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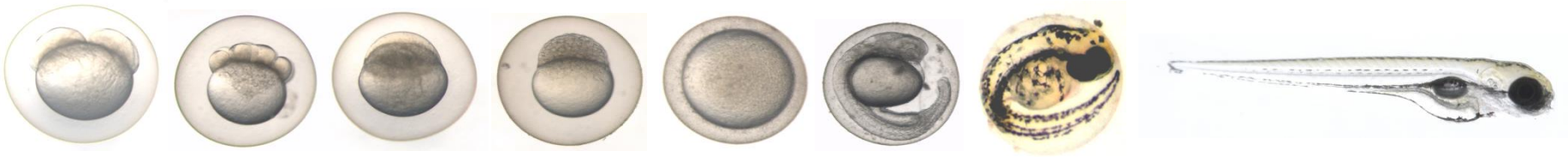
XX Congresso Farmacêutico de São Paulo

III SIMPÓSIO "FRONTEIRAS NAS CIÊNCIAS FARMACÊUTICAS"

10 e 11 de Outubro de 2019

Zebrafish

Sua Importância na Avaliação de Toxicidade



Mônica Lopes Ferreira

Modelo experimental para pesquisa cientifica

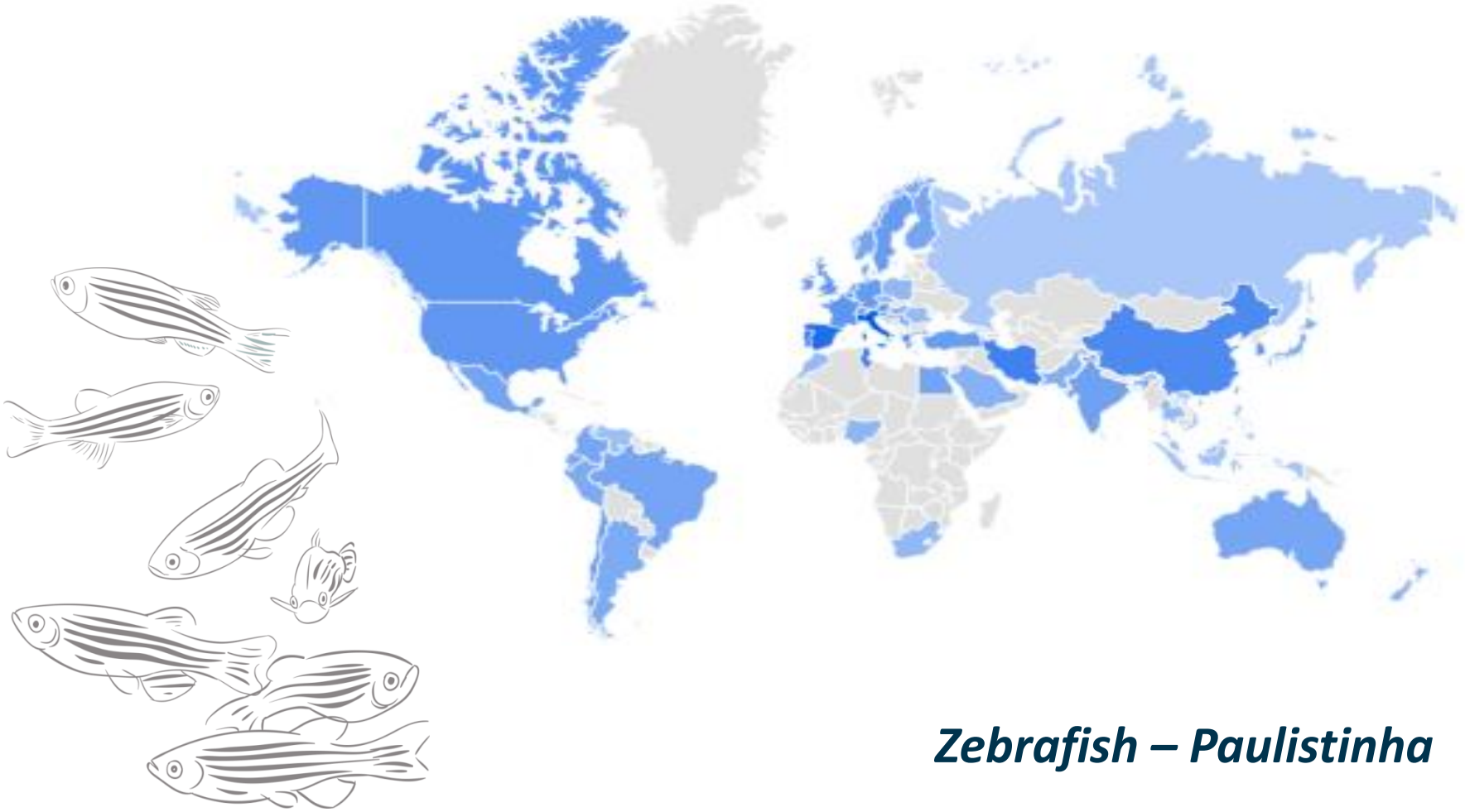
Em 1981 - George Streisinger, biologo, professor da Universidade de Oregon

Considerado o introdutor do zebrafish como modelo na pesquisa científica.

Mostrou vantagens na utilização do peixe para estudos genéticos

publicou na Nature, artigo considerado um marco na medicina translacional.

Danio rerio



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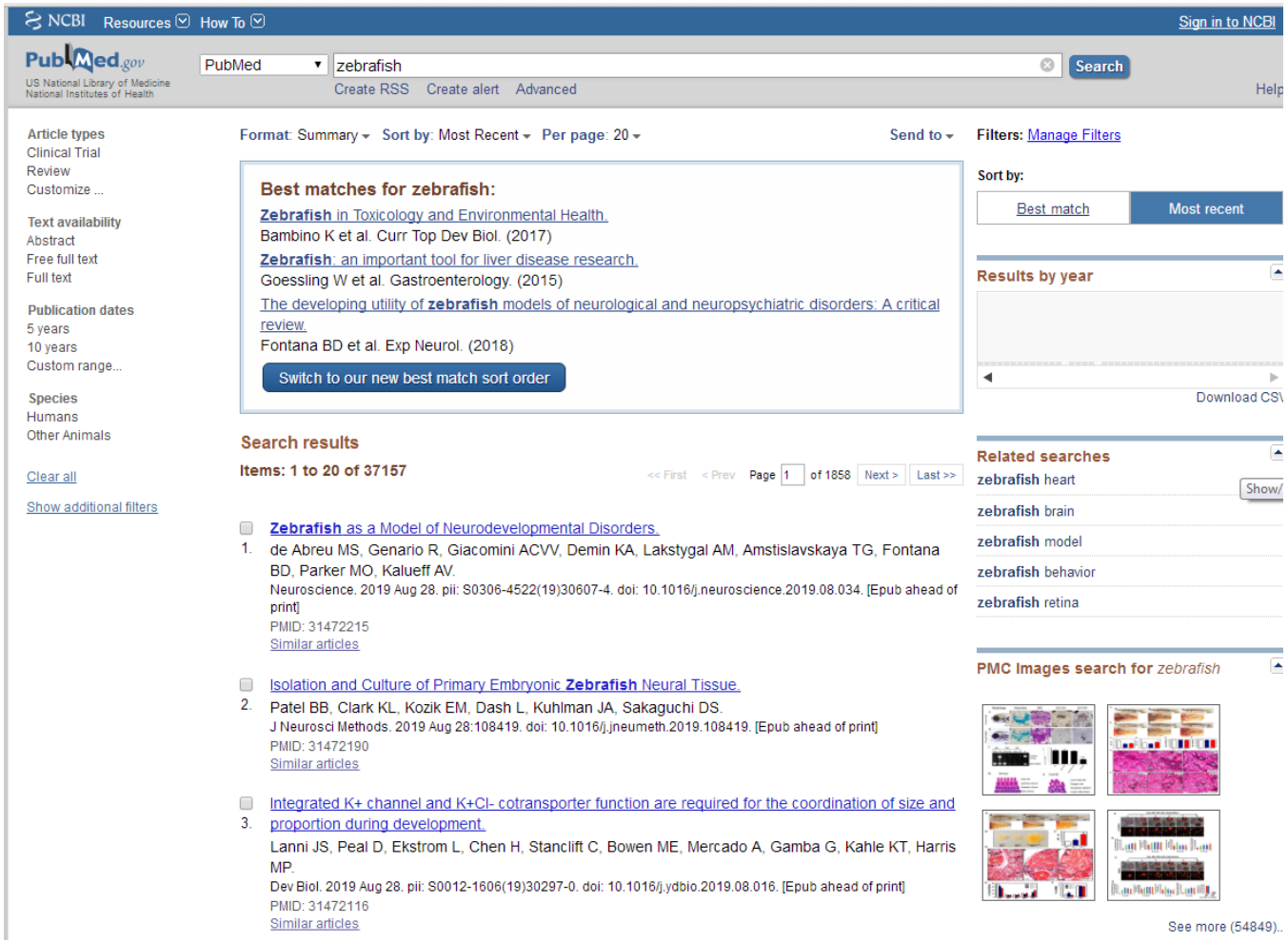

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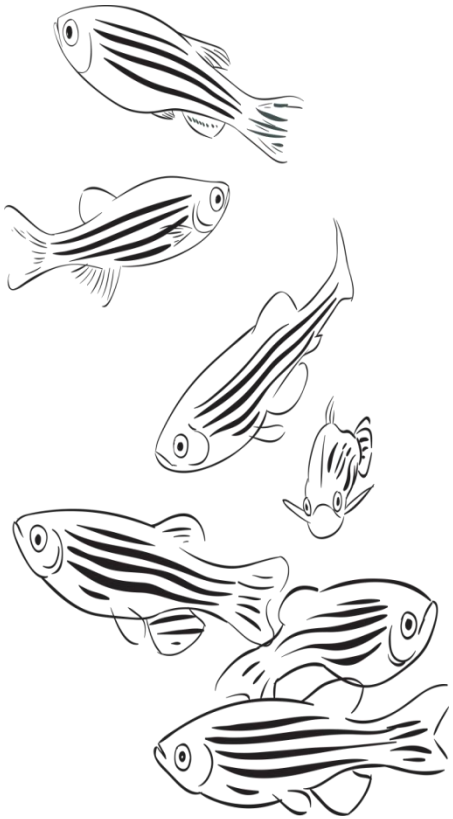

