with
795 free mature oocytes in the lumen and spermatogonia fixed to the wall of the tubule; 2)
796 ovarian follicles with spermatozoa in the lumen and oocytes in vitellogenesis fixed to
797 the wall of the follicle. However, only two hermaphrodites were observed (0.5%), and
798 their presence in the sample did not affect the sex ratio or classification of the gonadal
799 stages (Fig. 5).



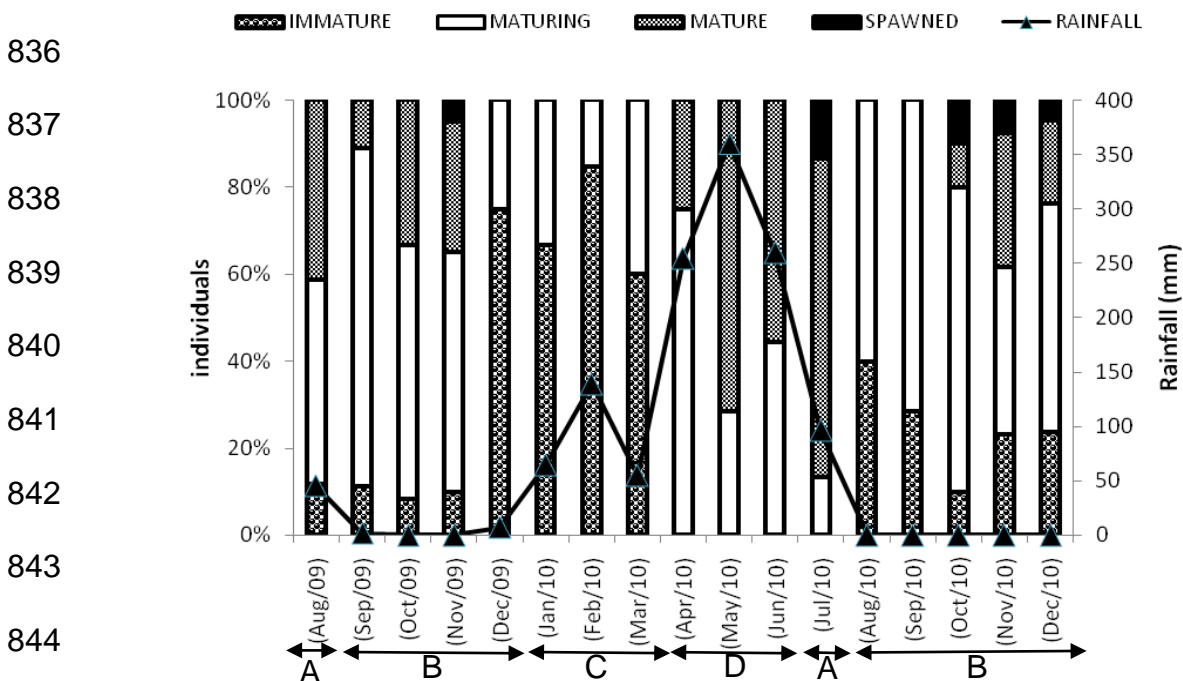
806 **Fig. 5:** Hermaphroditic gonad of *C. gasar*. A – Spermatic tubules containing mature
807 oocytes (O) and B – ovarian follicles with spermatozooids in the lumen and oocytes in
808 vitellogenesis.
809

810 Immature females were present only in the rainy-dry transition, dry season and
811 dry-rainy transition (January-March, 2010), whereas all other phases were observed
812 throughout the study period. Maturing females were present throughout the study
813 period, but were also more frequent during the dry season. Mature females were more
814 common during the rainy season and the rainy-dry transition (August, 2009, and July,
815 2010), while the highest frequency of the spawning phase was recorded in the dry
816 season (Fig. 6 and 7). The frequency of immature males was similar to that of immature
817 females, although they were relatively more abundant during the dry season. Maturing
818 males were observed throughout the study period except for the rainy-dry transition.
819 The highest frequencies of mature males were recorded in the rainy season and rainy-

820 dry transition. By contrast, spermiated males were relatively more common in the dry
 821 season and dry-rainy transition of 2009 (Fig. 8 and 9).



