Table 2.2. – Overview on research experiments using germ-free or gnotobiotic cnidarians, equinoderms and rotifers models (adapted from Marques *et al.*, 2006a).

Species	Procedures used to obtain axenic organisms				Methods to verify contamination	Research Field	References
	Method	Amount	Exposure	Target			
Cnidarians							
Hydra viridis *H. vulgaris*	RW+Pen+Stre+Neo+ Chl *or* Rif	100 µg.ml^{-1} each	48h	Adults	PP (33ºC)	Host-microbial relationships	Rahat & Diementman (1982)*
Equinoderms							
Strongylocentrotus purpuratus	Ethanol	95%	Immersion Bath	Adults	ZB (NRºC), AO	Nutritional requirements Metabolic functions	Manahan *et al.* (1983)*
Rotifers							
Philodina acuticornis	Kan S+Col Na+Cep Na	1+1+1 (mg.ml^{-1})	5 – 6 h	Resting Eggs	TSA, DA (NRºC)	Host-microbial relationships	Meadow & Barrows (1971)*
Brachionus plicatilis	Dhstre+Pen G+Pol B S+ Tet+Chl+Neo NaOCl	5+500 µ+0.15 µ+ 0.25 µ+0.05+0.25 (mg.ml^{-1}) 0.5+0.25 mg.L^{-1}	2 h + Bath *or* 60+ 30 min	Resting Eggs	STP (NRºC)	Host-microbial relationships	Hagiwara *et al.* (1994)*
B. plicatilis	OA+Kan+Ery+Pen G+ Stre	10+10+10+150+ 75 (mg.ml^{-1})	24 h	Resting Eggs	BHIB (1% NaCl) (20ºC)	Host-microbial relationships when feed to axenic turbot	Munro *et al.* (1995)
B. rotundiformis	NaOCl	5, 1, 0.5, *or* 0.05%	1, 3 *or* 5	Resting Eggs	MA (25ºC), DAPI	Host-microbial relationships	Douillet (2000)
B. plicatilis	Merthiolate *or* glutaraldhyde	100 mg.L^{-1} *or* 0.05 µ	10 min *or* 6 h	Resting Eggs	MA (28ºC)	Host-microbial relationships	Rombaut *et al.* (1999)
B. rotundiformis	Dhstre+Pen G+Pol B S+ Tet+Chl+Neo	5+5000 U+0.15 U+ 0.25+0.05+0.25 (mg.ml^{-1})	2 h + Bath	Resting Eggs	MA (NRºC)	Host-microbial relationships	Jung and Hagiwara (2001)*
B. plicatilis	Tri+Sx	100-500 µl	24 h	Resting Eggs	BHIB (30ºC)	Effect of antimicrobial treatments on the bacterial load and egg	Martínez-Díaz *et al.* (2003)*
B. plicatilis	Glutaraldehyde	50-100 mg.L^{-1}	1-2 h	Resting Eggs	MA (28ºC), MTT (RT)	Host-microbial relationships Axenic cultutes	Tinh *et al.* (2006)

* Reference *in* Marques (2006a); *AO*, acridine orange staining microscopy; *BHIB*, brain heart infusion broth; *Cep Na*, sodium cephalotin; *Chl*, chloramphenicol; *Col Na*, sodium colistimethate; *DA*, desoxychocolate agar; *DAPI,* 4'6-diamidino-2-phenylindole staining microscopy; *Dhstre*, dihydrostreptomycin; *Ery*, erythromycin; *Kan*, kanamycin; *Kan S*, kanamycin sulphate; *MA*, marine agar; *MTT*, 3-(4,5-dimethylthazol-2-yl)-2,5-dphenyl tetrazolium bromide staining; *NaCl*, sodium chlorite; *NaOCl*, sodium hypochlorite, *Neo*, neomycin, *NR*, no reference; *OA*, oxolinic acid; *Pen*, penicillin; *Pen G*, penicillin G; *Pol B S*, polymyxin B sulphate; *PP*, proteose peptone medium; *Rif*, rifampicin; *RT,* room temperature, *RW*, reasing, *STP*, seawater with trypticase, *Stre*, streptomycin, *Sx*, sulfametoxalose; *Tet*, tetracycline; *Tri*, trimethoprim; *TSA*, trypticase soy agar; *ZB*, Zobell broth.

Table 2.3. – Overview on research experiments using germ-free or gnotobiotic molluscs and crustaceans models (adapted from Marques *et al.*, 2006a).