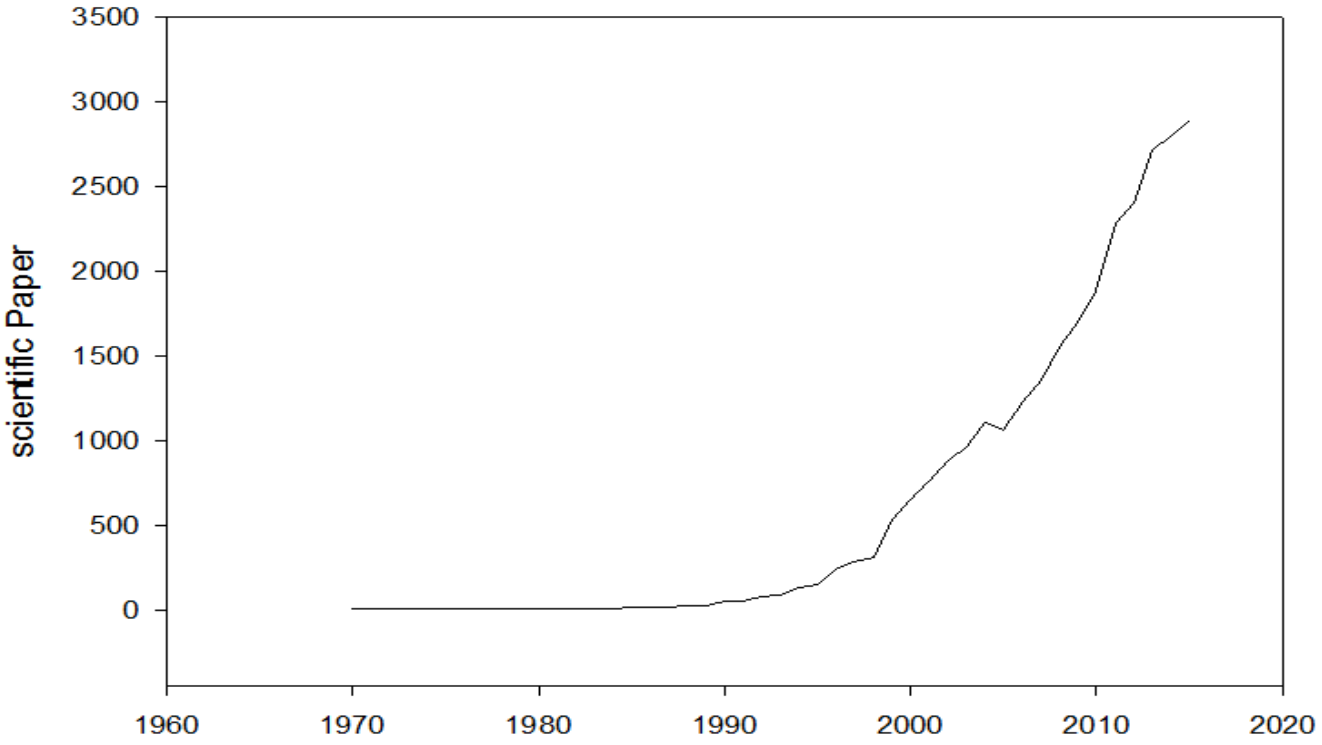

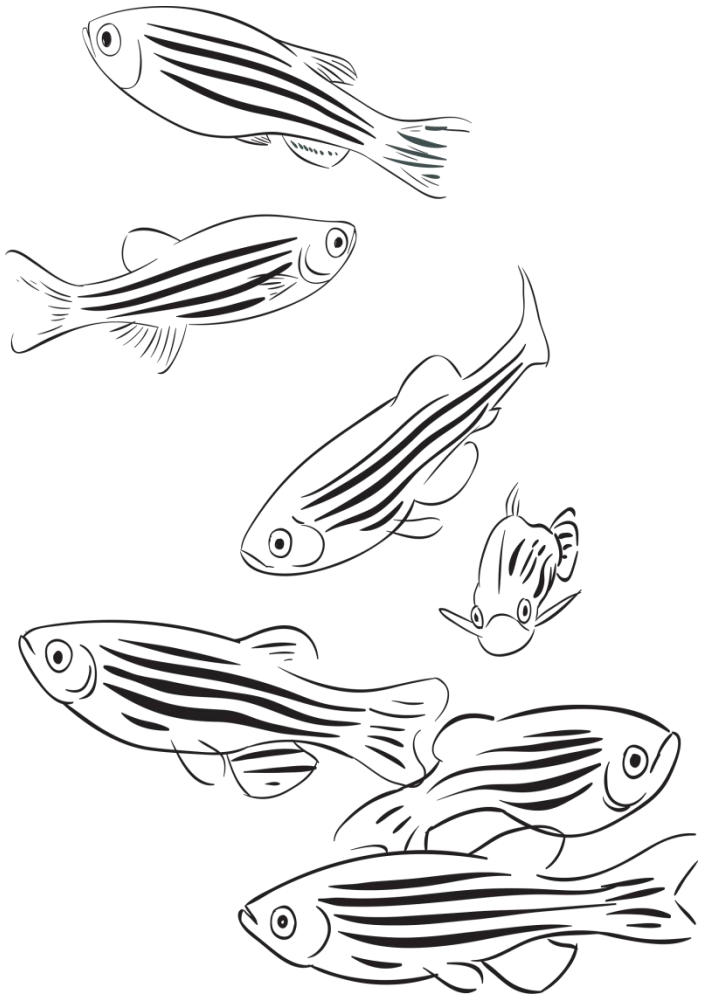

Zebrafish

e Toxicidade

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Zhang C et al. Chemosphere. (2017)

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Environ Pollut. 2019 Aug 6;254(Pt B):112997. doi: 10.1016/j.envpol.2019.112997. [Epub ahead of print]
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Environ Pollut. 2019 Jul 9;254(Pt B):112861. doi: 10.1016/j.envpol.2019.07.029. [Epub ahead of print]
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Data Brief. 2019 Aug 5;25:104351. doi: 10.1016/j.dib.2019.104351. eCollection 2019 Aug.
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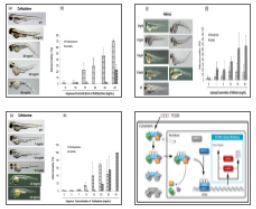

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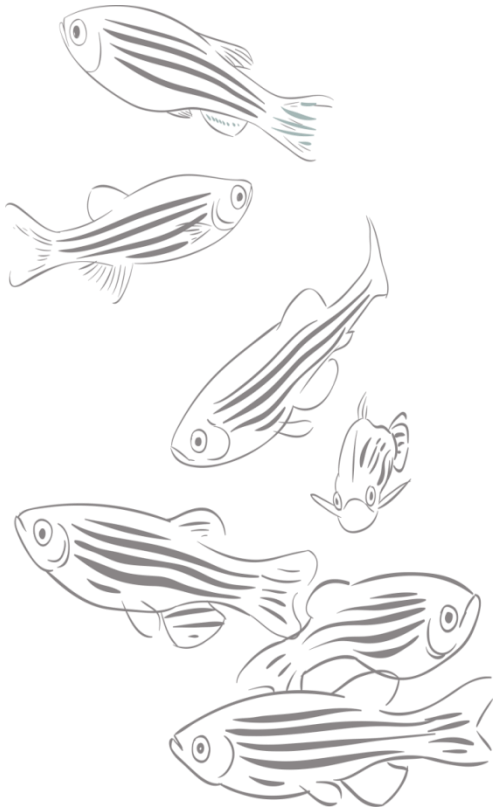

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J Control Release. 2019 Aug 22. pii: S0168-3659(19)30506-1. doi: 10.1016/j.jconrel.2019.08.022. [Epub ahead of print] **Review**.
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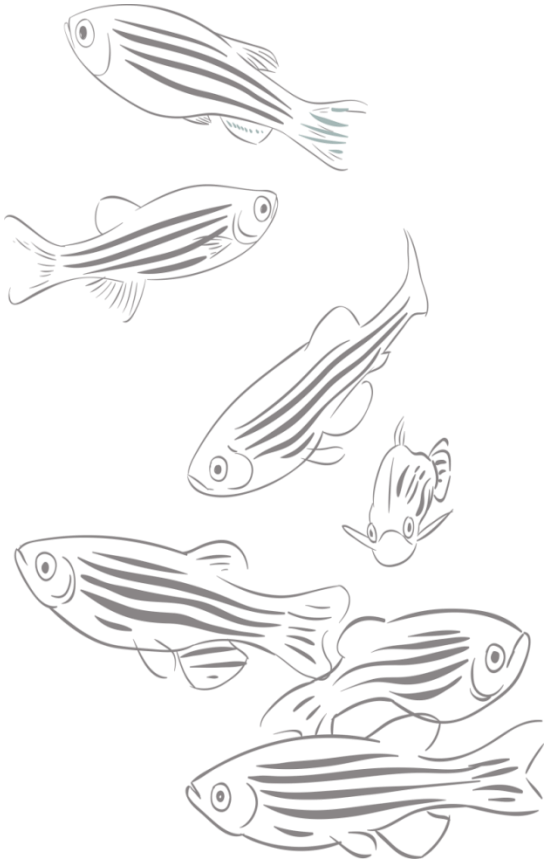

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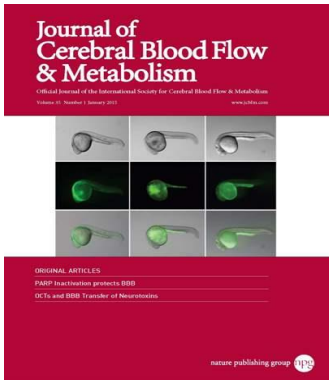

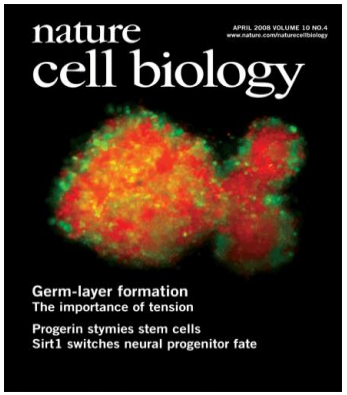

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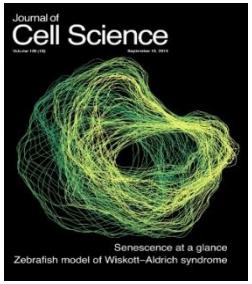