832 **Fig. 6:** Seasonal variation in salinity during the 2009 and 2010 reproductive cycles of *C.*
 833 *gasar* females A – dry-rainy transition period; B – dry season; C - rainy-dry transition
 834 period and D - rainy season.
 835



845 **Fig. 7:** Seasonal variation in rainfall during the 2009 and 2010 reproductive cycles of *C.*
 846 *gasar* females. A – dry-rainy transition period; B – dry season; C – rainy-dry transition
 847 period and D - rainy season.
 848

849

850

851

852

853

854

855

856

857

858

859

860

861

862

863

864

865

866

867

868

869

870

871

872

873

874

875

876

877

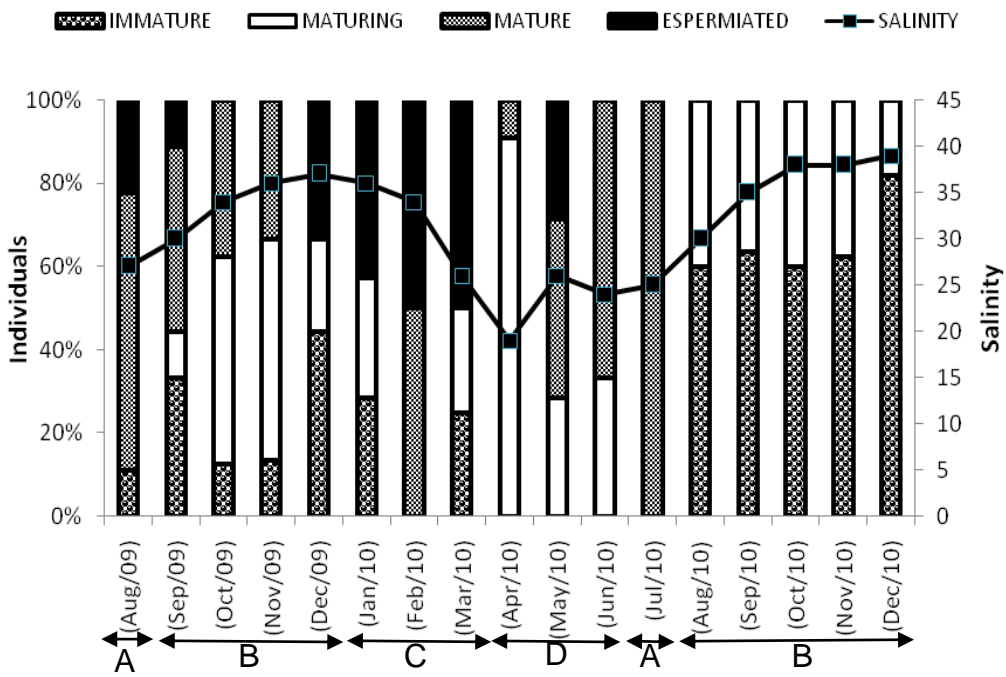


Fig. 8: Seasonal variation in salinity during the 2009 and 2010 reproductive cycles of *C. gasar* males. A – dry-rainy transition period; B – dry season; C – rainy-dry transition period and D - rainy season.

