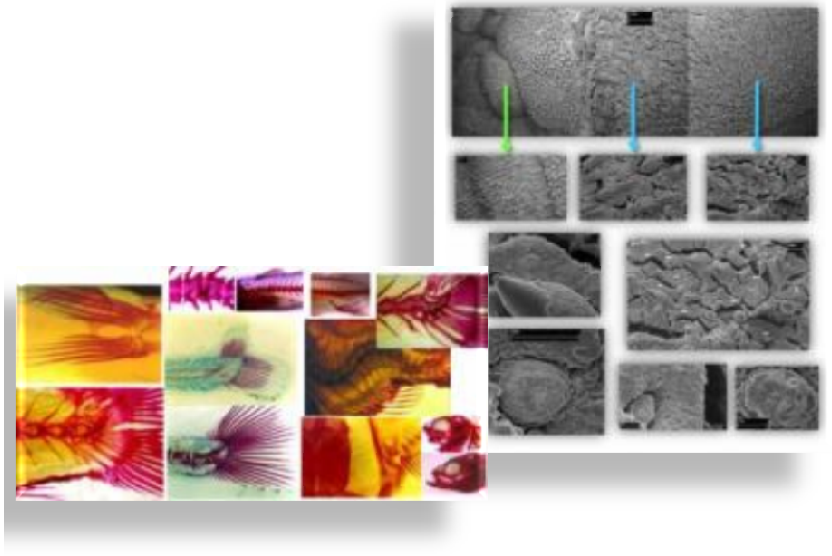
Università di Roma
Tor Vergata

OMICS International
International Conference on
Aquaculture & Fisheries
Park Regis North Quay, Brisbane, Australia July 20-22, 2015

Sensorial and skeletal ontogenesis: an useful tool for the optimization of larval rearing of new candidate species for aquaculture ….. *and not only!*



Clara Boglione

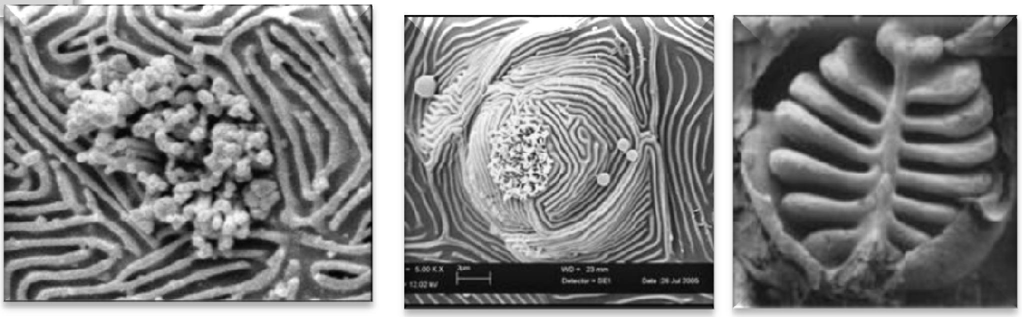
University of Rome Tor Vergata, Italy

….. *and not only!*

- Environmental monitoring
- Investigation on other threats than fishery on wild stocks
- Ecotoxicology
- Evolutionary aspect (i.e., domestication process; global climatic changes)
- Basic research: autoecology of different species and life stages
- Aquaculture of new candidate species

Basic research: autoecology of different species and life stages

Sense organs in Teleosts



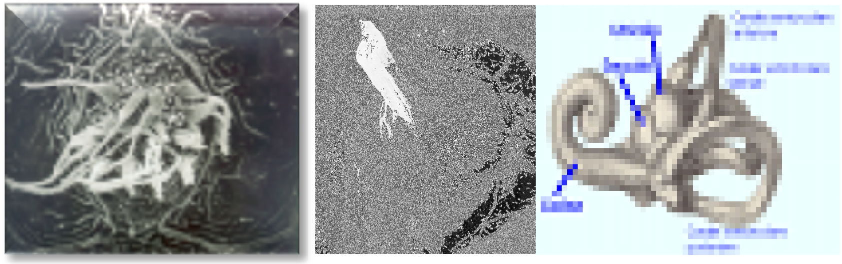
Organs for chemoreception:

taste buds

olfaction

solitary chemoreceptive cells

O_2- CO_2 receptors ??

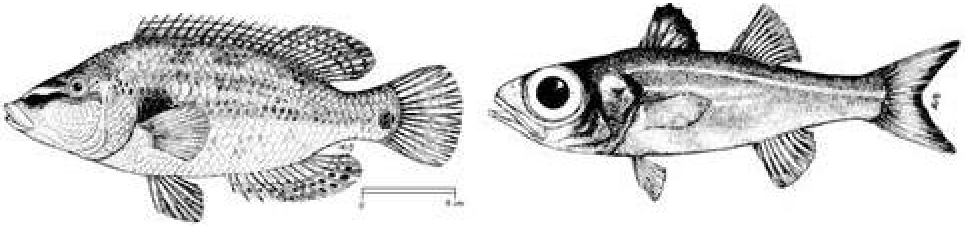


Organs for mechanoreception:

lateral line system

inner ear

cutaneous nociceptor ??

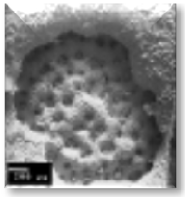


Organs for visual reception:

eye

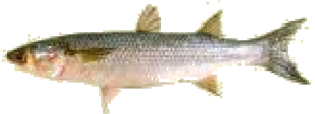
Organs for electroreception:

ampullary organs, tuberous organs



Environmental monitoring

Liza ramada

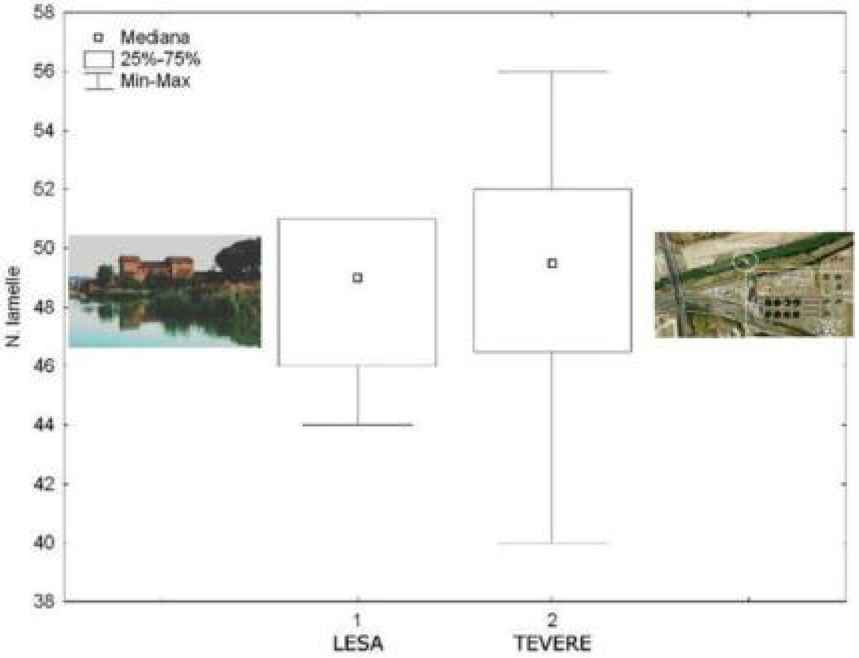


Boglione: unpub. data

7 mullets from the LESA' artificial lake

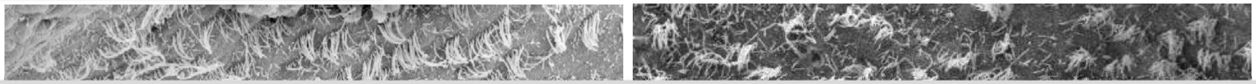
15 mullets from Tiber River (Rome) sampled downstream a municipal wastewater treatment plant outfall

- Significant (p<0,5) differences in the lamellae'numbers between the two groups
- Spearman's rank correlation coefficient highlighted **(R = 0.05)** no correlations between fish sizes and lamellae numbers, either between size of olfactory rosetta and lamellae number.