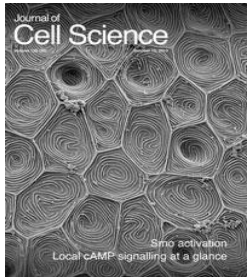

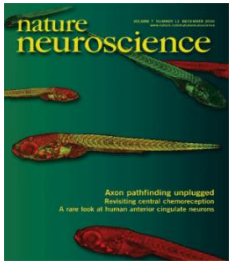

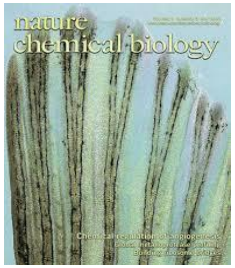

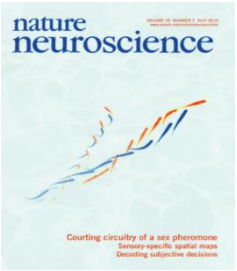

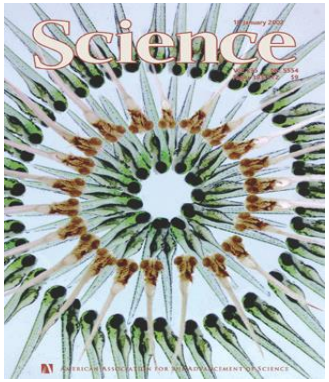

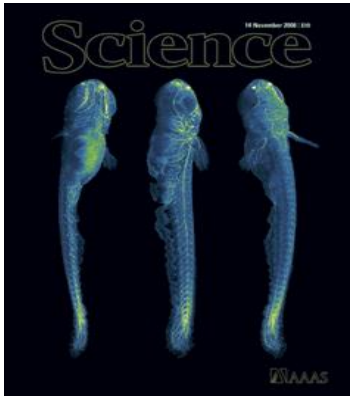

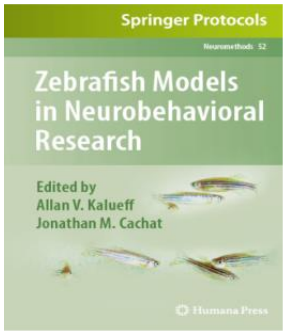
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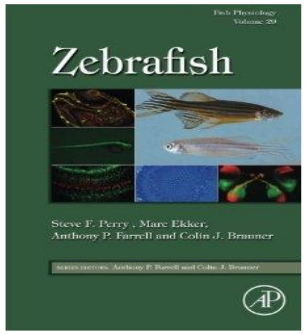
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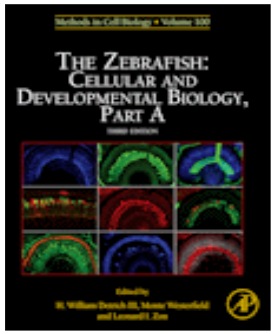
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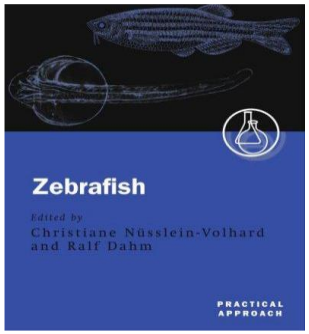
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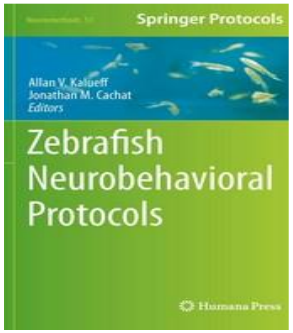
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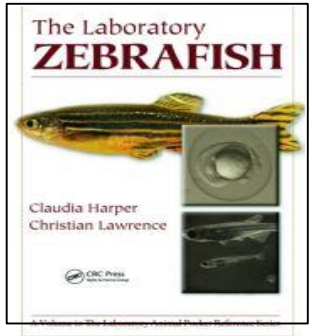
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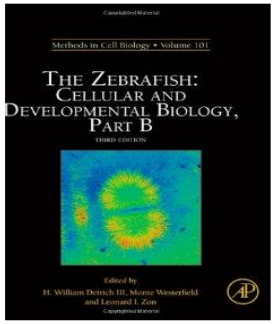
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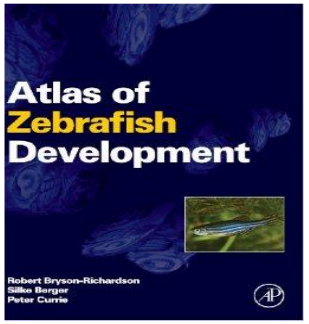
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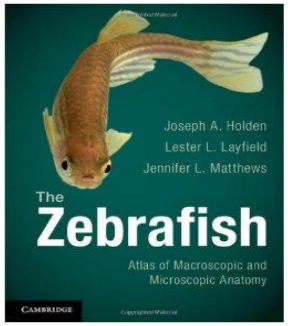
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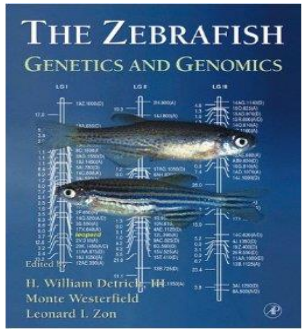
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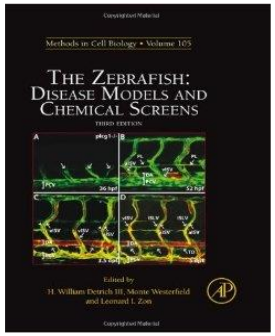
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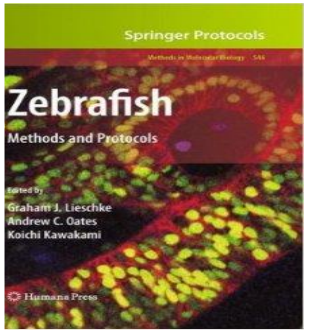
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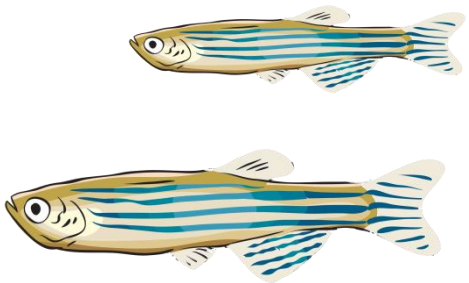

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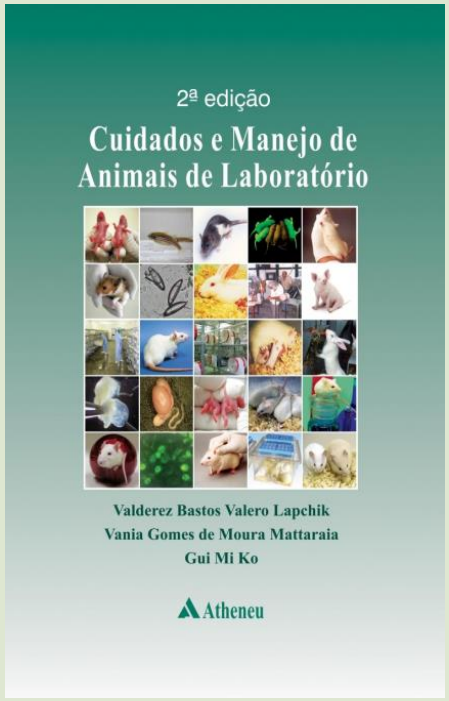

BIOTECNOLOGIA E SANIDADE DE ORGANISMOS AQUÁTICOS

ISBN 978-65-80723-00-3

Editora ABRAPOA

O uso do teleósteo *Danio rer*io como organismos modelo em estudo de sanidade aquícola

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Carla Lima
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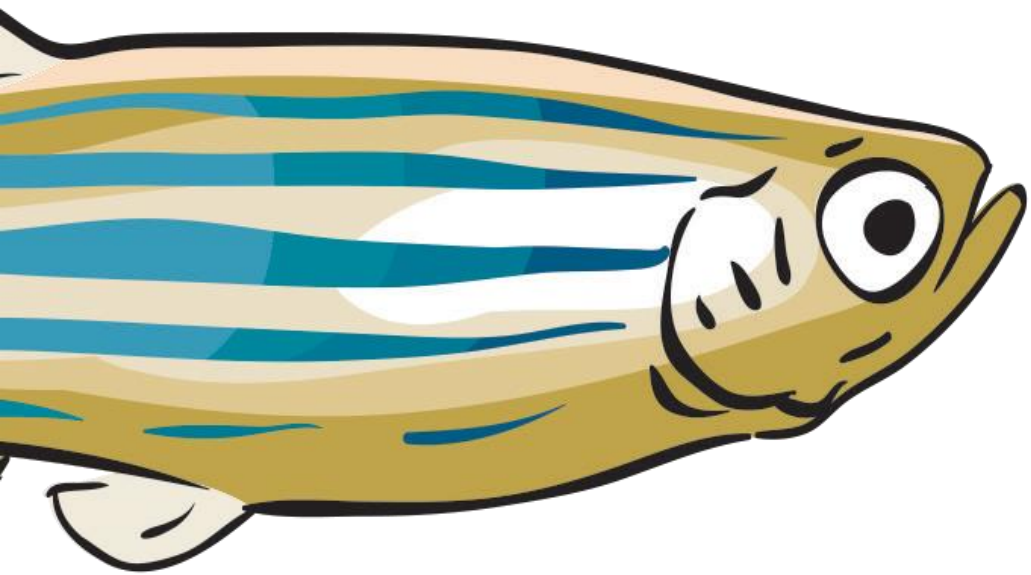


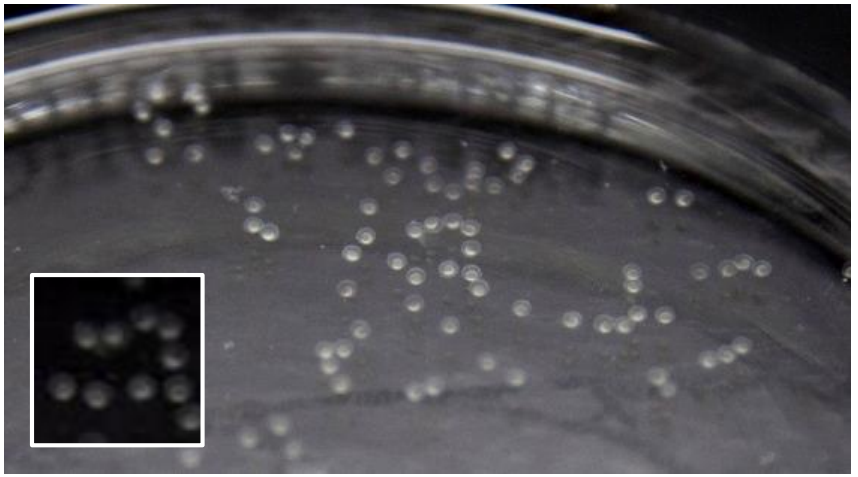
Vantagens como modelo experimental

Zebrafish: vantagens como modelo experimental

1. Alta taxa reprodutiva
2. Embriões transparentes
3. Rápido desenvolvimento
4. Pequeno porte
5. Uso na experimentação de todos os estágios de vida
6. Fácil manipulação
7. Econômicos para criação
8. Genoma sequenciado
9. Métodos e estratégias de avaliação genética e embriológica são aplicados com facilidade ao modelo
10. Importante número de mutantes

Zebrafish: vantagens como modelo experimental

1. Alta taxa reprodutiva: 100 a 200 embriões/dia



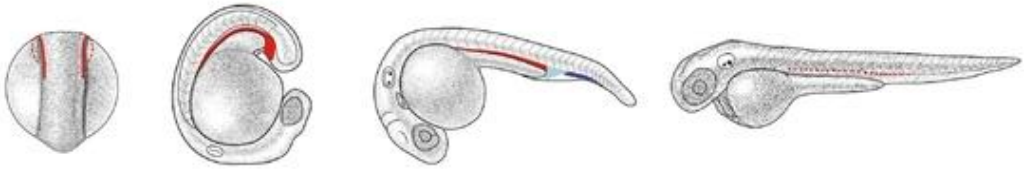
Zebrafish: vantagens como modelo experimental

2. Embriões transparentes

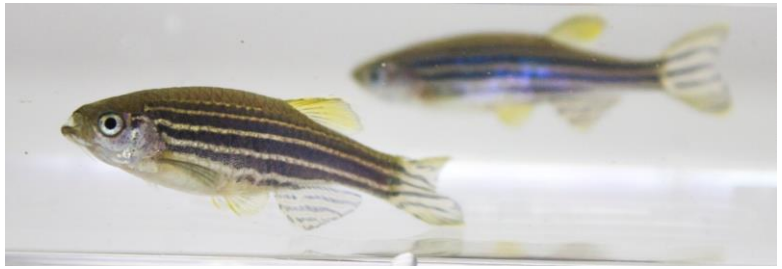
Zebrafish: vantagens como modelo experimental