863

864

865

866

867

868

869

870

871

872

873

874

875

876

877

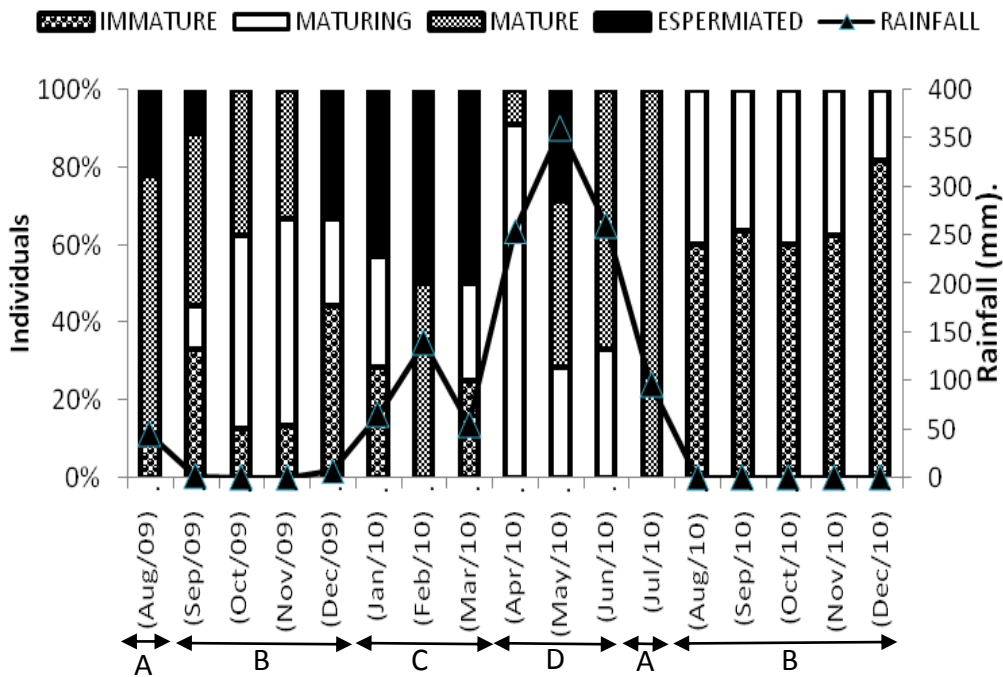


Fig. 9. Seasonal variation in rainfall during the 2009 and 2010 reproductive cycles of *C. gasar* males. A – dry-rainy transition period; B – dry season; C – rainy-dry transition period and D - rainy season.

878 As noted above, only salinity varied significantly during the study period,
 879 presenting an inverse relationship with rainfall. During periods of low rainfall and high
 880 salinity, all stages of the gametogenic cycle were observed in both male and female
 881 specimens, except mature stage. During periods of reduced salinity (high rainfall),
 882 however, the proportion of mature specimens of both sexes increased. While that of
 883 immature and spermiated/spawned individuals decreased.

884 The proportion of mature specimens peaked in the rainy-dry transition, with
 885 males reaching 100% of the sample and females, 73.37% (Table 1). In this case, the
 886 difference between males and females was significant.

887 Table 1: Relative frequency of gametogenic stages in male and female *C. gasar* during
 888 the different seasonal periods.
 889

Sex	Stage	Contribution (%) during the season:			
		Rainy-Dry	Dry	Dry-Rainy	Rainy
Female	Immature		22.68	71.76	-
	Maturing	26.63	65.47	28.24	49.53
	Mature	73.37	10.39	-	50.47
	Spawned	-	-	-	-
Male	Immature	-	52.86	14.07	-
	Maturing	-	47.14	14.07	60.35
	Mature	100.00	-	-	39.65
	Spermiated	-	-	71.85	-

890

891 4. Discussion

892 The histological analysis of the *C. gasar* specimens collected in the present
 893 study revealed a predominance of females in the population, although there was no
 894 significant deviation in the sex ratio. A similar situation was recorded by Lenz and
 895 Boehs (2011) in a Brazilian population of *C. rhizophorea*. Le Dantec (1968) has

896 suggested that the proportion of females tends to increase in areas that suffer oceanic
897 influences, which results in increased salinity. A similar scenario was observed in the
898 present study of *C. gasar*, even though the study area was an estuary, in which salinity
899 levels fluctuate considerably through evaporation, precipitation, and the inflow of
900 fluvial discharge.

901 Hermaphroditism – regarded as a transitional stage between the male and female
902 condition (Baghurst & Mitchell, 2002; Lango-Reynoso et al., 2006) – was observed
903 only rarely in the present study, and was characterized by the presence of spermatozoa
904 in the female gonads, and oocytes in the male gonads, confirming the transition from
905 male to female or female to male, respectively.