Species	Procedures used to obtain axenic organisms				Methods to verify contamination	Research Field	References
	Method	Amount	Exposure	Target			
Molluscs							
Mercenaria mercenaria	Pen G+Stre S+Chl	100+100+50 (ml.L^{-1})	24-36 h	Eggs	SWNB (NRºC)	Host-microbial relationships	Guillard (1959)*
Crassostrea gigas	NaOCl	1%	Immersion Bath	Adults	MB (25ºC), DAPI	Host-microbial relationships	Douillet & Langdon (1994)
C. gigas	OA+Kan+Ery+Stre S+ Flu+Pen	0.1+0.1+0.1+0.1 (ml.L^{-1})+ 30 µg.L^{-1}+100 units.L^{-1}	3 days	Eggs	Droop E6 (25ºC), AO	Host-microbial relationships	Renault *et al.* (1995)*
Haliotis midae	Amp+Chl+Cef	600+250+250 (µg L^{-1})	72 h	Juveniles	MA (22ºC), DAPI, SEM	Host-microbial relationships	Erasmus *et al.* (1997)
Crustaceans							
Artemia salina	Merthiolate+Aerosol OT	1 ppt+10% 2 ml.L^{-1}	10 min	Cyst	STP medium (22-6ºC)	Nutritional requirements, Metabolic functions	Provasoli & D'Agostino (1969)*
Daphnia magna *Moina macrocopa*	RW+ Standard aseptic conditions	-	Immersion Bath	Adults	TG (NRºC), M	Nutritional requirements	Murphy (1970)*
Artemia sp.	Merthiolate	1 ppt	10 min	Cyst	2% YEG (RT), STM (NRºC), DBM (NRºC), MA (NRºC)	Nutritional requirements, Host-microbial relationships	Douillet (1987)*
Artemia salina	NaOCl (2% Chlorine)	20%	15 min	Cyst	4% PP (33ºC)	Feed to axenic hydra	Rahat & Diementman (1982)*
A. franciscana	Decapsulation Chl+Tri+Sfm	NR 30+40+8 (mg.L^{-1})	NR 24 h	Cyst	MA, TCBS (30ºC)	Host-microbial relationships	Gomez-Gil *et al.* (1998)
A. franciscana	Merthiolate	1 g.L^{-1}	10 min	Cyst	MB, MA (28ºC)	Host-microbial relationships	Verschuere *et al.* (1999, 2000b)
A. franciscana	Cloralex (active chlorine)	60 g.L^{-1}	10 min	Cyst	MA, M (30ºC)	Host-microbial relationships	Orozco-Medina *et al.* (2002)*
A. franciscana	Decapsulation Chl+Tri+Sfm	NR 30+40+8 (mg.L^{-1})	NR 24 h	Cyst	TSA+2% NaCl (30ºC) DTAF	Host-microbial relationships	Soto-Rodriguez *et al.* (2003)*
A. franciscana	NaOCl+NaOh+HCl	10%+0.25 mol.L^{-1}+ 0.1 mol.L^{-1}	NR	Cyst	MA, MB (6ºC), DAPI	Host-microbial relationships, Feed to axenic halibut	Verner-Jeffreys *et al.* (2003)
A. franciscana	NaOCl+NaOh+$Na_2S_2O_3$ RW	50 ml (32%)+3 ml (50%) +90 ml 75 ml (10 g.L^{-1})+tap water	150 s Bath	Cyst	MA (28ºC) and MTT (RT) *or* MB (28ºC)	Nutritional requirements, Host-microbial relationships	Marques *et al.* (2004, 2005, 2006b), Defoird *et al.* (2005)

* References *in* Marques (2006a); *Amp*, ampicillin; *AO*, acridine orange staining microscopy; *Cef*, cefotaxime; *Chl*, chloramphenicol; *DAPI,* 4'6-diamidino-2-phenylindole staining microscopy; *DBM*, Difco broth mixture; *DTAF*, 5-(4,6-dichlorotriazin-2-yl) aminofluorescein staining microscopy; *Ery*, erythromycin; *Flu*, flumequine; *HCl*, chloridric acid; *Kan*, kanamycin; *M*, light microscopy; *MA*, marine agar; *MB,* marine broth; *MTT*, 3-(4,5-dimethylthazol-2-yl)-2,5-dphenyl tetrazolium bromide staining; *NaOH, sodium hydroxide*; $Na_2S_2O_3$, sodium thiosulphate; *NaCl*, sodium chlorite; *NaOCl*, sodium hypochlorite, *NR*, no reference; *OA*, oxolinic acid; *Pen*, penicillin; *Pen G*, penicillin G; *PP*, proteose peptone medium; *RT,* room temperature, *RW*, reasing; *SEM*, scanning electron microscopy, *Sfm*, sulfamethoxaxole; *STM*, sterily test medium; *STP*, seawater with trypticase, *Stre S*, streptomycin sulphate; *SWNB*, seawater nutrient broth; *TCBS*, thiosulfate citrate; *TG*, thioglycollate; *Tri*, trimethoprim; *TSA*, trypticase soy agar; *YEG*, yeast extract glucose solution.