3. Rápido desenvolvimento

48 a 72 horas: ovo ➡ larva



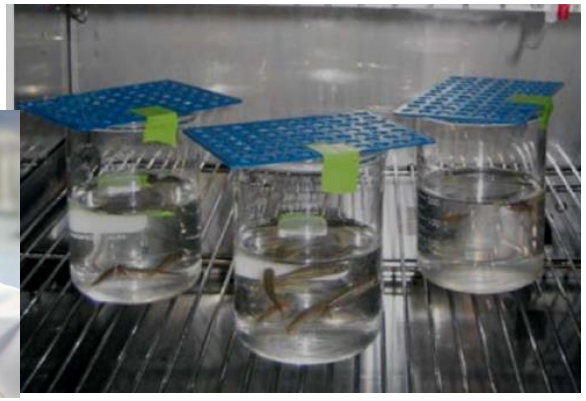
4. Pequeno porte: 3 a 5 cm quando adulto



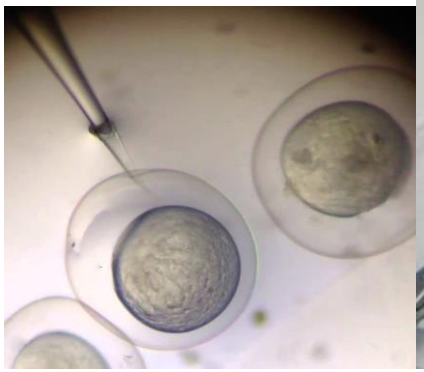
Zebrafish: vantagens como modelo experimental

6. Fácil manipulação

a) imersão do animal em uma solução com o agente a ser investigado



b) administração de substâncias pode ser feita por via oral, intramuscular, intravenosa e intraperitoneal



Zebrafish: vantagens como modelo experimental

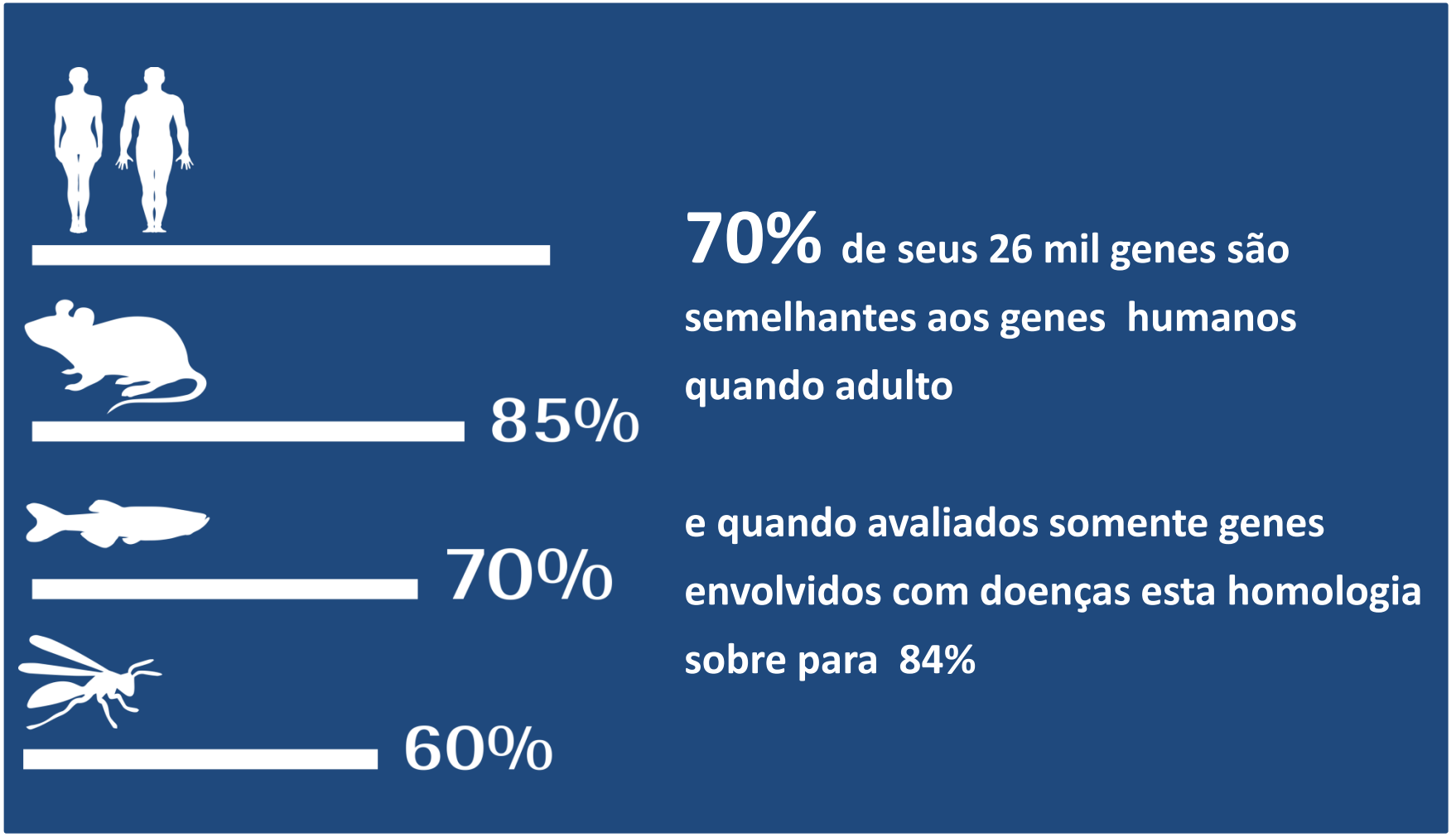
7. Econômicos para criação (recursos e objetivos)

		ZEBRAFISH	CAMUNDONGO
MANUTENÇÃO DIÁRIA	-	R$ 0,60	R$ 8,00

FONTE JOSÉ XAVIER NETO / LNBIO, MONICA RYFF VIANNA / PUC-RS E DENIS ROSEMBERG / UNCHAPECÓ

Zebrafish: vantagens como modelo experimental

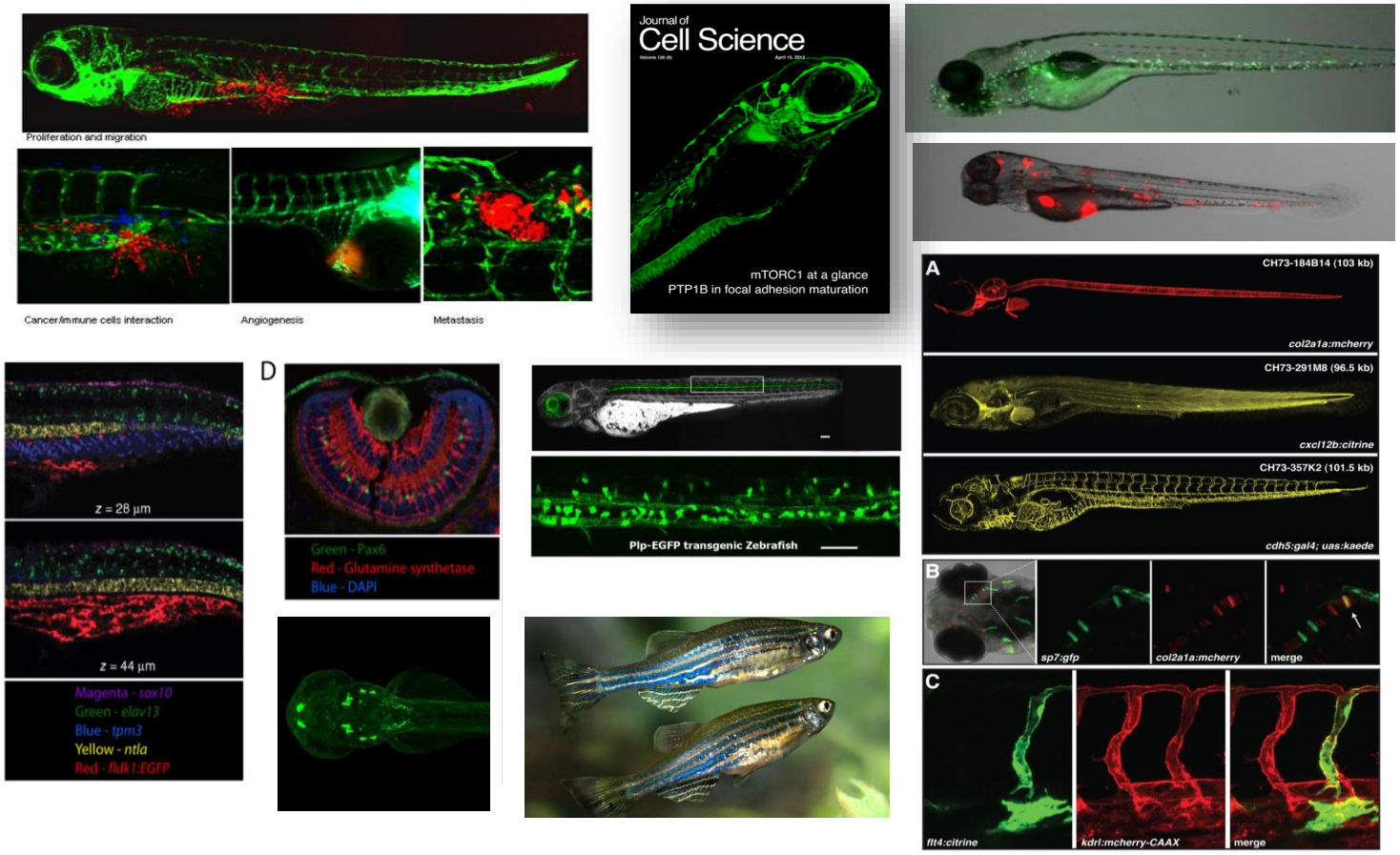
8. Genoma sequenciado: **70** % de seus 26 mil genes são semelhantes aos genes humanos