906 In general, gametogenesis in *C. gasar* follows the typical pattern of bivalve
907 mollusks, with the exception of the undifferentiated stage reported in *C. corteziensis*
908 (Rodriguez-Jaramillo et al., 2008), *C. gigas* (Enríque-Díaz et al., 2009), and *Ostrea*
909 *edullis* (Cano et al., 1997). In *C. gasar*, it was necessary to employ TEM in order to
910 characterize the gametic cells, and thus permit the identification of ultra-structural
911 differences between males and females.

912 The gonadal development of *C. gasar* was classified in four stages (immature,
913 maturing, mature, and spawned/spent). There is some divergence in the number of
914 gonadal stages identified by other authors – in *C. gigas*, for example, and Lango-
915 Reynoso et al. (2000) and Chávez-Villalba et al. (2007) describe four stages in females
916 (initial gametogenesis, gonad growth, mature, and degeneration), but only three in the
917 males (initial gametogenesis, gonad growth and mature), although these authors
918 described the same histological characteristics of gonadal development as those
919 observed in the present study.

920 The increase in the frequency of mature oysters during the rainy and rainy-dry
921 transition (low salinity) may be related to the higher concentrations of organic matter
922 found in the water during this period (Dittmar and Lara, 2001; Dittmar et al., 2001).
923 While this parameter was not quantified in the present study, a number of studies of the
924 Amazon coast have shown phytoplankton biomass increases during the rainy season.
925 The data suggest that rainfall influences the composition, density, biomass, and
926 diversity of phytoplankton (Costa et al., 2011). Given the observed pattern, we suggest
927 that organic matter in suspension is the primary source of food for *C. gasar* and that
928 these nutrients are necessary for gonadal maturation, given that the production of
929 gametes requires a considerable input of energy (Galtsoff, 1964; Andrews, 1979;
930 Lunetta and Grotta, 1982).

931 The present study found no evidence of fluctuations in water temperature that
932 may have affected reproductive patterns in *C. gasar*. This contrasts with findings from
933 temperate regions, where well-defined seasonal variation in temperature appears to
934 affect reproductive patterns in *C. gigas* (Massapina et al., 1999; Fabioux et al., 2005;
935 Chávez-Villalba et al., 2007; Enríque-Díaz et al., 2009), *C. virginica* (Lango-Reynoso et
936 al., 2000), *C. corteziensis* (Mazón-Suástegui et al., 2002; Rodríguez-Jaramillo et al.,
937 2008), and *P. carnea* (Narváez et al., 2000). By contrast, the results of the present study
938 indicated that increased salinity during the dry season was a factor in the release of *C.*
939 *gasar* gametes. This conclusion is reinforced by the relative frequency of immature and
940 spawned/spermiated specimens in the dry season and dry-rainy transition period.
941 However, Lenz and Boehs (2011) observed spawned and spermiated individuals
942 throughout the year in a Brazilian population of *Crassostrea rhizophorae*.

943 The results of the present study also indicate a degree of synchronization
944 between males and females in all stages of gonadal maturation. Massapina et al. (1999)

945 recorded a similar pattern in *C. gigas*, which appears to favor the probability of the
946 reproduction process in these oysters.

947 The reproductive cycle of *C. gasar* is regulated by the typical tropical
948 seasonality of the Amazon region, in which the marked increase in precipitation during
949 the rainy season provokes a decrease in salinity levels in estuarine regions, such as that
950 of the study area. The results of the present study indicate that the dry season or dry-
951 rainy transition months is the optimum period for the collection of embryos or larvae for
952 cultivation, given the higher frequency of spawned/spermiated individuals found during
953 these months.