Environmental monitoring

Boglione: unpub. data



*Co
N
N m

Use of olfactory organ as benchmark

- for monitoring program?
- for sub lethal toxicological text for chemical pollutants ?

Sam l

conce A.,

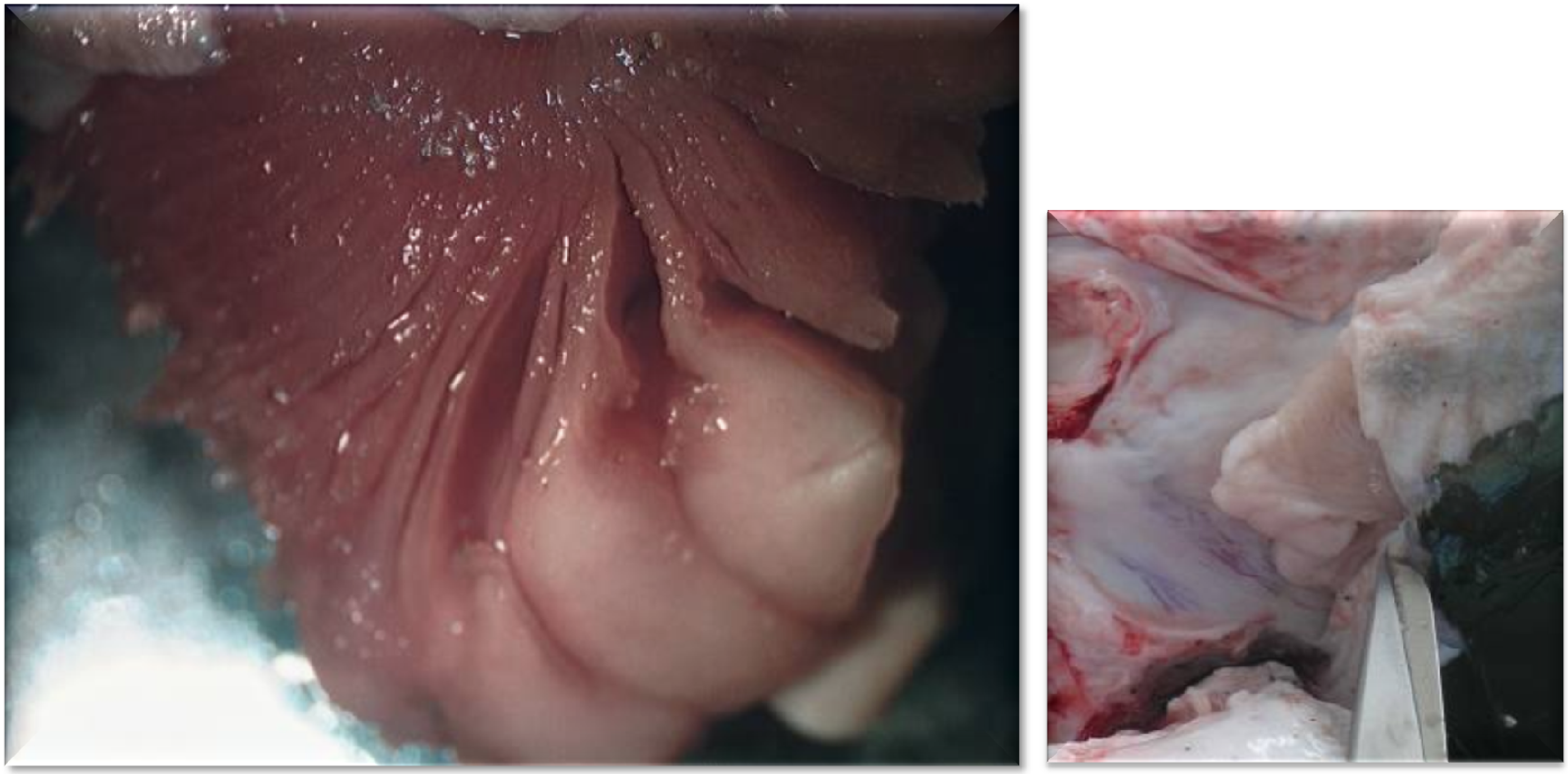
2002. Dis. Aquat. Org. Vol. 51: 37–47)

Investigation on other threats than fishery on wild stocks

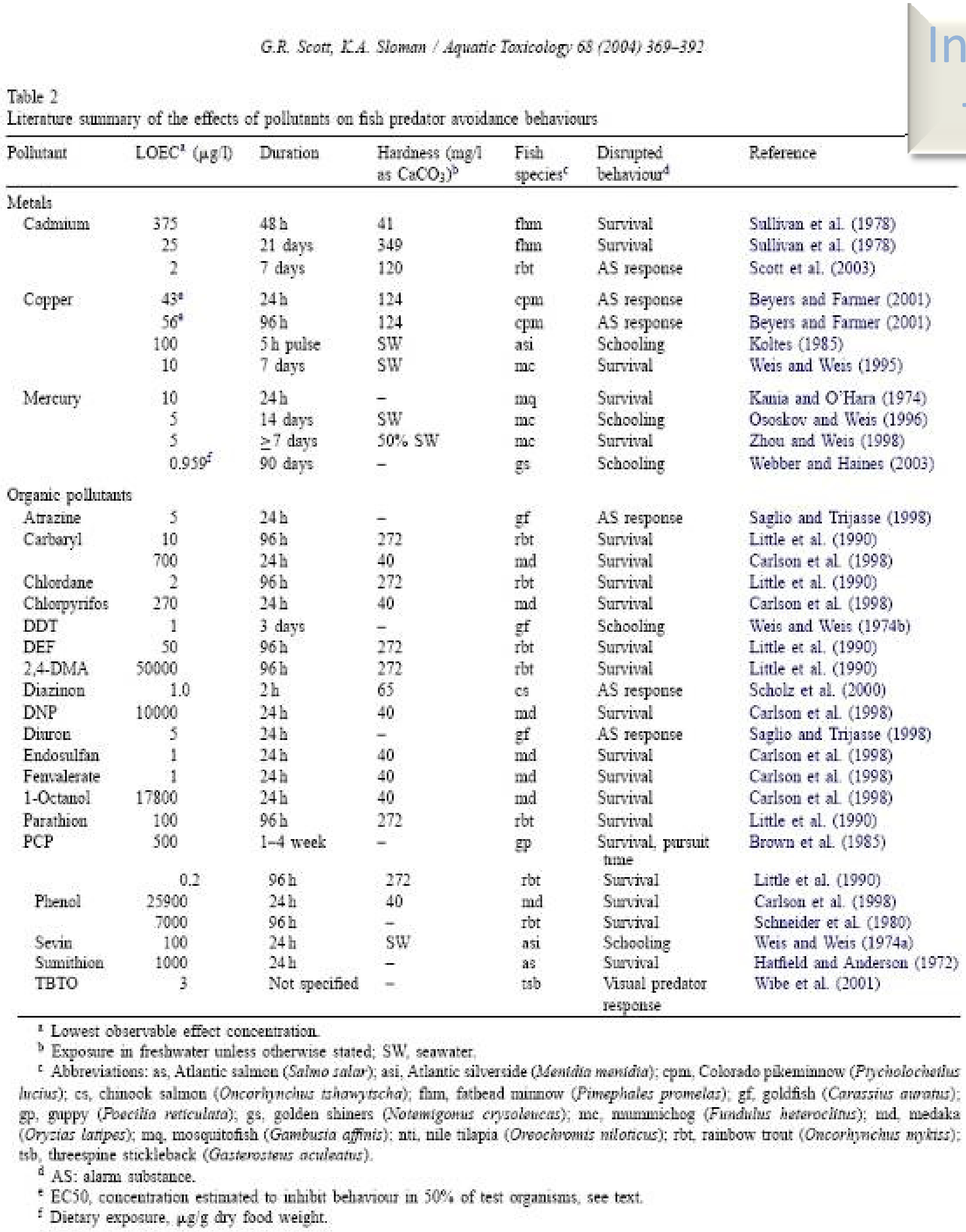
Chemical spying

Boglione: unpub. data

Thunnus thynnus thynnus
2.3 m SL



Investigation on other threats than fishery on wild stocks



G.R. Scott, K.A. Sloman / Aquatic Toxicology 68 (2004) 369–392

Table 2
Literature summary of the effects of pollutants on fish predator avoidance behaviours