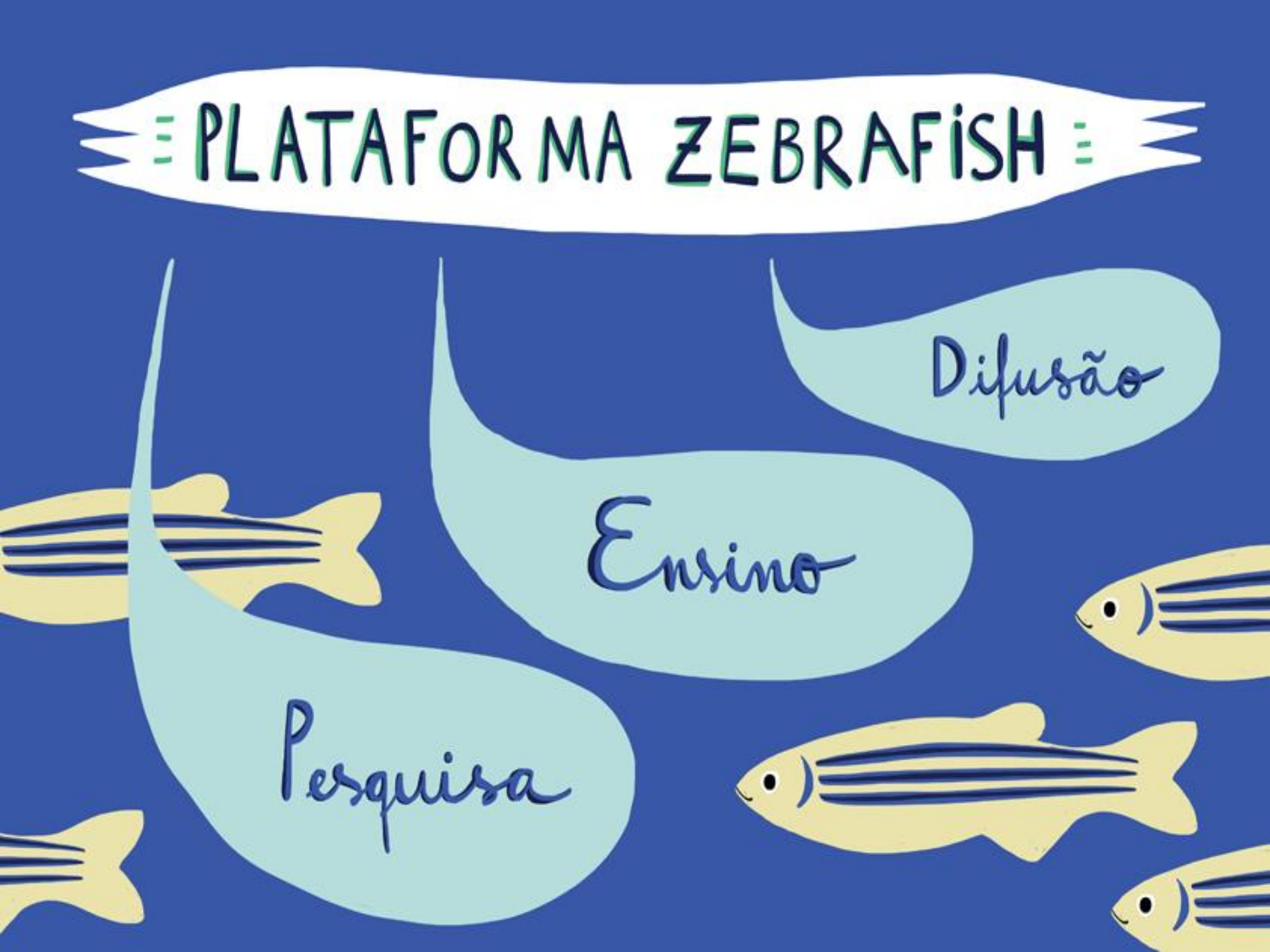


Zebrafish: vantagens como modelo experimental

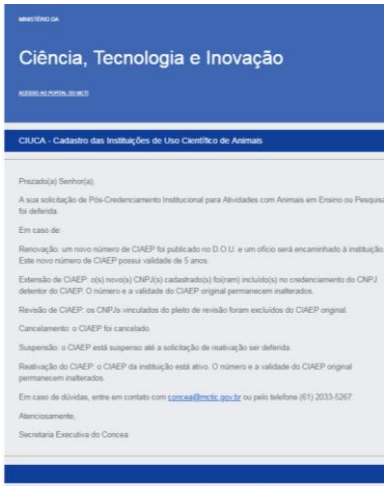
9. Métodos e estratégias de avaliação genética e embriológica são aplicados com facilidade ao modelo

10. O desenvolvimento de técnicas especiais de clonagem, mutagênese e transgênese permite a identificação de um número importante de mutantes




PLATAFORMA ZEBRAFISH
Difusão
Ensino
Pesquisa

Plataforma Zebrafish



Ciência, Tecnologia e Inovação

CIUCA - Cadastro das Instituições de Uso Científico de Animais

Prezado(a) Senhor(a),

A sua solicitação de Pós-Credenciamento Institucional para Atividades com Animais em Ensino ou Pesquisa foi deferida.

Em caso de:

Renovação: um novo número de CIAEP foi publicado no D.O.U. e um ofício será encaminhado à instituição. Este novo número de CIAEP possui validade de 5 anos.

Extensão de CIAEP: o(s) novo(s) CNPJ(s) cadastrado(s) foi(ram) incluído(s) no credenciamento do CNPJ detentor do CIAEP. O número e a validade do CIAEP original permanecem inalterados.

Revisão de CIAEP: os CNPJs vinculados do pleito de revisão foram excluídos do CIAEP original.

Cancelamento: o CIAEP foi cancelado.

Suspensão: o CIAEP está suspenso até a solicitação de reativação ser deferida.

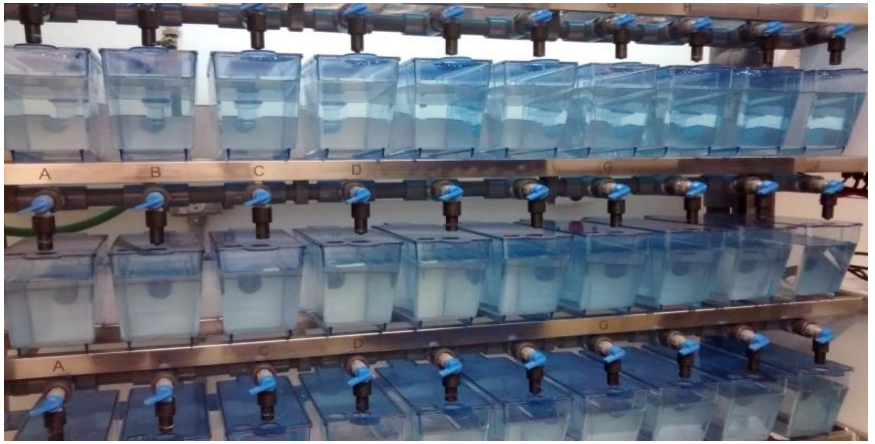
Reativação do CIAEP: o CIAEP da instituição está ativo. O número e a validade do CIAEP original permanecem inalterados.

Em caso de dúvidas, entre em contato com concea@mctic.gov.br ou pelo telefone (61) 2033-5267.

