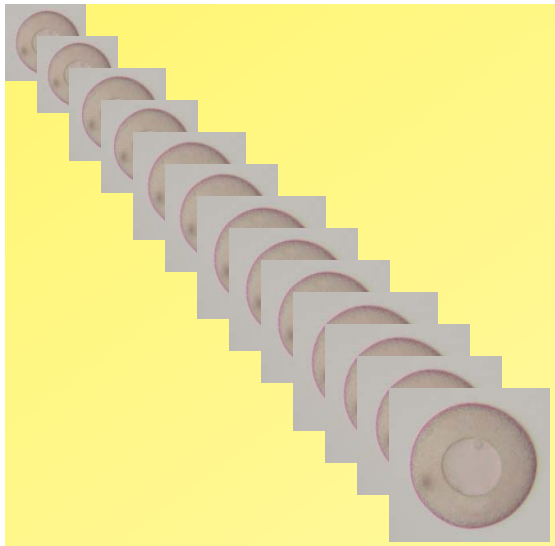
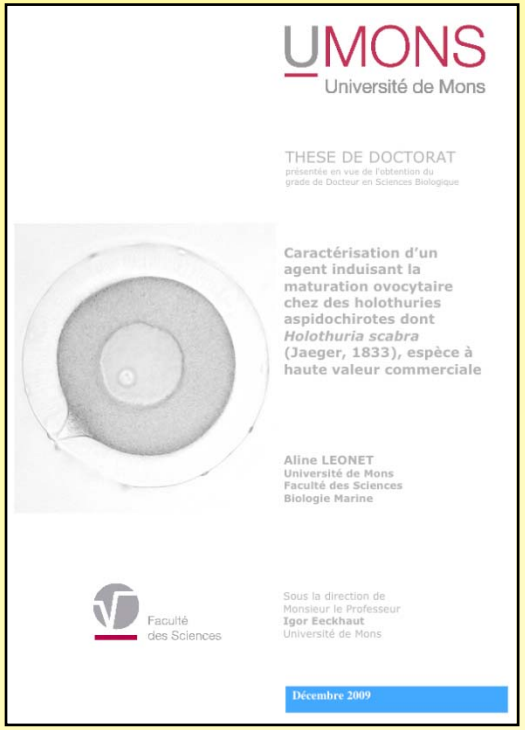




UMONS
Université de Mons

THESE DE DOCTORAT
présentée en vue de l'obtention du grade de Docteur en Sciences Biologique

Caractérisation d'un agent induisant la maturation ovocytaire chez des holothuries aspidochirotes dont *Holothuria scabra* (Jaeger, 1833), espèce à haute valeur commerciale

Aline LEONET
Université de Mons
Faculté des Sciences
Biologie Marine

Faculté des Sciences

Sous la direction de
Monsieur le Professeur
Igor Eeckhaut
Université de Mons

Décembre 2009

In vitro fertilization of sea cucumbers, a new method for boosting aquaculture production

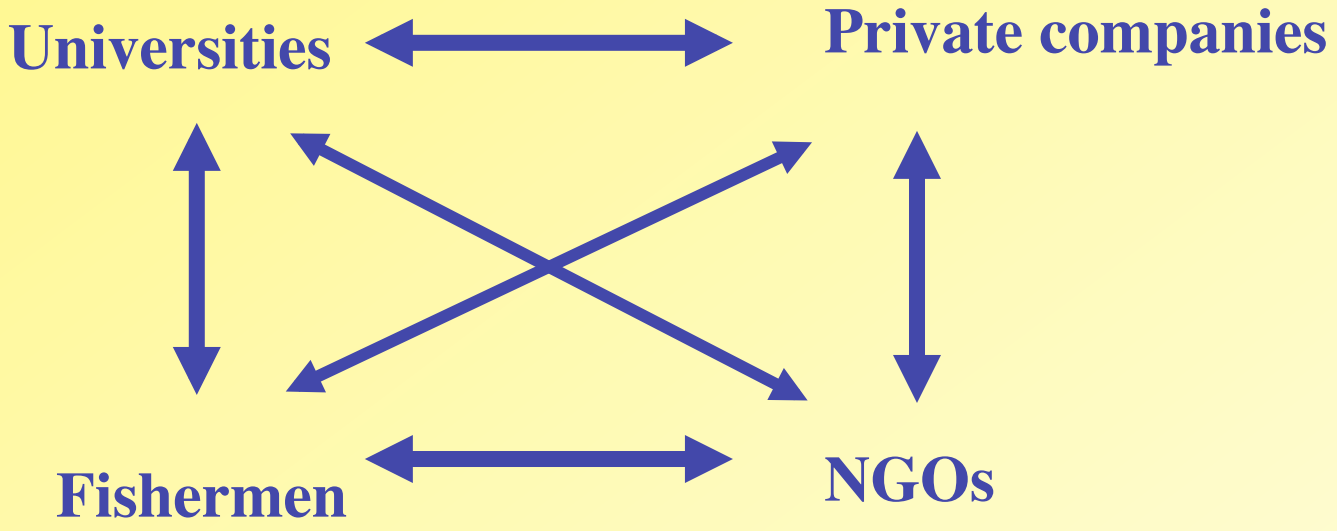
Igor Eeckhaut, Aline Léonet, Ruddy Wattiez, Michel Jangoux, Thierry Lavitra and Richard Rasolofonirina