Pollutant	LOEC[a] (μg/l)	Duration	Hardness (mg/l as $CaCO_3$)[b]	Fish species[c]	Disrupted behaviour[d]	Reference
Metals						
Cadmium	375	48 h	41	fhm	Survival	Sullivan et al. (1978)
	25	21 days	349	fhm	Survival	Sullivan et al. (1978)
	2	7 days	120	rbt	AS response	Scott et al. (2003)
Copper	43[e]	24 h	124	cpm	AS response	Beyers and Farmer (2001)
	56[e]	96 h	124	cpm	AS response	Beyers and Farmer (2001)
	100	5 h pulse	SW	asi	Schooling	Koltes (1985)
	10	7 days	SW	mc	Survival	Weis and Weis (1995)
Mercury	10	24 h	–	mq	Survival	Kania and O'Hara (1974)
	5	14 days	SW	mc	Schooling	Ososkov and Weis (1996)
	5	≥7 days	50% SW	mc	Survival	Zhou and Weis (1998)
	0.959[f]	90 days	–	gs	Schooling	Webber and Haines (2003)
Organic pollutants						
Atrazine	5	24 h	–	gf	AS response	Saglio and Trijasse (1998)
Carbaryl	10	96 h	272	rbt	Survival	Little et al. (1990)
	700	24 h	40	md	Survival	Carlson et al. (1998)
Chlordane	2	96 h	272	rbt	Survival	Little et al. (1990)
Chlorpyrifos	270	24 h	40	md	Survival	Carlson et al. (1998)
DDT	1	3 days	–	gf	Schooling	Weis and Weis (1974b)
DEF	50	96 h	272	rbt	Survival	Little et al. (1990)
2,4-DMA	50000	96 h	272	rbt	Survival	Little et al. (1990)
Diazinon	1.0	2 h	65	cs	AS response	Scholz et al. (2000)
DNP	10000	24 h	40	md	Survival	Carlson et al. (1998)
Diuron	5	24 h	–	gf	AS response	Saglio and Trijasse (1998)
Endosulfan	1	24 h	40	md	Survival	Carlson et al. (1998)
Fenvalerate	1	24 h	40	md	Survival	Carlson et al. (1998)
1-Octanol	17800	24 h	40	md	Survival	Carlson et al. (1998)
Parathion	100	96 h	272	rbt	Survival	Little et al. (1990)
PCP	500	1–4 week	–	gp	Survival, pursuit time	Brown et al. (1985)
	0.2	96 h	272	rbt	Survival	Little et al. (1990)
Phenol	25900	24 h	40	md	Survival	Carlson et al. (1998)
	7000	96 h	–	rbt	Survival	Schneider et al. (1980)
Sevin	100	24 h	SW	asi	Schooling	Weis and Weis (1974a)
Sumithion	1000	24 h	–	as	Survival	Hatfield and Anderson (1972)
TBTO	3	Not specified	–	tsb	Visual predator response	Wibe et al. (2001)

[a] Lowest observable effect concentration.

[b] Exposure in freshwater unless otherwise stated; SW, seawater.

[c] Abbreviations: as, Atlantic salmon (*Salmo salar*); asi, Atlantic silverside (*Menidia menidia*); cpm, Colorado pikeminnow (*Ptycholochetlus lucius*); cs, chinook salmon (*Oncorhynchus tshawytscha*); fhm, fathead minnow (*Pimephales promelas*); gf, goldfish (*Carassius auratus*); gp, guppy (*Poecilia reticulata*); gs, golden shiners (*Notemigonus crysoleucas*); mc, mummichog (*Fundulus heteroclitus*); md, medaka (*Oryzias latipes*); mq, mosquitofish (*Gambusia affinis*); nti, nile tilapia (*Oreochromis niloticus*); rbt, rainbow trout (*Oncorhynchus mykiss*); tsb, threespine stickleback (*Gasterosteus aculeatus*).

[d] AS: alarm substance.

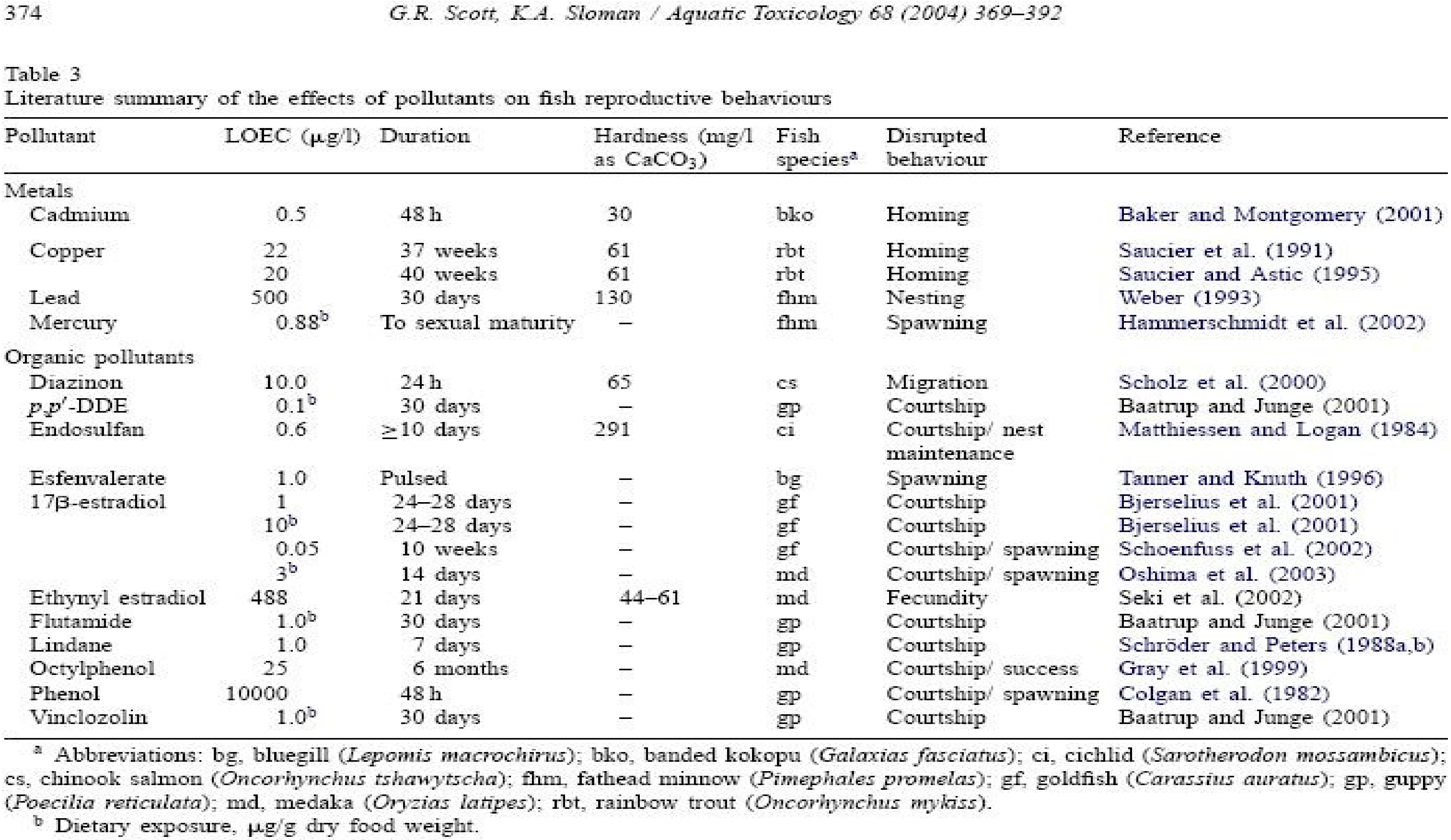
[e] EC50, concentration estimated to inhibit behaviour in 50% of test organisms, see text.