Table 2.4. – Overview on research experiments using germ-free or gnotobiotic freshwater and marine fish models (adapted from Marques *et al.*, 2006a).

Species	Procedures used to obtain axenic organisms				Methods to verify contamination	Research Field	References
	Method	Amount	Exposure	Target			
Freshwater fishes							
Salmo irideus *Salmo fario* *Savelinus fontinalis*	Acriflavine *or* sulfo-merthiolate	0.015% 0.185 %	30 min	Eggs	FIB (RT)	Effect of antimicrobial treatments on the bacterial load and egg	Gee & Sarles (1942)*
Oncorhynchus kisutch *O. keta* *O. nerka* *Salmo aquabonito* *Salmo gairdneri* *Salvelinus frontinalis*	Povidone-iodine Standard aseptic conditions	1% active iodine	Immersion Bath	Adult Eggs	BHIB, GFB, TSB (13 and 30ºC)	Germ-free culture	Trust (1974)
Danio rerio	Betadine	10%	2 min	Adults	BHIB, NB, SDB (28ºC and 37ºC)	Germ-free culture Host-microbial relationships Morphology	Rawls *et al.* (2004)
	Amp+Kan+Amphotericin B	100 µg+5 µg+ 250 ng (ml^{-1})	6 h	Eggs			
	Betadine	0.1%	2 min	Eggs			
	NaOCl	0.003%	20 min	Eggs			
Marine fishes							
Gadus morhua *Hippoglossus hippoglossus*	Standard aseptic conditions	NR	NR	Eggs	NR	Egg bacteria colonization	Hansen & Olafsen (1989)
Sciaenops oceallatus *Ocyurus chrysurus* *Cynoscion nebulosus*	Hydrogen peroxide	3%	5 min	Eggs	ZA, ZB, TSPSB, TSPSA (NR ºC), DAPI	Egg disinfection efficiency, obtaining bacteria-free larvae	Douillet & Holt (1994)
Hyppoglossus hippoglossus *Gadus morhua* *Pleuronectes platessa*	Glutaraldehyde *or* NaOCl (5% active cloride)	25% (800 mg) 100 mg (ml^{-1})	2.5 – 10 min	Eggs	M-65 SWA (1ºC)	Effect of antimicrobial treatments on the bacterial load and egg	Salvesen & Vadstein (1995)
Scophthalmus maximus	OA+Kan+Ery+Pen G+Stre	10+10+10+150+ 75 ($mg.ml^{-1}$)	24 h	Eggs	BHIB (1% NaCl) (20ºC)	Host-microbial relationships	Munro *et al.* (1995)
Hipoglossus hippoglossus	Glutaraldehyde OA+Kan+Ery+Pen G+Stre Peracetic Acid	25% 10+10+10+150+ 75 ($mg.ml^{-1}$) 0.02%	10 min	Eggs	MA, MB (6ºC), DAPI	Host-microbial relationships	Verner-Jeffreys *et al.* (2003)

* Reference *in* Marques (2006a); *Amp*, ampicillin; *BHIB*, brain heart infusion broth; *DAPI,* 4'6-diamidino-2-phenylindole staining microscopy; *Ery*, erythromycin; *FIB*, fish infusion broth; *GFB*, glucose fermentation broth; *Kan*, kanamycin; *MA*, marine agar; *MB,* marine broth; *NaCl*, sodium chlorite; *NaOCl*, sodium hypochlorite, *NB*, nutrient broth, *NR*, no reference; *OA*, oxolinic acid; *Pen G*, penicillin G; *RT,* room temperature, *SDB*, Sabouraud dextrose broth; *Stre*, streptomyci; *SWA*, seawater agar; *TSB*, trypticase soy broth; *TSPSA,* tryptic soy plus salt agar; *TSPSB,* tryptic soy plus salt broth; *ZA*, Zobell agar; *ZB*, Zobell broth.