954

955 **Acknowledgments**

956 We are especially grateful to FAPESPA, for their financial support of the research,
957 CAPES for granting a scholarship, and to Mrs. Lia Sogabe, technician at the UFPA
958 Histological Techniques

959

960 **References**

961

962 Andrews, J.D., 1979. Pelecypoda: Ostreidae. In: *Reproduction of Marine Invertebrates*.

963 Giese, A. C.; Pierse, J. S. (eds.). New York, Academic Press, 293- 341.

964 Baghurst, B., Mitchell, J.G., 2002. Sex specific growth and condition of the Pacific
965 oyster *Crassostrea gigas* (Thunberg 1793). *Aquaculture Research* 33, 1253–1263.

966 Cano, J., Rosique, M. J., Rocamora, J., 1997. Influence of environmental parameters on
967 reproduction of the European flat oyster (*Ostrea edulis*) in a coastal lagoon (Mar
968 Menor, south eastern Spain). *Journal of Molluscan Studies* 63, 187–196.

969 Chávez-Villalba, J., Villelas-Ávila, F., Cáceres-Martínez, C., 2007. Reproduction,
970 condition and mortality of the Pacific oyster *Crassostrea gigas* (Thunberg 1793)
971 along coastal Sonora, Mexico. *Aquaculture Research* 38, 268–278.

972 Cintrón-Molero, G., Schaeffer-Novelli, Y. 1999. Brazilian Mangroves: a historical
973 ecology. *Ciência e Cultura* 51, (3/4): may/august.

974 Christo, S.W., Absher, T.M., 2008. Crescimento da prodissoconch de ostras do gênero
975 *Crassostrea Sacco*, 1897 (Bivalvia, Ostreidae). *Boletim do Instituto de Pesca* 34, 71-
976 77.

977 Costa, V.B., Sousa, E.B., Pinheiro, S.C.C., Pereira, L.C.C., Costa, R.M., 2011. Effects
978 of a high energy coastal environment on the structure and dynamics of phytoplankton
979 communities (Brazilian Amazon littoral). *Journal of Coastal Research* 64, 354-358.

980 Dittmar, T., Lara R.J. (2001). Driving forces behind nutrient and organic matter
981 dynamics in a mangrove tidal creek in North Brazil. *Estuar. Coastal Shelf Sci.* **52**,
982 249–259.

983 Dittmar, T., Lara R.J., Kattner, G. (2001). River or mangrove? Tracing major organic
984 matter sources in tropical brazilian coastal Waters. *Mar. Chemistry.* **73**, 252-271.

985 Enríquez-Díaz, M., Pouvreau, S., Chávez-Villalba, J., LePenneec, M., 2009.
986 Gametogenesis, reproductive investment and spawning behavior of the Pacific giant
987 oyster *Crassostrea gigas*: evidence of an environment-dependent strategy.
988 *Aquaculture Institute* 17, 491-506.

989 Fabioux, C., Huvet, A., Le Souchu, P., LePenneec, M., Pouvreau, S., 2005. Temperature
990 and photoperiod drive *Crassostrea gigas* reproductive internal clock. *Aquaculture*
991 250, 458-470.