Working groups

UMons
ULB
UTul

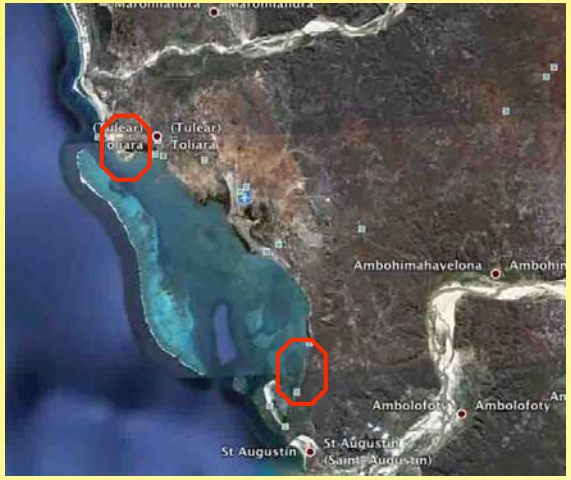
Spin Off: *Madagascar Holothurie S.A.*

Copefrito S.A.
IRDP S.A.

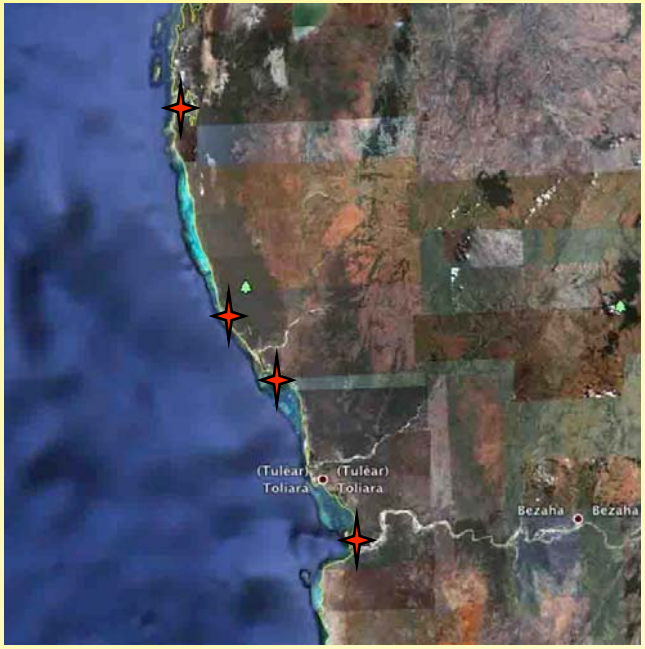


Sarodrano
Fiherenamasay
Andrevo
Tampolove
…

Transmad
Blue Venture


(Tulear)
Toliara
Ambohimahavelona
Ambolofoty
St Augustin
(Saint-Augustin)


Iles Glorieuses
Moroni
Mamoudzou
Mayotte
Ile Tromelin
Ile Juan de Nova
Antananarivo
Madagascar
Port Louis
Saint-Denis
Réunion
Bassas da India
Ile Europa


(Tulear)
Toliara
(Tulear)
Toliara
Bezaha
Bezaha


MADAGASCAR HOLOTHURIE S.A.