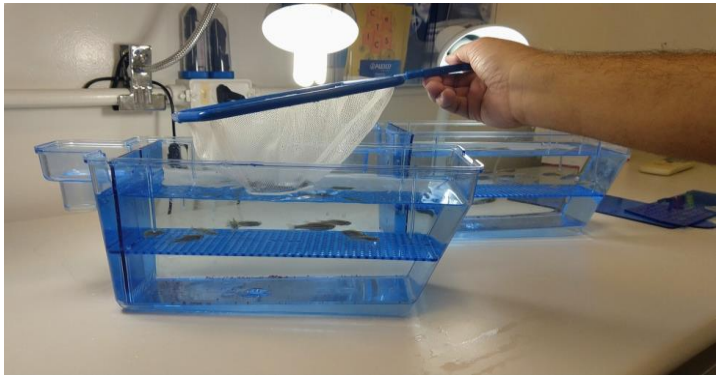
Atenciosamente,

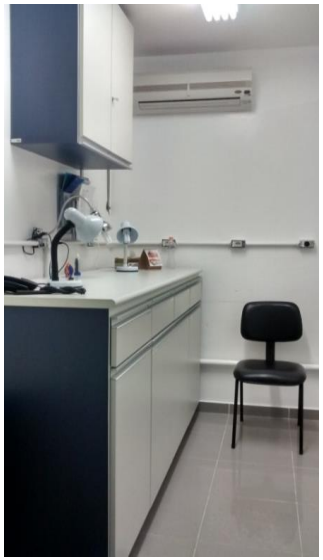
Secretaria Executiva do Concea

Plataforma Zebrafish

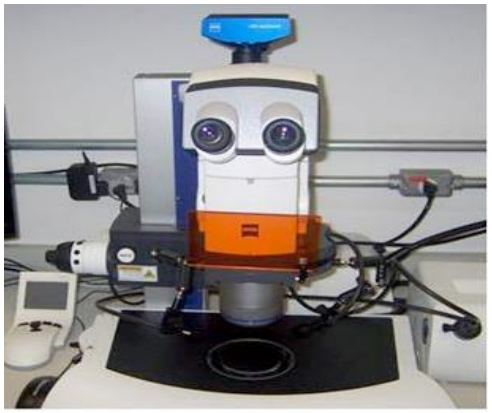


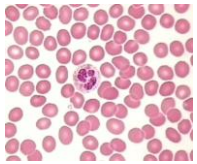
Plataforma Zebrafish

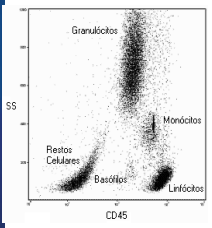


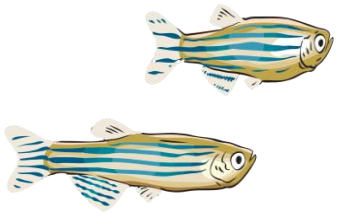


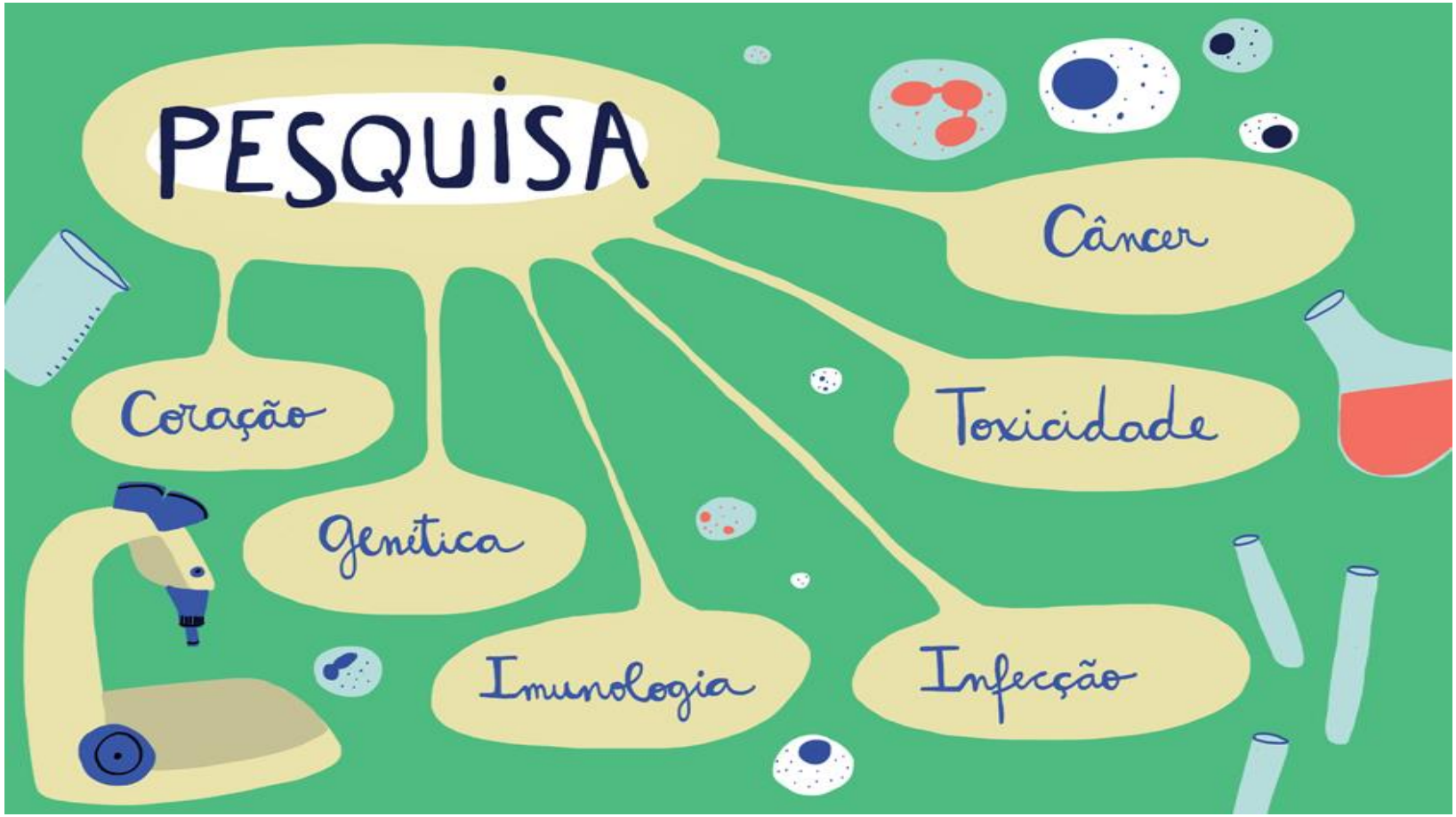
Plataforma Zebrafish

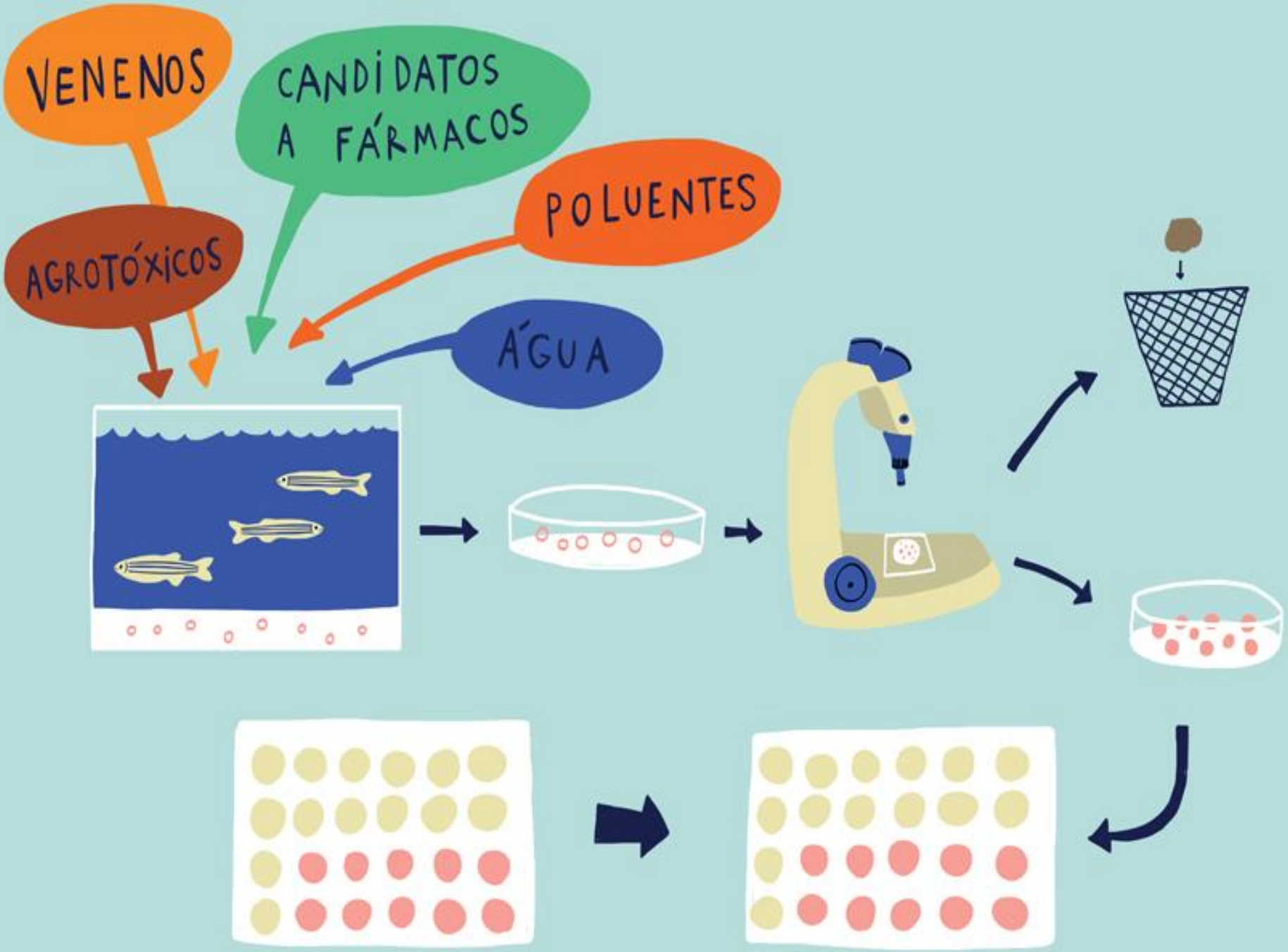









PESQUISA
Câncer
Coração
Toxicidade
Genética
Imunologia
Infecção


VENENOS
CANDIDATOS A FÁRMACOS
POLUENTES
AGROTÓXICOS
ÁGUA

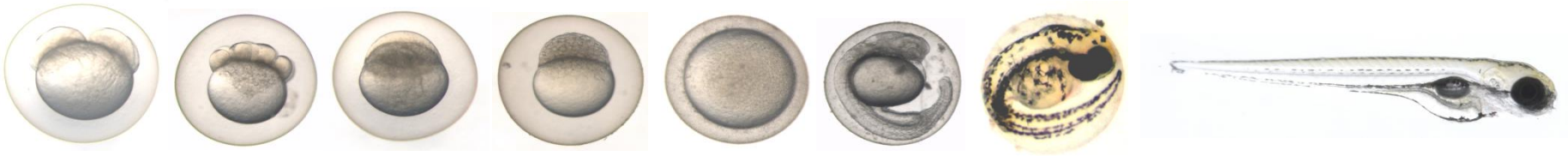
Toxicidade

Teste Toxicidade em embriões de peixes

“Zebrafish Embryotoxicity Test” - ZET

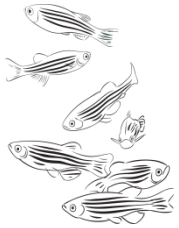
Danio rerio

OECD 236



Características Importantes para o Teste ZET (*Danio rerio*)

* Fertilização externa
* Baixo custo
* Necessidade de pequenas quantidades de moléculas
* Tempo de exposição reduzido
* Embriões e larvas transparentes, o que facilita a visualização de seu desenvolvimento
* Ciclo de vida curto
* Rápido desenvolvimento embrionário rápido
* Descoberta rápida de fenótipo
* Rastreabilidade genética
* Eticamente aceitável
*Grande sensibilidade quando exposto a produtos químicos por ser capaz de absorver de forma rápida os compostos que são diretamente adicionados na água e acumulá-los em diferentes tecidos (principalmente no Sistema Nervoso Central)
* Permite a avaliação de respostas crônicas, teratogenicidade, cardiotoxicidade, genotoxicidade, distúrbios musculares e ósseos
* Toxicidade neurocomportamental, toxicidade específica de órgãos (ex. hepatotoxicidade e nefrotoxicidade), toxicidade reprodutiva, ruptura endócrina, estresse oxidativo e avaliação de risco ambiental



OECD/OCDE **236**

Adopted:
26 July 2013

OECD GUIDELINES FOR THE TESTING OF CHEMICALS

PRINCIPLE OF THE TEST

3. Newly fertilised zebrafish eggs are exposed to the test chemical for a period of 96 hrs. Every 24 hrs, up to four apical observations are recorded as indicators of lethality (6): (i) coagulation of fertilised eggs, (ii) lack of somite formation, (iii) lack of detachment of the tail-bud from the yolk sac, and (iv) lack of heartbeat. At the end of the exposure period, acute toxicity is determined based on a positive outcome in any of the four apical observations recorded, and the LC_{50} is calculated.

Embriões de zebrafish recém-fertilizados (no estágio da blástula ≤3 hpf) são expostos a produtos químicos em teste **por um período de 96 horas**.
A cada 24 horas, até quatro observações são registradas **como indicadores de letalidade**: (1) coagulação de ovos fertilizados, (2) falta de formação de somito, (3) falta de descolamento da cauda e (4) ausência de batimento cardíaco.

Fig. 2: **Scheme of the zebrafish embryo acute toxicity test procedure (from left to right):** production of eggs, collection of the eggs, pre-exposure immediately after fertilisation in glass vessels, selection of fertilised eggs with an inverted microscope or binocular and distribution of fertilised eggs into 24-well plates prepared with the respective test concentrations/controls, n = number of eggs required per test concentration/control (here 20), hpf = hours post-fertilisation.