[f] Dietary exposure, μg/g dry food weight.

Effects of pollutants on **fish avoidance behaviours** (*reaction to alarm substances, response to predators, survival, schooling*)

Investigation on other threats than fishery on wild stocks

Effects of heavy metals and organic pollutants on **fish reproductive behaviours** (*homing, nesting, spawning, courtship, fecundity*)



374 G.R. Scott, K.A. Sloman / *Aquatic Toxicology 68 (2004) 369–392*

Table 3
Literature summary of the effects of pollutants on fish reproductive behaviours

Pollutant	LOEC (µg/l)	Duration	Hardness (mg/l as $CaCO_3$)	Fish species[a]	Disrupted behaviour	Reference
Metals						
Cadmium	0.5	48 h	30	bko	Homing	Baker and Montgomery (2001)
Copper	22	37 weeks	61	rbt	Homing	Saucier et al. (1991)
	20	40 weeks	61	rbt	Homing	Saucier and Astic (1995)
Lead	500	30 days	130	fhm	Nesting	Weber (1993)
Mercury	0.88[b]	To sexual maturity	–	fhm	Spawning	Hammerschmidt et al. (2002)
Organic pollutants						
Diazinon	10.0	24 h	65	cs	Migration	Scholz et al. (2000)
p,p′-DDE	0.1[b]	30 days	–	gp	Courtship	Baatrup and Junge (2001)
Endosulfan	0.6	≥10 days	291	ci	Courtship/ nest maintenance	Matthiessen and Logan (1984)
Esfenvalerate	1.0	Pulsed	–	bg	Spawning	Tanner and Knuth (1996)
17β-estradiol	1	24–28 days	–	gf	Courtship	Bjerselius et al. (2001)
	10[b]	24–28 days	–	gf	Courtship	Bjerselius et al. (2001)
	0.05	10 weeks	–	gf	Courtship/ spawning	Schoenfuss et al. (2002)
	3[b]	14 days	–	md	Courtship/ spawning	Oshima et al. (2003)
Ethynyl estradiol	488	21 days	44–61	md	Fecundity	Seki et al. (2002)
Flutamide	1.0[b]	30 days	–	gp	Courtship	Baatrup and Junge (2001)
Lindane	1.0	7 days	–	gp	Courtship	Schröder and Peters (1988a,b)
Octylphenol	25	6 months	–	md	Courtship/ success	Gray et al. (1999)
Phenol	10000	48 h	–	gp	Courtship/ spawning	Colgan et al. (1982)
Vinclozolin	1.0[b]	30 days	–	gp	Courtship	Baatrup and Junge (2001)

[a] Abbreviations: bg, bluegill (*Lepomis macrochirus*); bko, banded kokopu (*Galaxias fasciatus*); ci, cichlid (*Sarotherodon mossambicus*); cs, chinook salmon (*Oncorhynchus tshawytscha*); fhm, fathead minnow (*Pimephales promelas*); gf, goldfish (*Carassius auratus*); gp, guppy (*Poecilia reticulata*); md, medaka (*Oryzias latipes*); rbt, rainbow trout (*Oncorhynchus mykiss*).

[b] Dietary exposure, µg/g dry food weight.

Investigation on other threats than fishery on wild stocks

Effects of heavy metals and organic pollutants on **fish non-reproductive social behaviours** *(agonism, dominance, territoriality)*

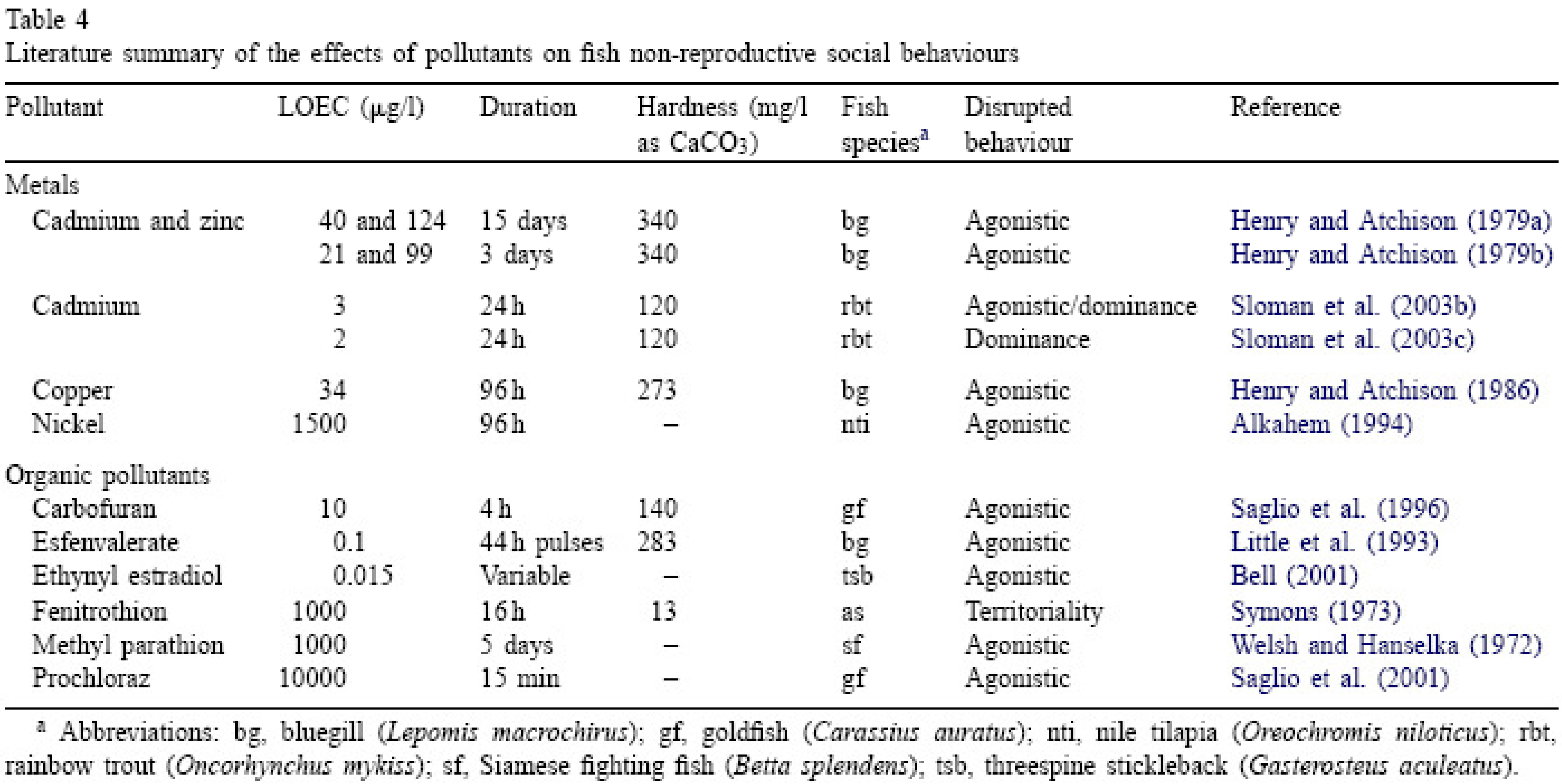
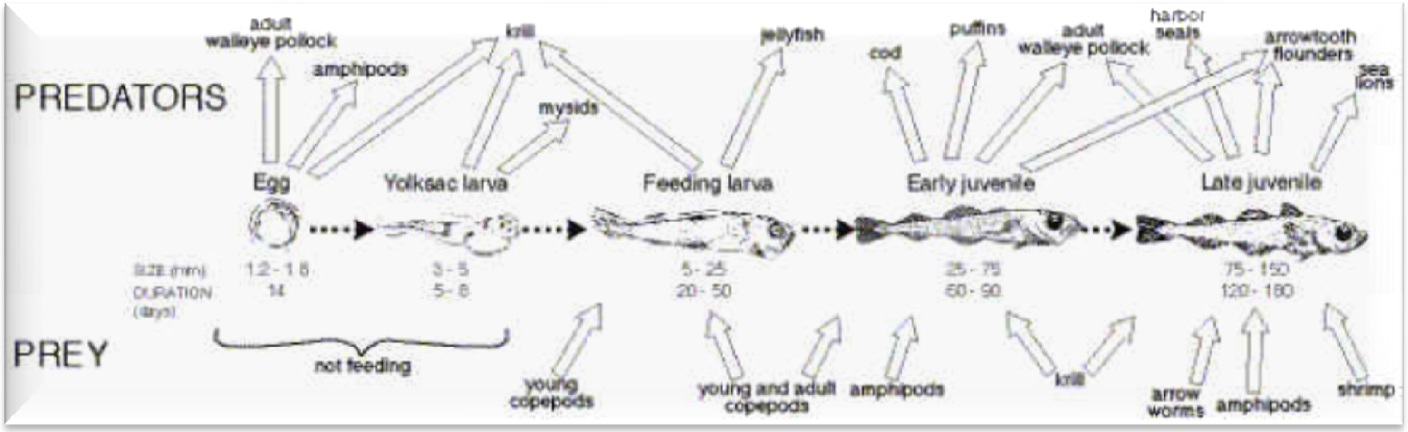