Larval development

(and early postmetamorphic development)

Postmetamorphic development

Pregrowing

Growing

90 days

60 days

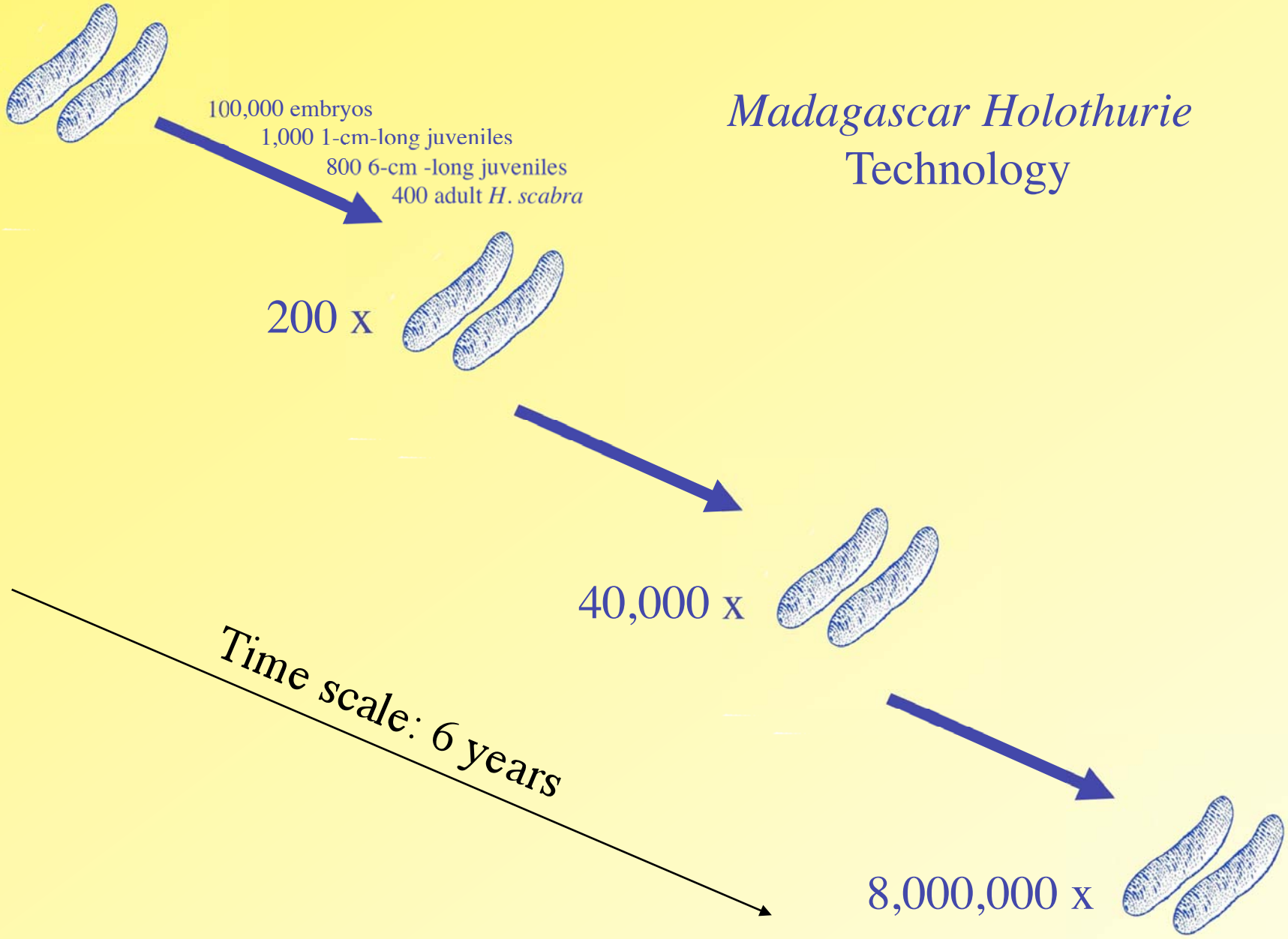
8 months

Hatchery

Ponds

Fences

Madagascar Holothurie Technology



Thermal shocks

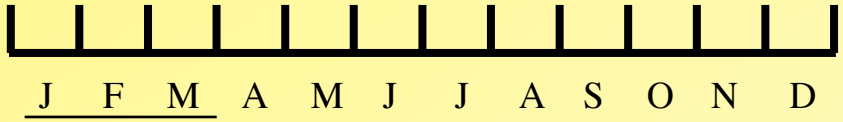
Output with 15 tanks/year: 1 x 15 x 1,000 juveniles