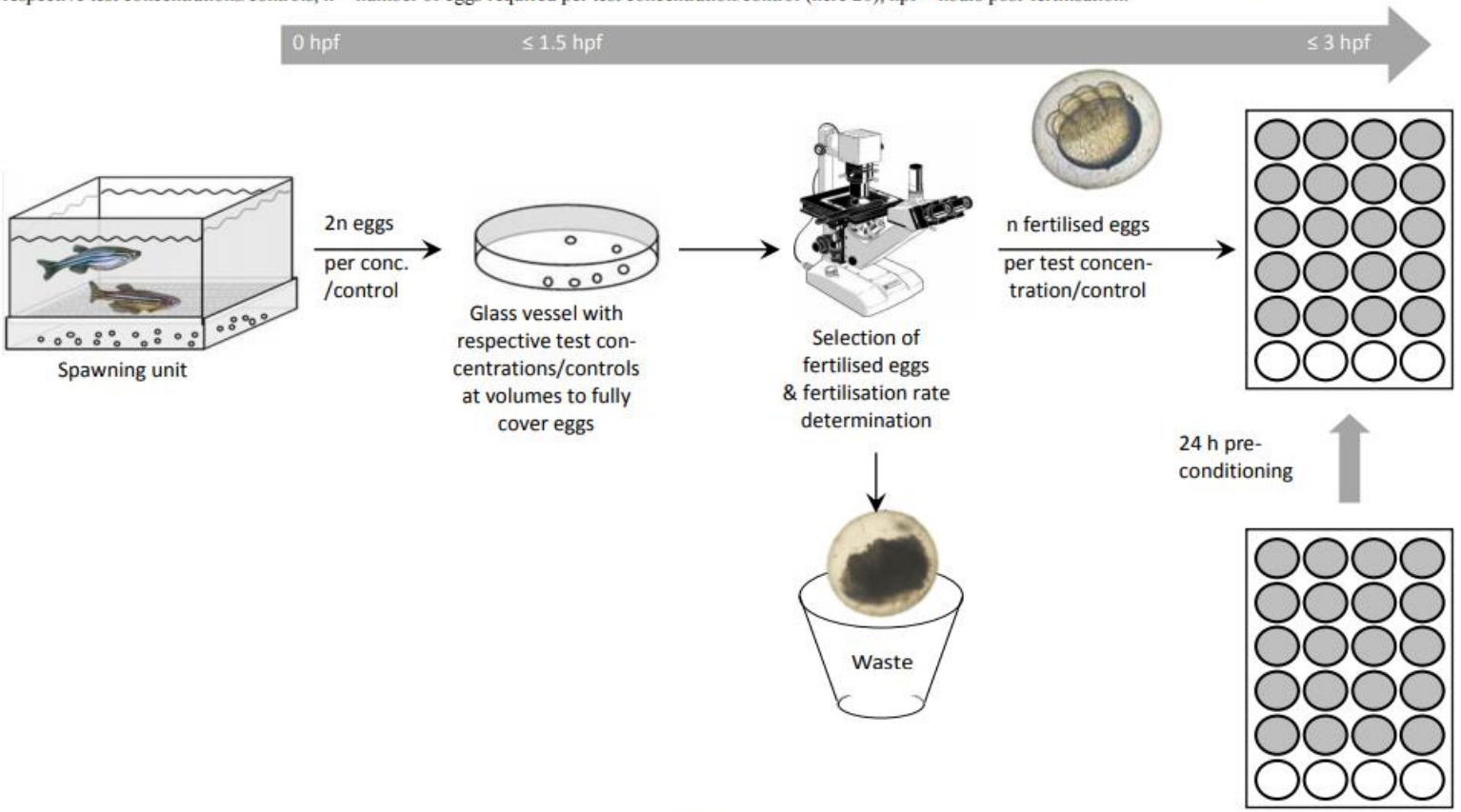


Table 1. Apical observations of acute toxicity in zebrafish embryos 24 - 96 hrs post fertilisation.

	Exposure times			
	24 hrs	48 hrs	72 hrs	96 hrs
Coagulated embryos	+	+	+	+
Lack of somite formation	+	+	+	+
Non-detachment of the tail	+	+	+	+
Lack of heartbeat		+	+	+

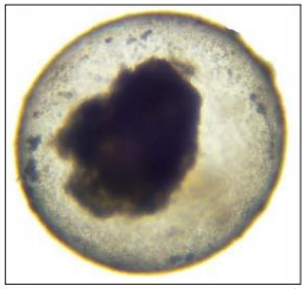


Fig. 1: **Coagulation of the embryo**: Under bright field illumination, coagulated zebrafish embryos show a variety of intransparent inclusions.

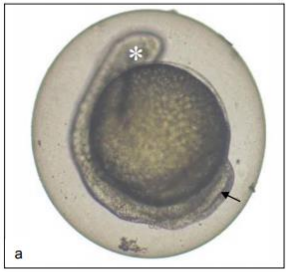


Fig. 3: **Non-detachment of the tail** bud in lateral view (a: →; 96 hrs old zebrafish embryo). Note also the lack of the eye bud (*).

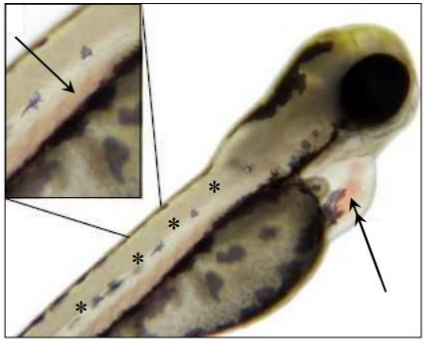


Fig. 4: **Lack of heartbeat** is, by definition, difficult to illustrate in a micrograph. Lack of heartbeat is indicated by non-convulsion of the heart (double arrow). Immobility of blood cells in, *e.g.*, the aorta abdominalis (→ in insert) is not an indicator for lack of heartbeat. Note also the lack of somite formation in this embryo (*, homogenous rather than segmental appearance of muscular tissues). The observation time to record an absence of heartbeat should be at least of one minute with a minimum magnification of 80×.

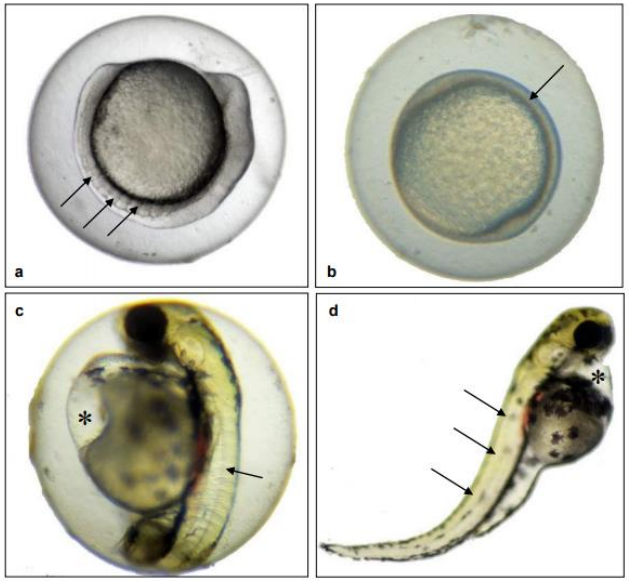
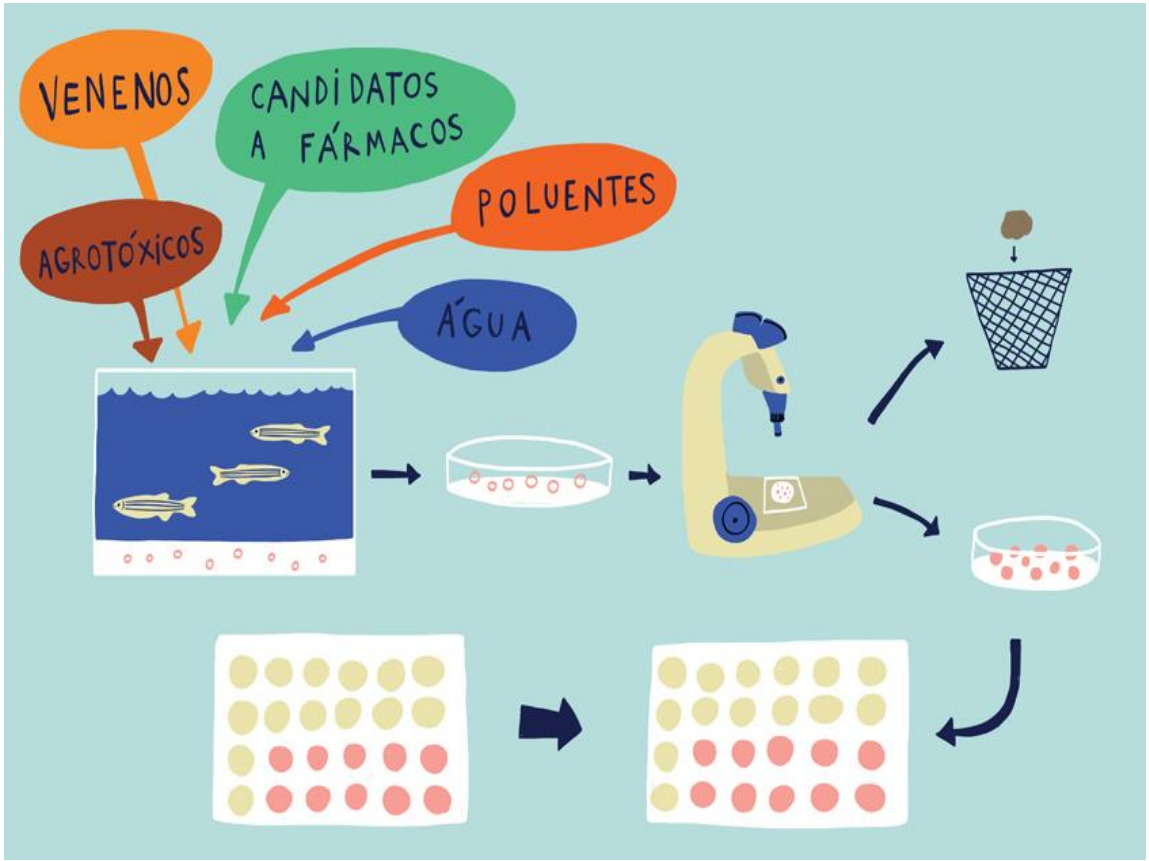
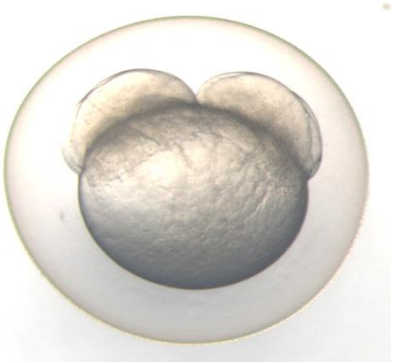
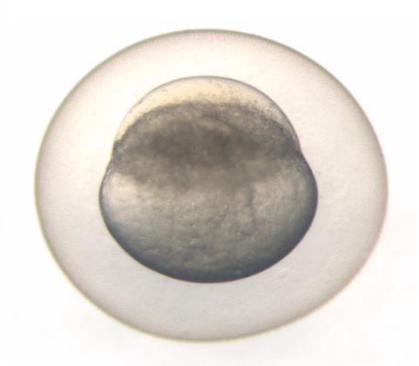


Fig. 2: **Lack of somite formation**: Although retarded in development by approx. 10 hrs, the 24 hrs old zebrafish embryo in (a) shows well-developed somites (→), whereas the embryo in (b) does not show any sign of somite formation (→). Although showing a pronounced yolk sac oedema (*), the 48 hrs old zebrafish embryo in (c) shows distinct formation of somites (→), whereas the 96 hrs old zebrafish embryo depicted in (d) does not show any sign of somite formation (→). Note also the spinal curvature (scoliosis) and the pericardial oedema (*) in the embryo shown in (d).