Table 4

Literature summary of the effects of pollutants on fish non-reproductive social behaviours

Pollutant	LOEC (µg/l)	Duration	Hardness (mg/l as $CaCO_3$)	Fish species[a]	Disrupted behaviour	Reference
Metals						
Cadmium and zinc	40 and 124	15 days	340	bg	Agonistic	Henry and Atchison (1979a)
	21 and 99	3 days	340	bg	Agonistic	Henry and Atchison (1979b)
Cadmium	3	24 h	120	rbt	Agonistic/dominance	Sloman et al. (2003b)
	2	24 h	120	rbt	Dominance	Sloman et al. (2003c)
Copper	34	96 h	273	bg	Agonistic	Henry and Atchison (1986)
Nickel	1500	96 h	–	nti	Agonistic	Alkahem (1994)
Organic pollutants						
Carbofuran	10	4 h	140	gf	Agonistic	Saglio et al. (1996)
Esfenvalerate	0.1	44 h pulses	283	bg	Agonistic	Little et al. (1993)
Ethynyl estradiol	0.015	Variable	–	tsb	Agonistic	Bell (2001)
Fenitrothion	1000	16 h	13	as	Territoriality	Symons (1973)
Methyl parathion	1000	5 days	–	sf	Agonistic	Welsh and Hanselka (1972)
Prochloraz	10000	15 min	–	gf	Agonistic	Saglio et al. (2001)

[a] Abbreviations: bg, bluegill (*Lepomis macrochirus*); gf, goldfish (*Carassius auratus*); nti, nile tilapia (*Oreochromis niloticus*); rbt, rainbow trout (*Oncorhynchus mykiss*); sf, Siamese fighting fish (*Betta splendens*); tsb, threespine stickleback (*Gasterosteus aculeatus*).



behaviour links physiological function with ecological processes

- Water pollution
- Aquaculture
- Pollution + Aquaculture

real and/or ostensible ‘ecological death’

G. R. Scott, K. A. Sloman, 2004. *The effects of environmental pollutants on complex fish behaviour: integrating behavioural and physiological indicators of toxicity*
Aquatic Toxicology, 68: 369–392

OPEN ACCESS Freely available online

March 2013 | Volume 8 | Issue 3 | e59162

PLOS | ONE

Differences in Lateral Line Morphology between Hatchery- and Wild-Origin Steelhead

Andrew D. Brown[1¤], Joseph A. Sisneros[2], Tyler Jurasin[3], Chau Nguyen[4], Allison B. Coffin[4,5*]

- Wild juveniles were found to possess primarily normal, aragonite-containing otoliths, while hatchery-reared juveniles possessed a high proportion of crystallized (vaterite) otoliths.
- Wild juveniles had significantly more superficial neuromasts than hatchery juveniles
- Hair cell number per neuromast did not differ across groups
- Reduced brain weight in hatchery-origin juveniles

Nippon Suisan Gakkaishi 55(12), 2079-2083 (1989)

These differences together predict reduced sensitivity to biologically important hydrodynamic and acoustic signals from natural biotic (predator, prey, conspecific) and abiotic (turbulent flow, current) sources among hatchery-reared steelhead, in turn predicting reduced survival fitness after release.

Mechanoreceptors dev earli in the reared larvae, whereas the eyes and tast ds de lo arlier in the wild larvae. Mechanisms involved in t rtained in this study. The formation of rs coincids, respectively, with th with the change from pelagic taste buds are formed, wild larva zooplankton *Paracalanus parvus*.

DOMESTICATION PROCESSES IN COURSE ?

FAILURE OF RESTOCKING PROGRAMS ?

Food search, localization and evaluation

Long range searching:

> 100 m → *olfaction*

100 - 25 m → *olfaction, hearing*

Medium range searching:

25 - 5 m → *olfaction, hearing, sight*

5 - 1 m → *olfaction, hearing, sight*

Aiming and Seizing:

1 - 0.25 m → *olfaction, hearing, sight, lateral line, outer chemoreception*

<0.25 m → *sight , lateral line, chemoreception, electroreception, touch*

Oral processing + Evaluating quality

0 m → inner chemoreceptors, touch

Pavlov et al.,1990

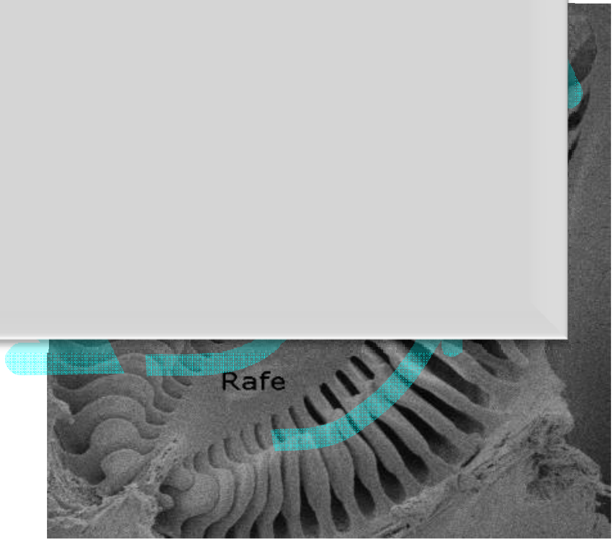
Basic research: autoecology of different life stages

Chemoreception: olfaction

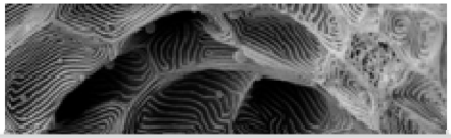
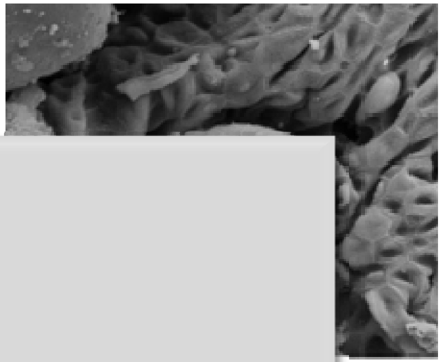
Feeding success depends on the progressive development of anatomical characteristics and physiological functions and on the availability of suitable food items throughout larval development

Ol

1.
2.
3.
4. Nares → juvenile
5. Olfactory rosetta → all along the life?