15,000 juveniles/year

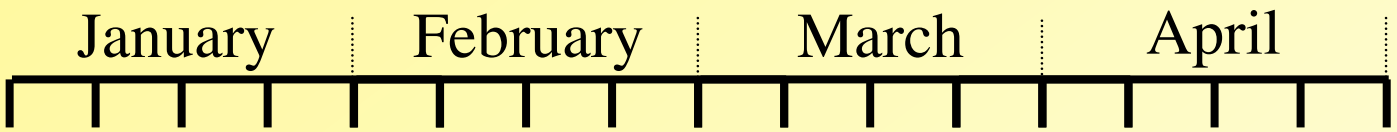
In vitro fertilization method

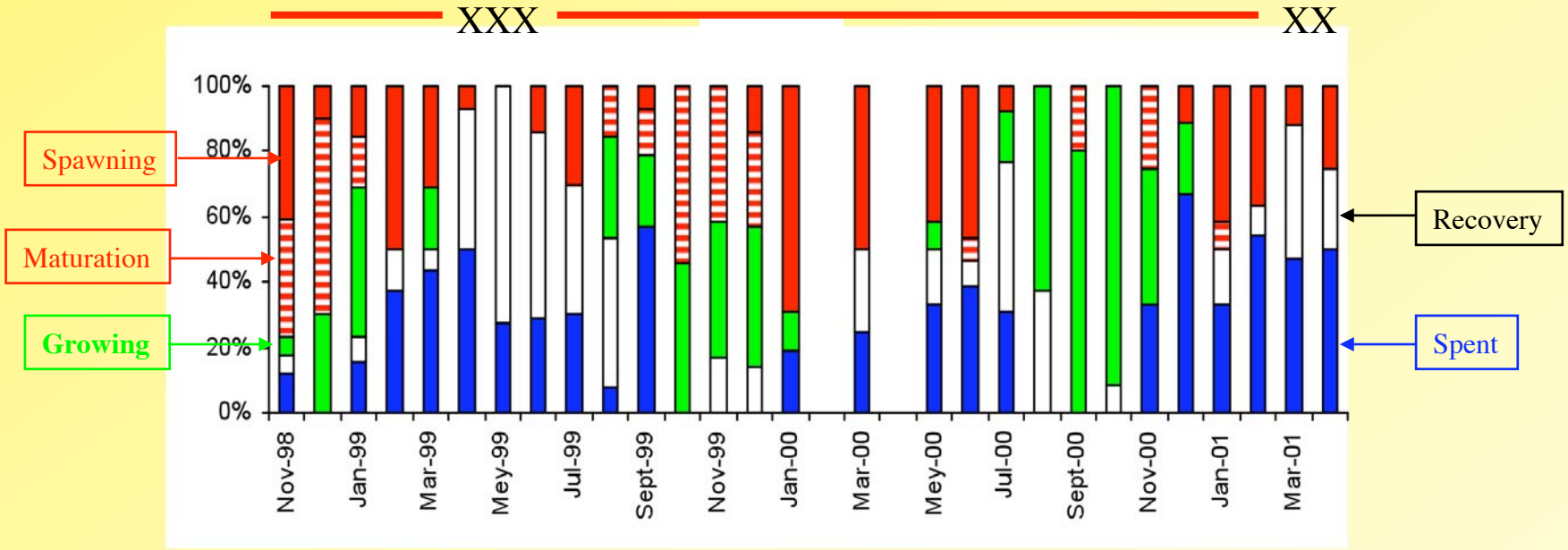
Output with 15 tanks/year: 4 x 15 x 1,000 juveniles

60,000 juveniles/year



Thermal shocks

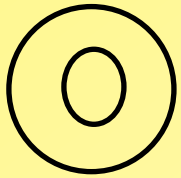




More than 30% of individuals have fertilizable oocytes in 23 months out of the 28-months period