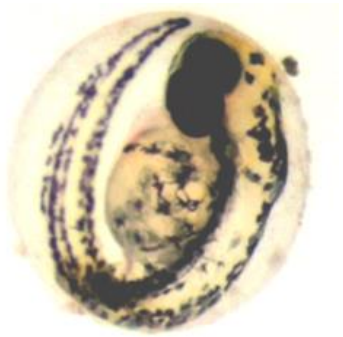
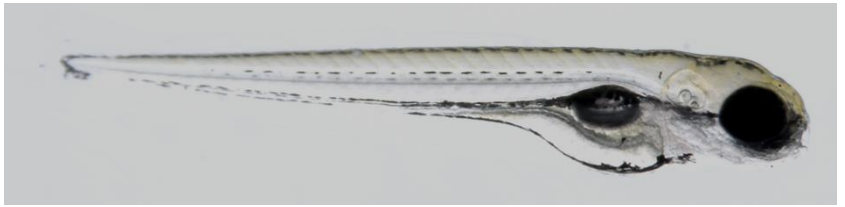
Plataforma Zebrafish





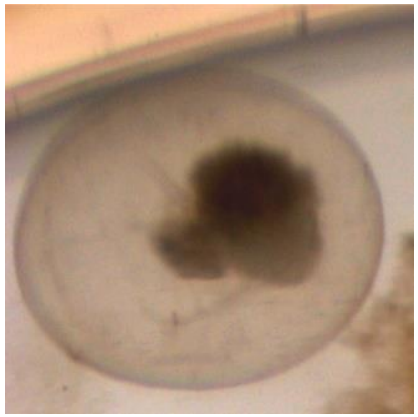


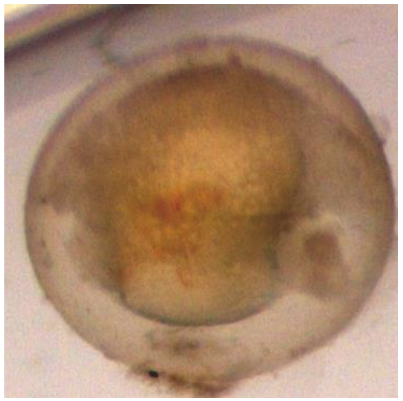
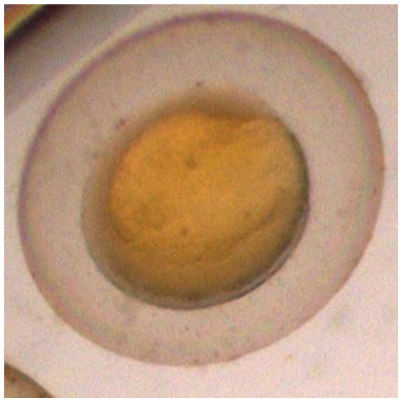
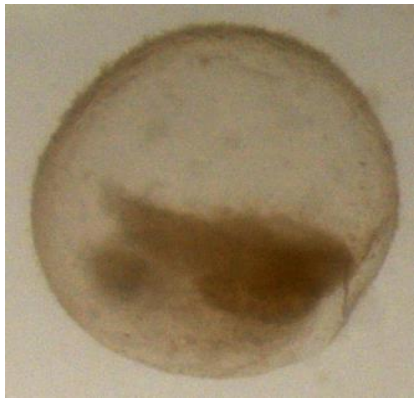
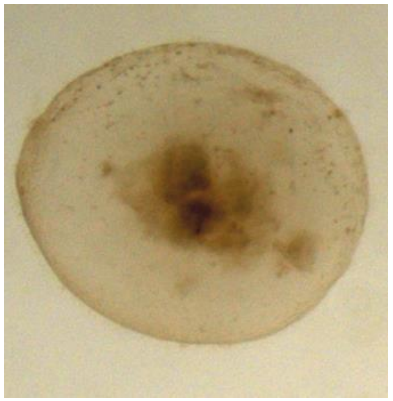
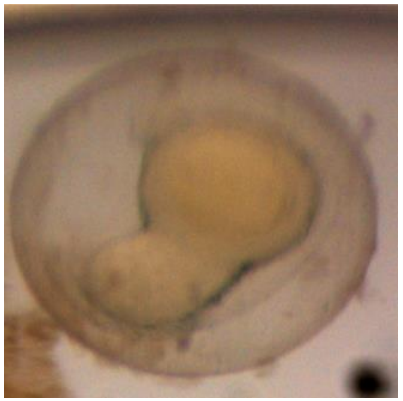
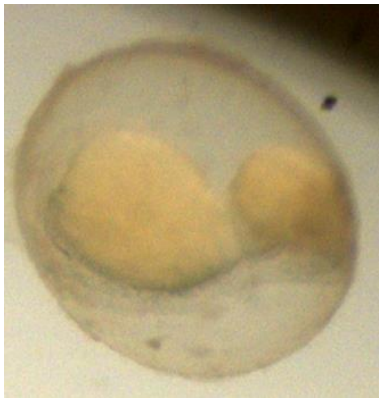


Plataforma Zebrafish

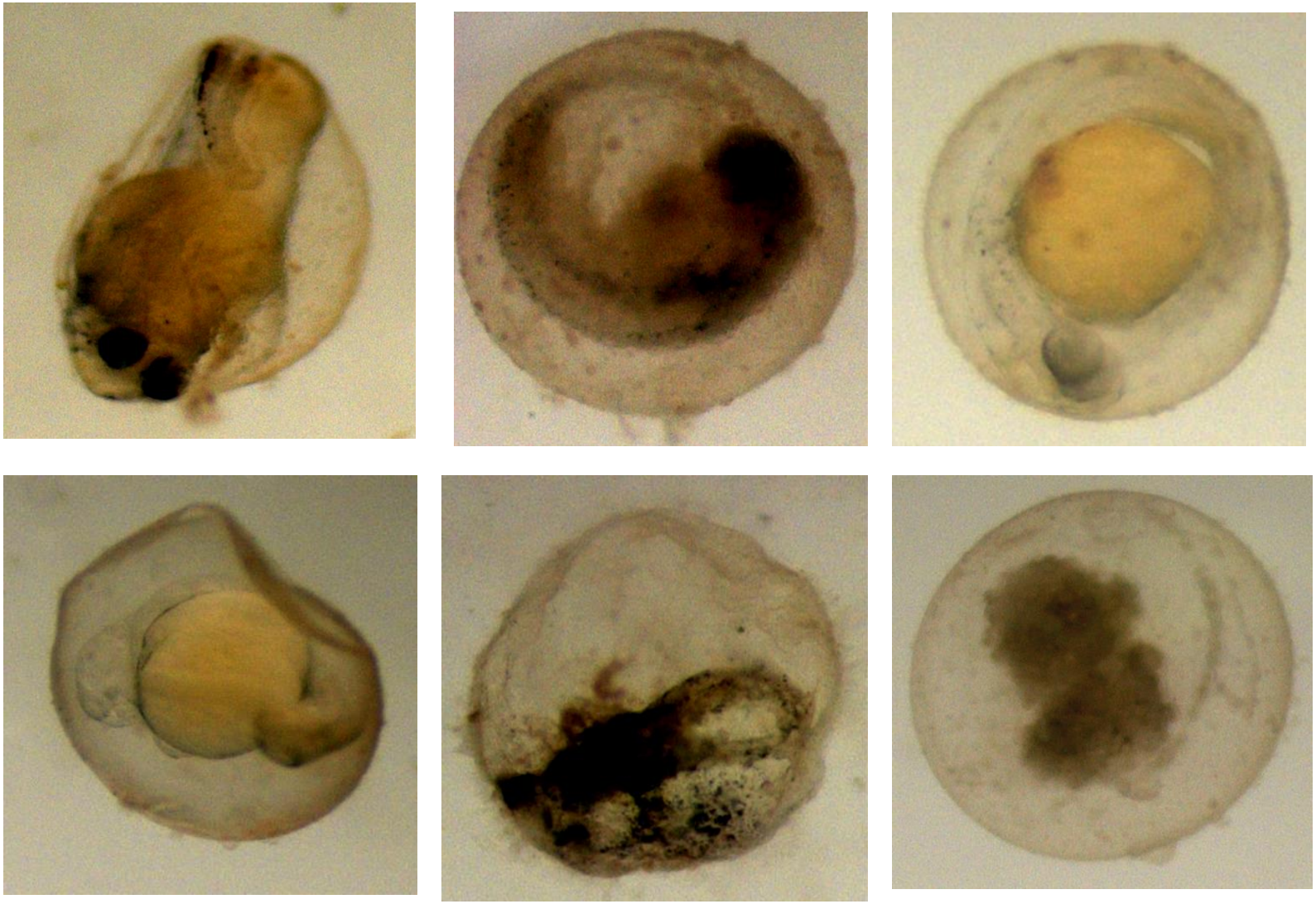
Toxicidade



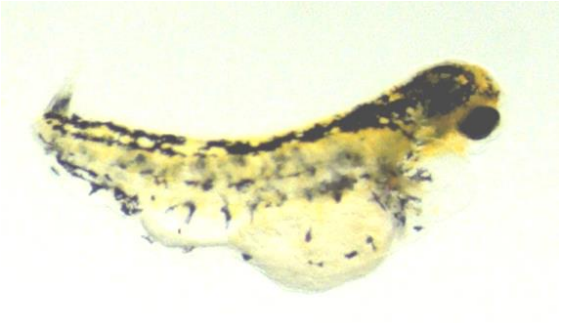
Plataforma Zebrafish

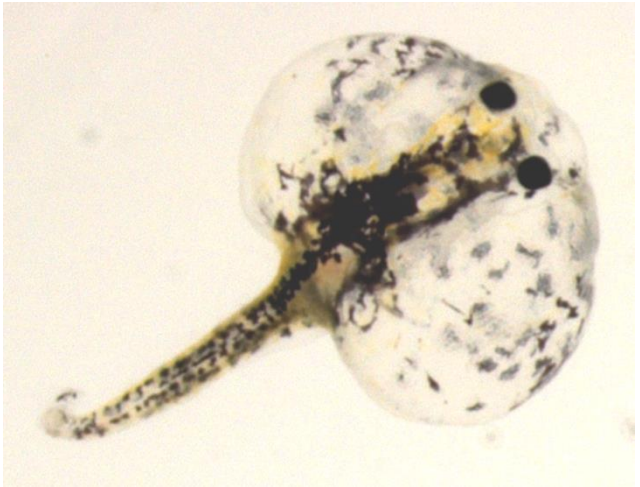
Toxicidade



Plataforma Zebrafish

Toxicidade

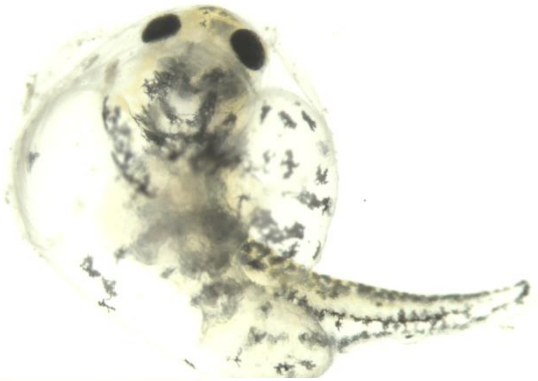




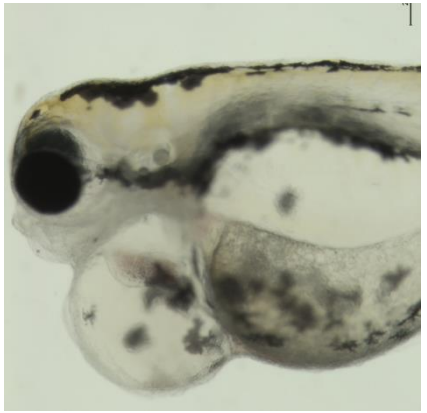
Toxicidade











Plataforma Zebrafish


Muito obrigada!!
monica.lopesferreira@butantan.gov.br