REVIEWS IN Aquaculture

Reviews in Aquaculture (2013) **5** (Suppl. 1), S59–S98 doi: 10.1111/raq.12010

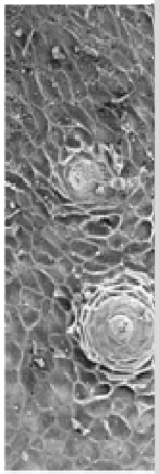
Feeding behaviour and digestive physiology in larval fish: current knowledge, and gaps and bottlenecks in research

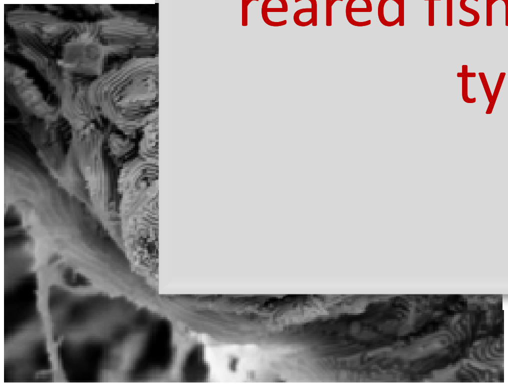
Ivar Rønnestad[1], Manuel Yúfera[2], Bernd Ueberschär[3], Laura Ribeiro[4], Øystein Sæle[5] and Clara Boglione[6]

Different typologies reflect functional differences (i.e., only gustatory, also tactile)

The localization indicates the role plaid (aiming, sizing, final evaluation) and the range of detection.

Very few data exist on the ontogenesis of taste buds in reared fish, and the individual ontogenic stages of different types of taste bud still need to be defined.


BG II TIP


BG III TIPO


BG IV TIPO

Pulcini and Boglione: unpub. data


BG IV TIPO

Boglione: unpub. data

The dorsal pharynx is a reliable indicator of the trophic ecology of Teleost fish ?

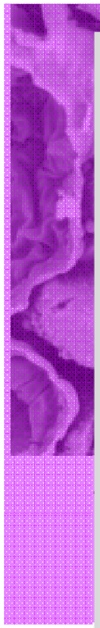
S. aurata

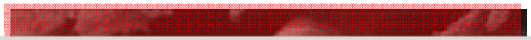
M. cephalus

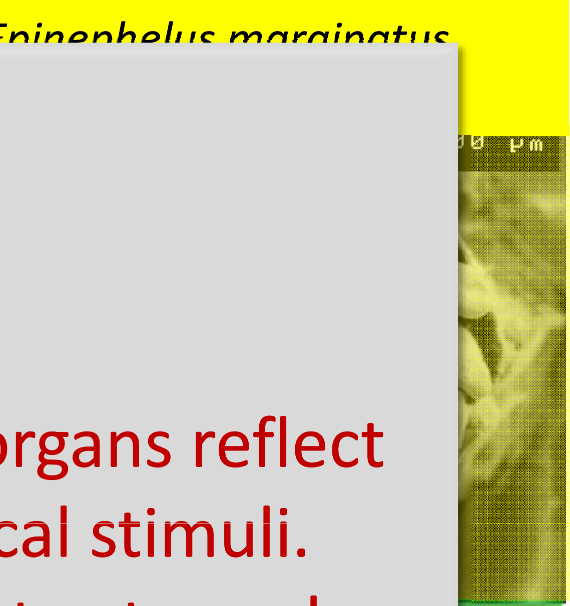
M. merluccius

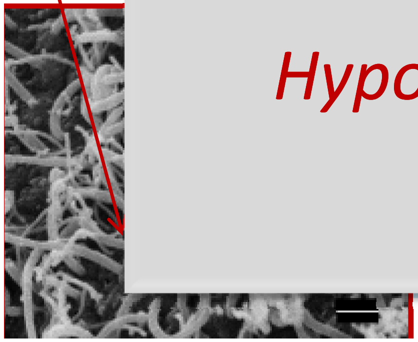
Basic research: autoecology of different species

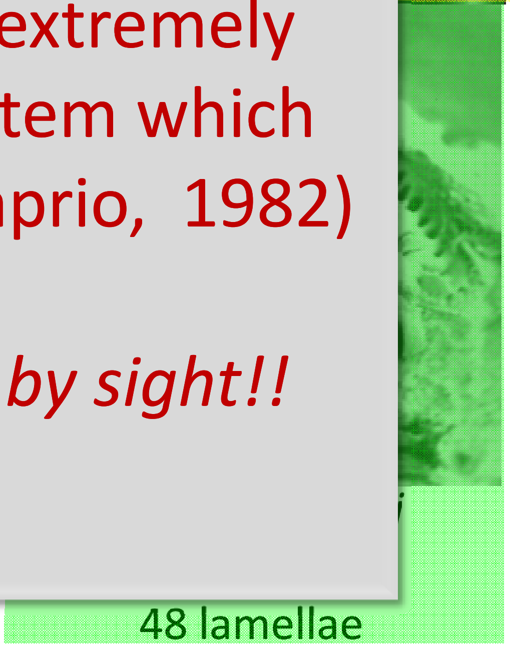
Differences in the olfactory organs mainly reflect the developmental stage, autoecology and osmatic capabilities of the species ......











REVIEWS IN Aquaculture

Reviews in Aquaculture (2013) 5 (Suppl. 1), S59–S98 doi: 10.1111/raq.12010

Feeding behaviour and digestive physiology in larval fish: current knowledge, and gaps and bottlenecks in research

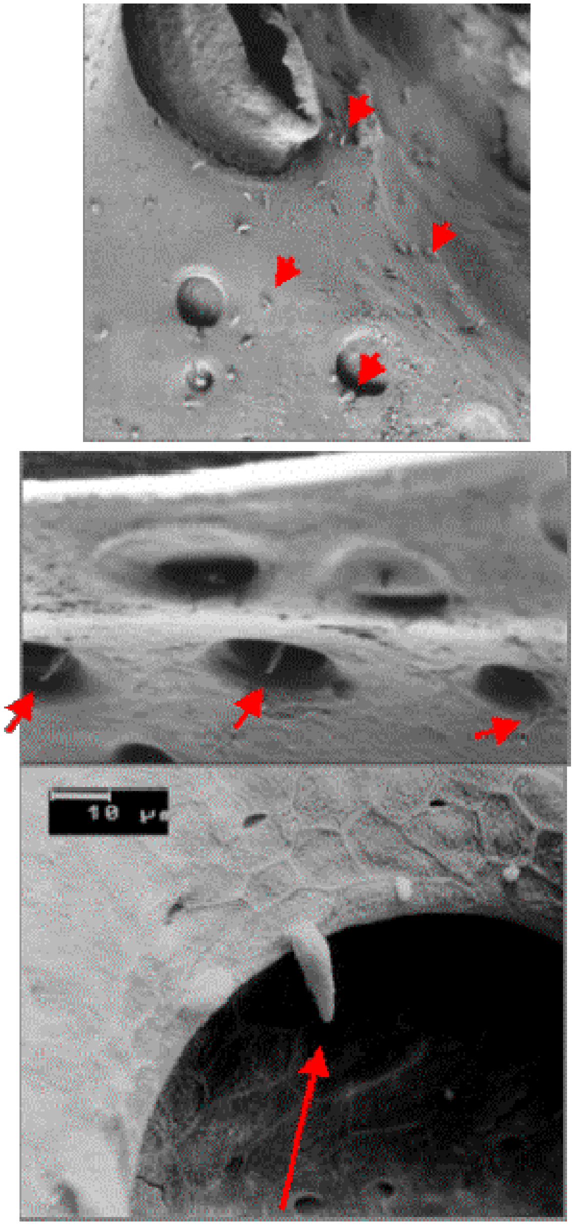
Ivar Rønnestad[1], Manuel Yúfera[2], Bernd Ueberschär[3], Laura Ribeiro[4], Øystein Sæle[5] and Clara Boglione[6]