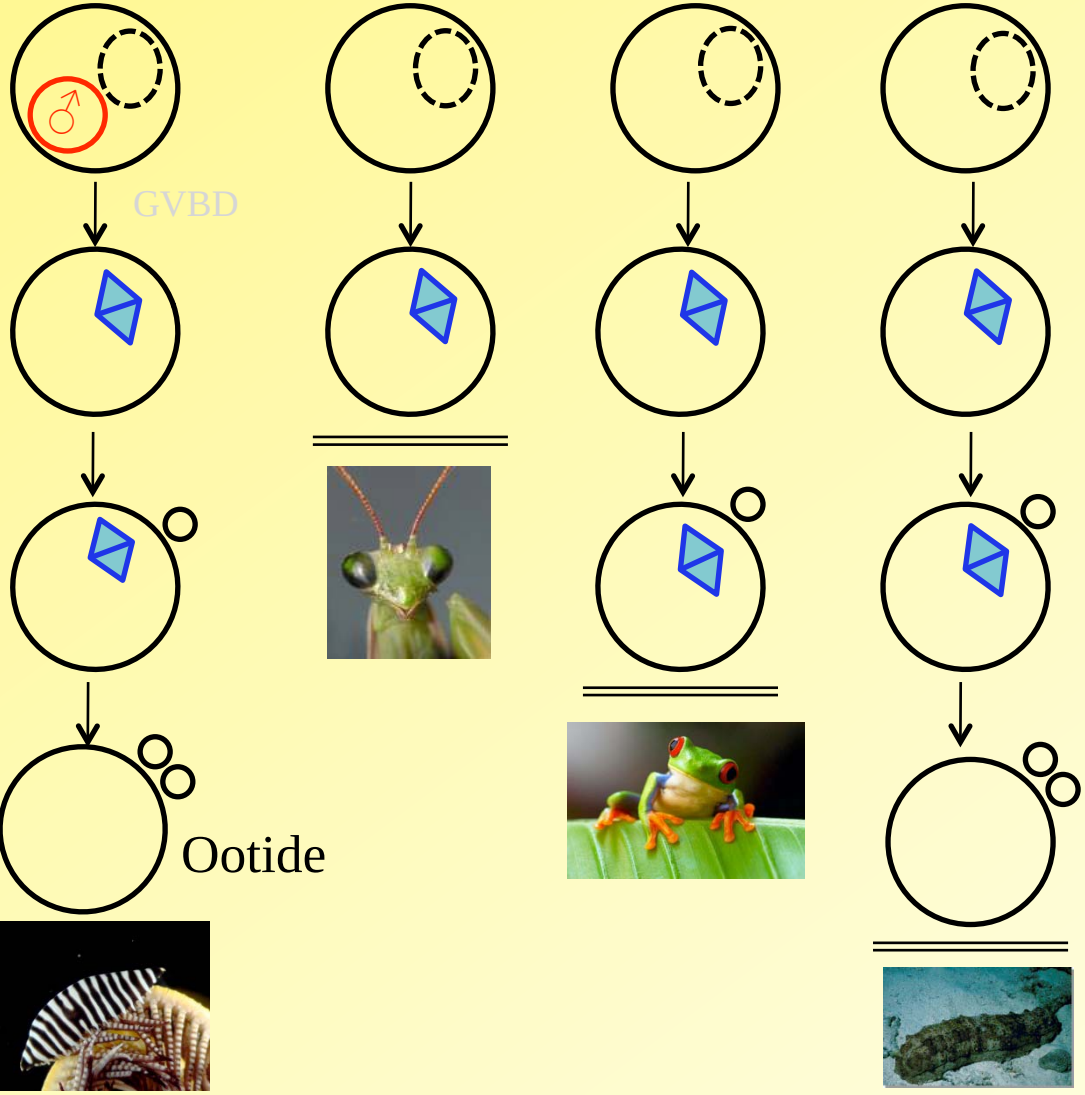
Richard Rasolofonirina, Devarajen Vaïntiligon, Igor Eeckhaut, M. Jangoux 2005

Prophase I



Universal blocking

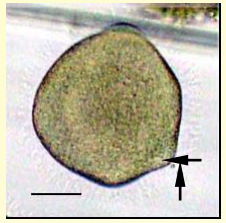
Fertilization | Ecdysteroïd | Progesteron | « Spawning »