992 Gosling, E., 2003. Bivalve mollusks: *Biology, Ecology and Culture*. News Books,
993 Oxford-UK, 438p.

- 994 Galstoff, P.S., 1964. The American oyster *Crassostrea virginica* (Gmelin) Fish Bulletin
995 United States 64, 1–480.
- 996 Instituto Nacional de Meteorologia. Monitoramento das Estações Convencionais.
997 Disponível em: <<http://www.inmet.gov.br/sim/sonabra/convencionais.php>>.
998 acesso em janeiro de 2010.
- 999 Instituto Nacional de Meteorologia. Monitoramento das Estações Convencionais.
1000 Disponível em: <[http://www.inmet.gov.br/sim/sonabra/convencionais](http://www.inmet.gov.br/sim/sonabra/convencionais.php)
1001 [.php](http://www.inmet.gov.br/sim/sonabra/convencionais.php)>. acesso em janeiro de 2011.
- 1002 Lango-Reynoso, F., Chávez-Villalba, J., Cochard, J.C., LePennec M., 2000.
1003 Oocytesize, a means to evaluate the Gametogenic development of the Pacific oysters
1004 *Crassostrea gigas* (Thunberg 1793). Aquaculture 190, 183–199.
- 1005 Lango-Reynoso, F., Chávez-Villalba, J., Le Pennec, M., 2006. Reproductive patterns of
1006 the pacific oyster *Crassostrea gigas* in France. Invertebrate Reproduction and
1007 Development 49, 41-50.
- 1008 Le Dantec, J., 1968. Reproduction em *Crassostrea angulata* (Lamarck 1819) dans le
1009 Basin D' Arcachon. Quelques comparaisons avec les huitres de la Gironde. Revue
1010 des Travaux de Intitut Pêches Maritimes 32, 300-362.
- 1011 Lenz, T., Boehs, G., 2011. Ciclo reproductivo Del ostion de manglar *Crassostrea*
1012 *rhizophorae* (bivalvia: ostreidae) em La Bahia de Camamu, Bahia, Brasil. Revista de
1013 Biologia Tropical 59, 137-149.
- 1014 Lunetta, J.E., Grotta, M., 1982. Influência de fatores exógenos e endógenos sobre a
1015 reprodução de moluscos marinhos. Boletim de Fisiologia Animal 6, 191-204.
- 1016 Massapina, C., Joaquim, S., Matias, D., Devauchelle, N., 1999. Oocyte and embryo
1017 quality in *Crassostrea gigas* (Portuguese strain) during a spawning period in
1018 Algarve, South Portugal. Aquatic Living Resources 12, 327-333.

- 1019 Mazon-Suastegui, J.M., Robles-Mungaray, M., Flores-Higuera, F., Aviles-Quevedo,
1020 M.A., 2002. Experiencias em La produccion de semilla de ostion de placer
1021 *Crassostrea corteziensis* em el laboratorio. IV Simposio Nacional de Acuicultura y
1022 Pesca (Book of Abstracts). Antigua, Guatemala. 16–18.
- 1023 Melo, A.G.C., Varela, E.S., Beasley, C.R., Schneider, H., Sampaio, I., Gaffney, P.M.,
1024 Reece, K.S., Tagliaro, C.H., 2010. Molecular identification, phylogeny and
1025 geographic distribution of Brazilian mangrove oyster (*Crassostrea*). Genetics and
1026 Molecular Biology 33, 564-572.
- 1027 Narvaez, C., Lodeiros, C., Freitas, L., Nunez, M., Pico, D., Prieto, A., 2000.
1028 Abundancia de juveniles y crecimiento de *Pinna carnea* (Mytiloide: Pinnacea)
1029 encultivo suspendido. Revista de Biologia Tropical 48, 785-797.
- 1030 National Oceanic and Atmospheric Administration (NOAA),
1031 <http://www.ngdc.noaa.gov/mgg/shorelines/shorelines.html>
- 1032 Pereira, O.M., Henriques, M.B., Machado, I.C., 2003. Estimativa da curva de
1033 crescimento da ostra *Crassostrea brasiliiana* em bosques de mangue e proposta para
1034 sua extração ordenada no estuário de Cananéia, SP, Brasil. Boletim do Instituto de
1035 Pesca 29, 19-28.
- 1036 Rodríguez–Jaramillo, C., Hurtado, M.A., Romero–Vivas, E., Ramírez, J. L., Manzano,
1037 M., Palacios, E., 2008. Gonadal development and histochemistry of the tropical
1038 oyster, *Crassostrea corteziensis* (Hertlein, 1951.) during an annual reproductiva
1039 cycle. Journal of Shellfish Research 27, 1129 – 1141.
- 1040 Varela, E.S., Colin, R.B., Schneider, H., Sampaio, I., Marques-Silva, N.S., Tagliaro,
1041 C.H., 2007. Molecular phylogeny os mangrove oysters (*Crassostrea*) from Brazil.
1042 Journal of Molluscan Studies 73, 229-234.