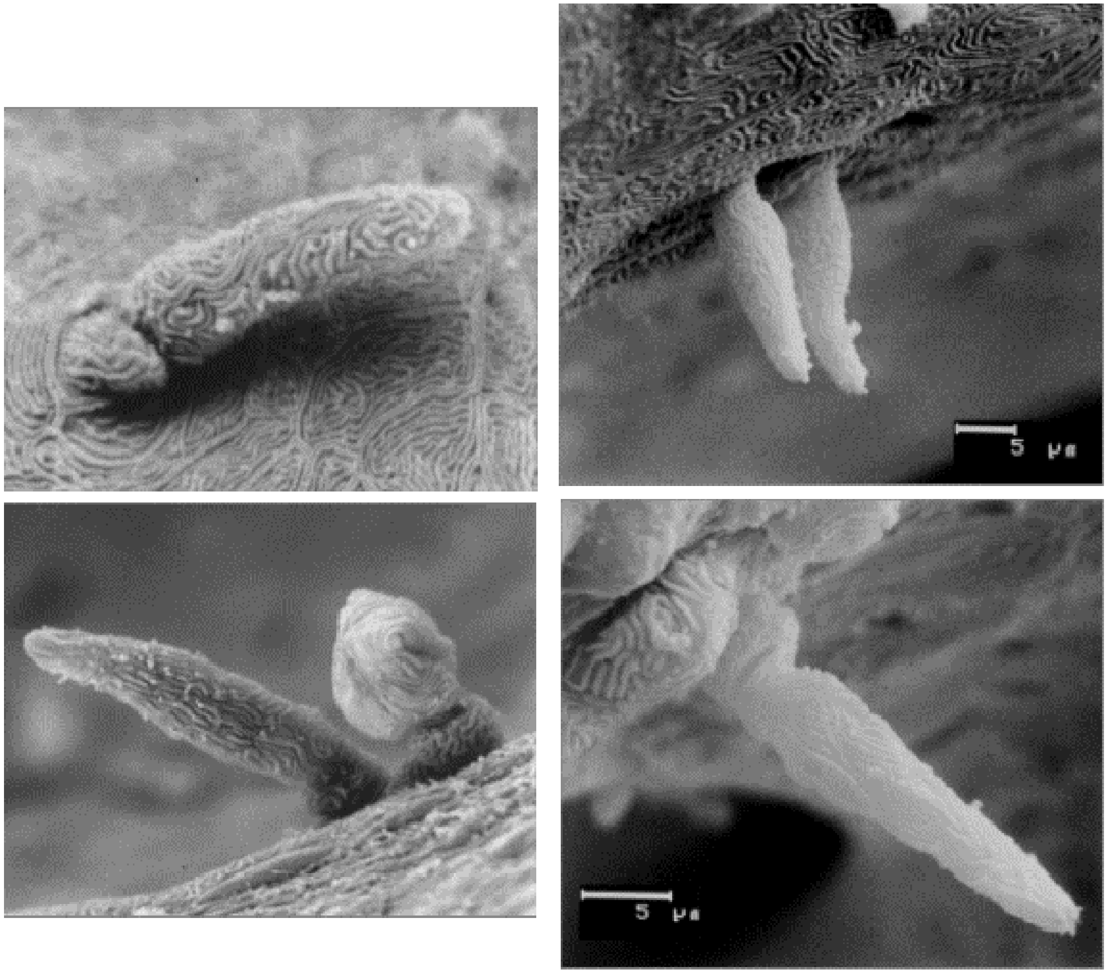
A different complexity of olfactory organs reflect different responsiveness a chemical stimuli. Olfaction is more effective to detect extremely diluted substances than gustatory system which demands for higher concentrations (Caprio, 1982)

Hyposmatic fish mainly localize food by sight!!

New questions:

1. All the sense organs have been described in Teleosts?

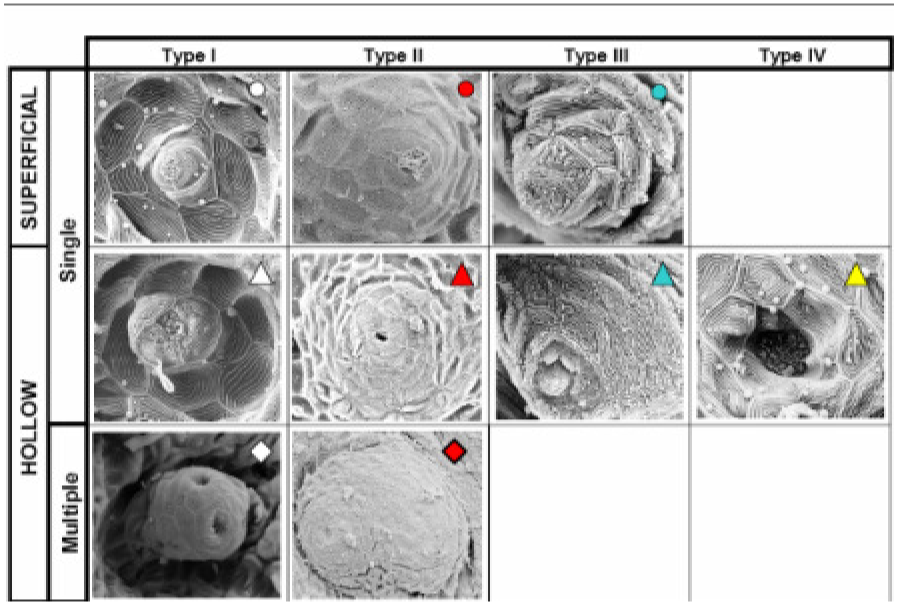




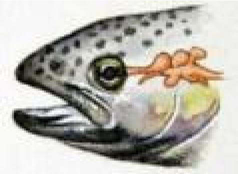
Boglione: unpub. data

2. Is the O_2- or the CO_2 receptions carried out by different organs or is a function carried out by taste buds ?

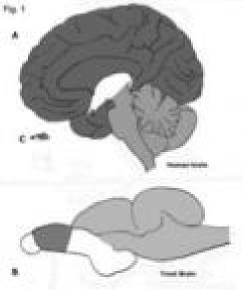
- Taste buds: already ascertained chemo- or chemo- and mechano-reception (Reutter, 1973) for feeding and protective functions
- In Salmonids and Cyprinids: the exposition of dorsal pharynx (rich in taste buds) to high CO_2 or H^+ levels (hypercapnia, anoxia) determines an immediate increase of respiratory movements (Jobling, 1995).



3. Do fish possess nociceptors ?



Neuroanatomical evidences highlight that fish have nocireception but not a neocortex: homologous or different organs ?