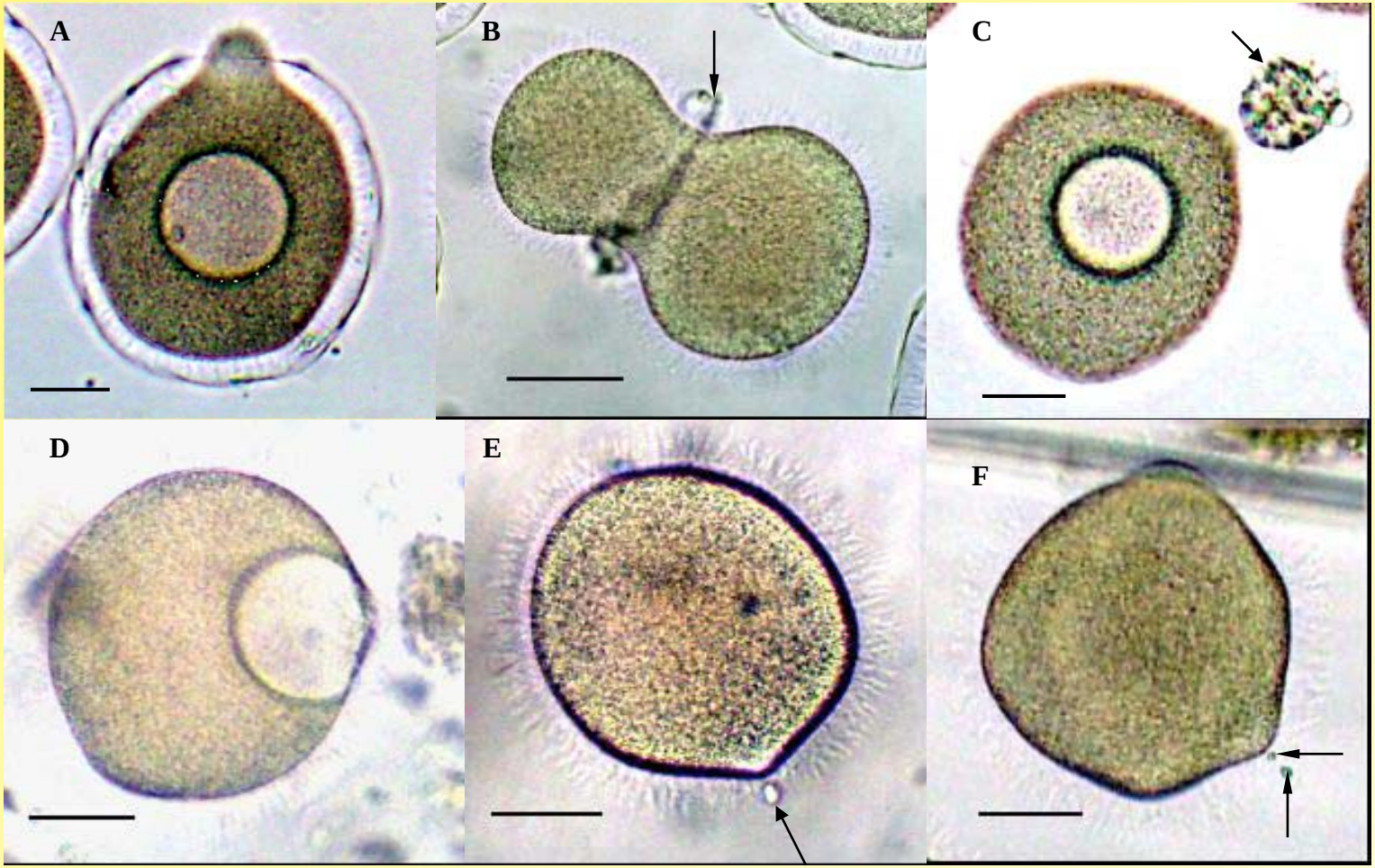


Prophase I

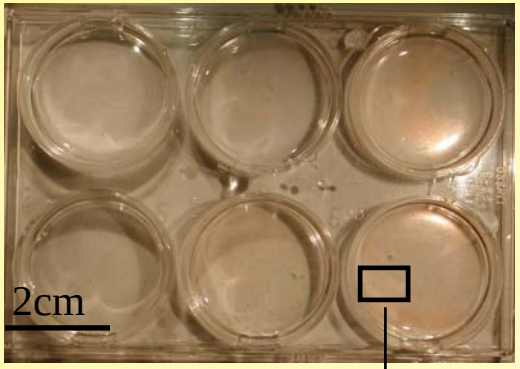
Métaphase I

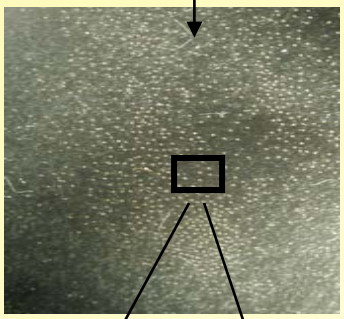
Métaphase II

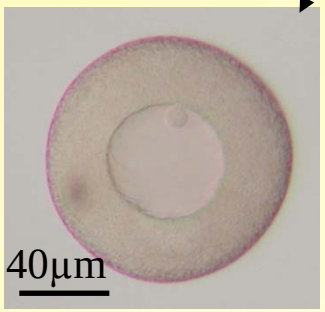


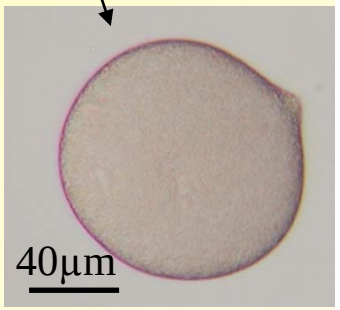


Scale bar=50μm



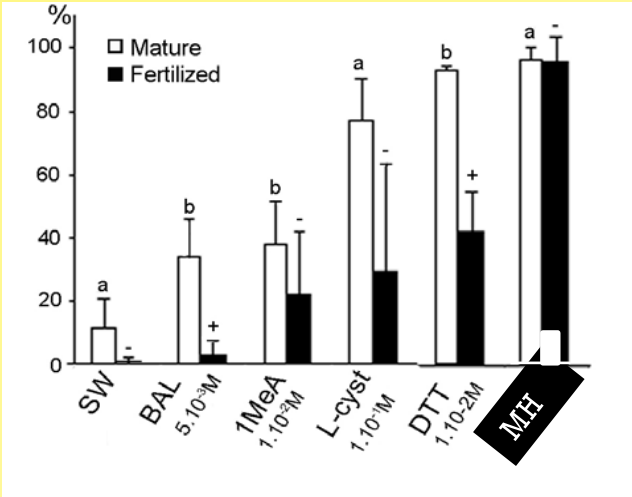






-MH inductor, other inductors
-Sea water

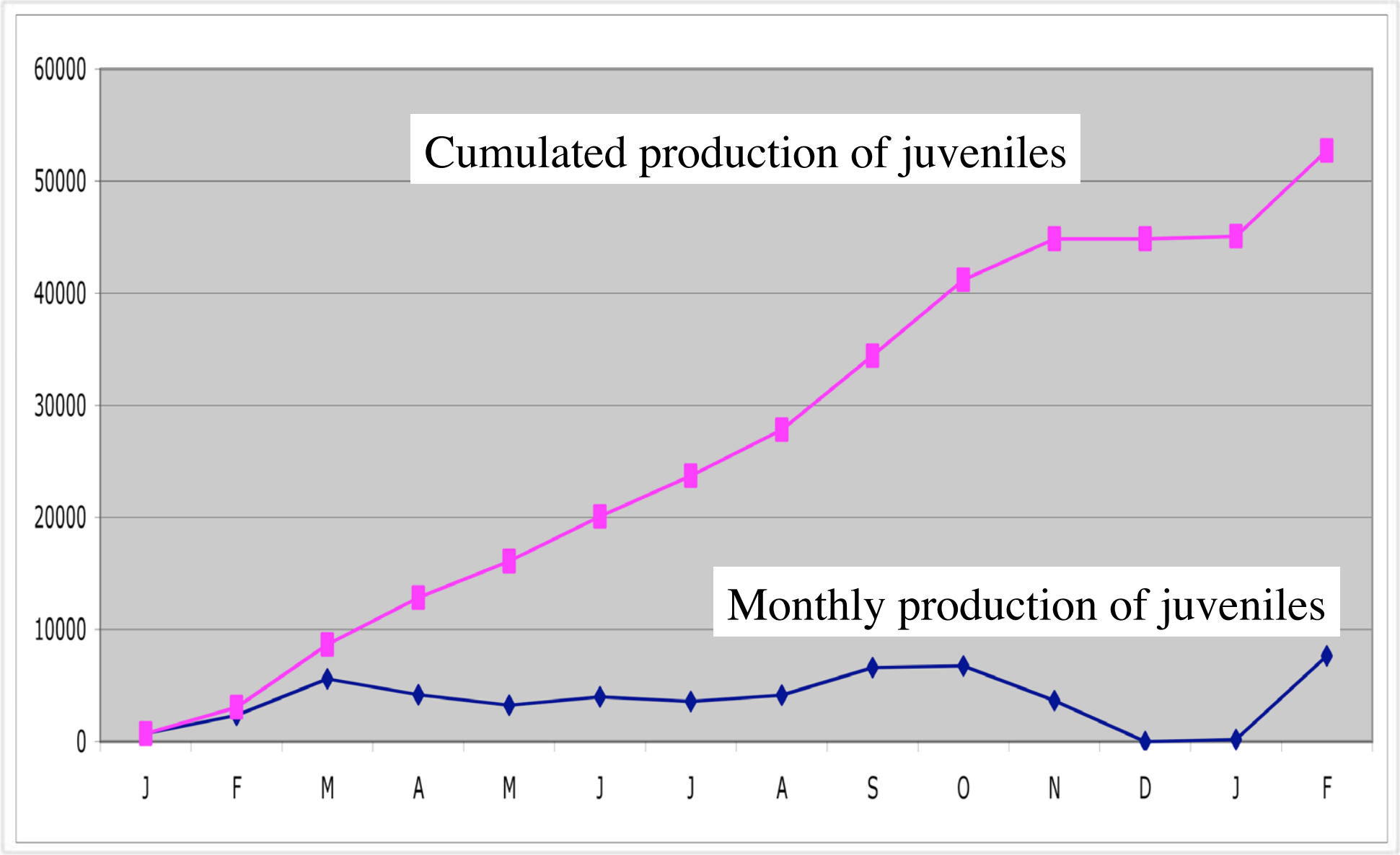
Properties of MH inductor



- Induces more than 95% of maturation
- More than 90% of the oocytes are fertilizable
- Does not induce any larval malformation
- Is more efficient than the other known inductors

Species	%maturation in MH	%maturation in sea water
Actinopyga echinites (n=3)	81	31
Bohadschia subrubra (n=2)	99	9
Bohadschia vitiensis (n=4)	87.42	9.65
Holothuria cinerascens (n=3)	92.6	12.3
Holothuria edulis (n=2)	92	11
Holothuria forskali (n=2)	94.5	7
Holothuria fuscogilva (n=1)	75	10
Holothuria leucospilota (n=4)	70.25	6
Holothuria maculosa (n=6)	63.35	9.2
Holothuria scabra (n=4)	92.25	15.75
Holothuria tubulosa (n=4)	82	24.25
Peasonothuria graeffei (n=3)	92	32
Thelenota ananas (n=3)	79.33	32.66

Production of juveniles in the hatchery of *Madagascar Holothurie S.A.*



Methods	details	Useless outside spawning period	Reliability	Risk of infestation	Risk of larval malformations	Tested species	References
Spawning stimulation							
Thermal shoks	Transfert into tanks of various t¡	-	random	+	-	*Astichopus.japonicus* *Australostichopus mollis*	Hamel *et al.*, 2002 Morgan and,AndrewDavid, 2009
PCF	Injection into coelomic cavity	-	-	+	-	*Bohadschia argus* *B.marmorata* *Holothuria leucospilota* *H. atra*	Mercier and Hamel, 2002
Cubifrine	Injection into coelomic cavity	-	+	+	-	*A.japonicus*	Fujiwara *et al.*, 2010 Kato *et al.*, 2009
Gonad incubation							
Aj-GSSL	Incubation with gonadal tubules	-	+	-	-	*A.japonicus*	Katow *et al.*, 2009
Cubifrine	Incubation with gonadal tubules	-	+	-	-	*A.japonicus*	Kato *et al.*, 2009
Oocyte incubation							
MH inductor	Incubation with oocytes	+	+	-	-	12 species	LŽonet *et al.*, 2009
DTT	Incubation with oocytes	+	+	-	+	*H. scabra* *A. japonicus* *H. leucospilota* *H. pardalis*	LŽonet *et al.*, 2009 Keraseva and Khotimchenko 1995 Maruyama, 1980
Other inductors (BAL, L-cystŽine, 1MŽA)	Incubation with oocytes	-	-	-	+	*H. scabra* *H. leucospilota* *H. pardalis*	LŽonet *et al.*, 2009 Maruyama, 1980

Thanxxx for your attention!