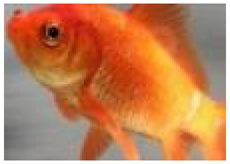


■ Forebrain (cerebrum, telencephalon)

▨ Hind brain (brainstem)

□ Mid brain (diencephalon)

Rose, 2002.



Behavioral evidences highlight that fish do evade, if they can, any pain. Further, they are able to evaluate negative and positive stimuli and modulate consequently their behavior.

TASTE BUDS

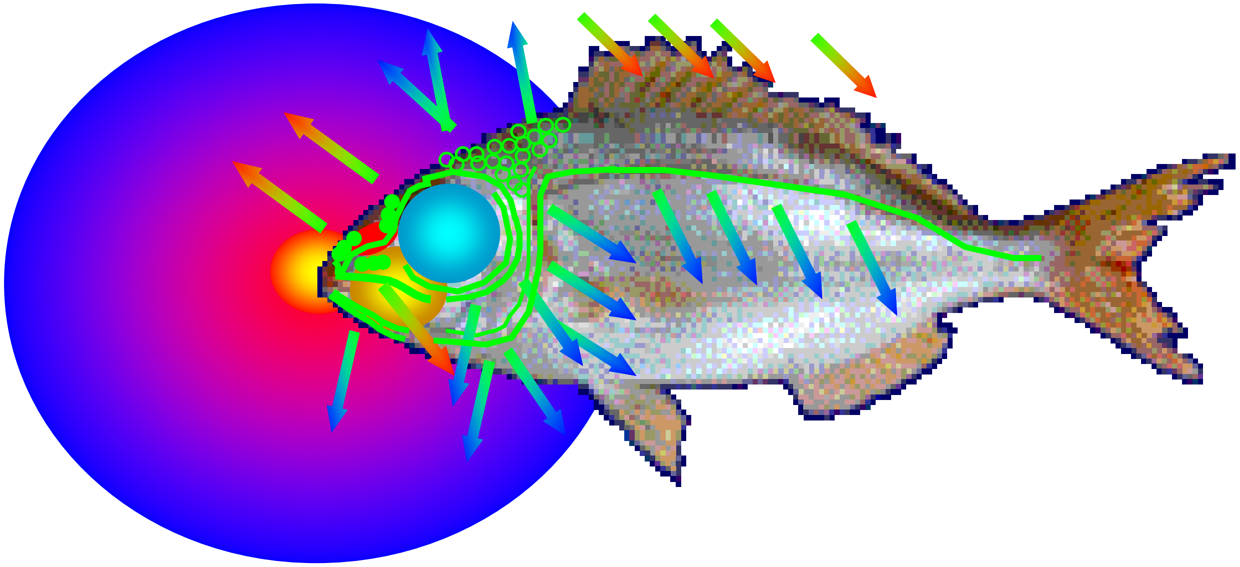
Different typologies reflect functional differences (i.e., only gustatory, also tactile)

The localization indicates the role plaid (aiming, sizing, final evaluation)

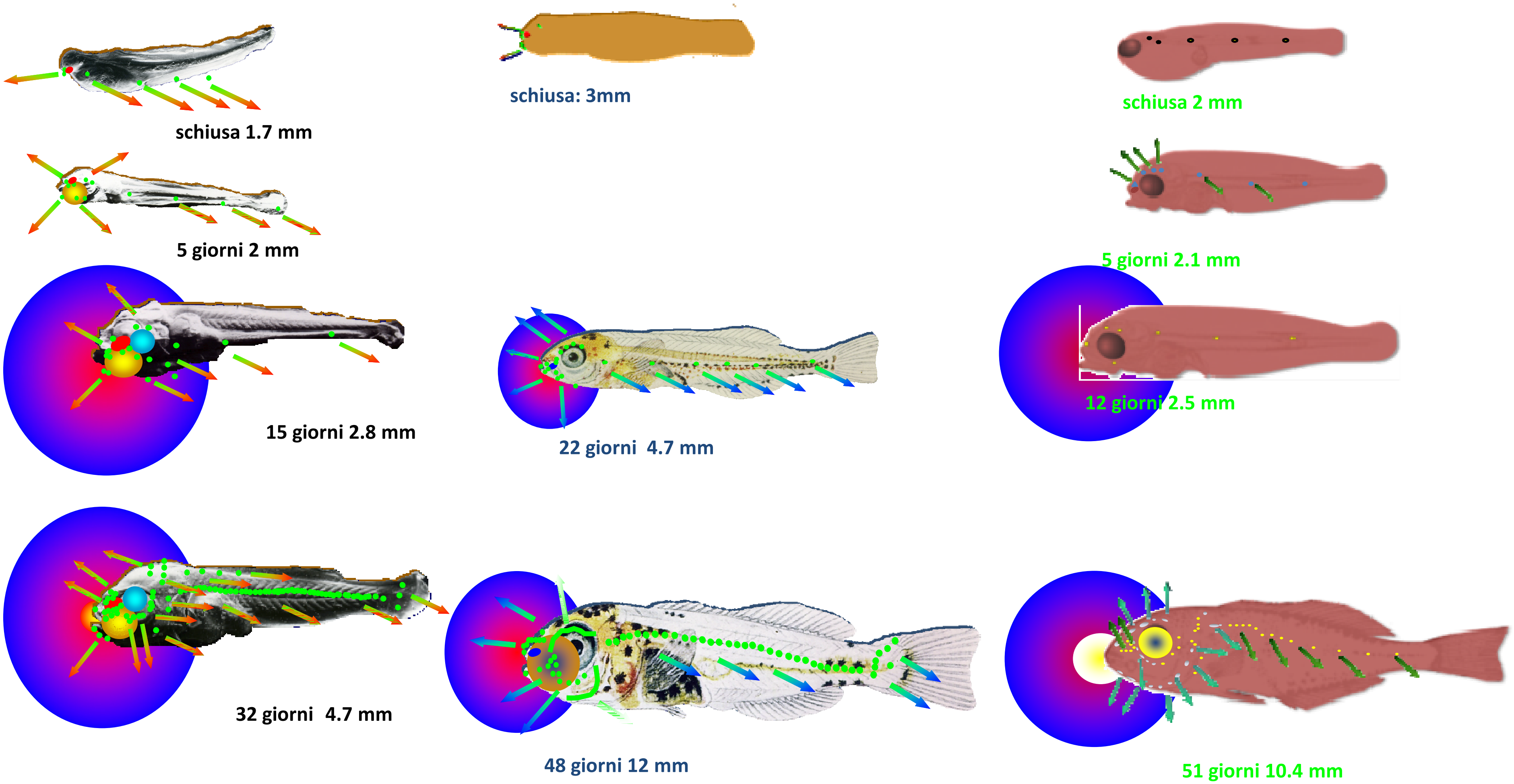
Food uptake follows rules defined by feeding behavior that determines the kind and quantity of food ingested by fish larvae as well as how live prey and food particles are detected, captured and ingested.

Very few data exist on the ontogenesis of taste buds in reared fish.

olfaction	100 - 0.25 m
free neuromasts	1 – 0.25 m
sight	1-0.25 m
canalized neuromasts	< 0.25 m
chemoreceptors	<0.25m



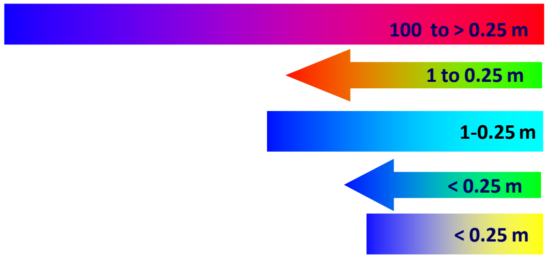
Boglione: unpub. data



Pagellus erythrinus (Linnaeus, 1758)

Diplodus puntazzo (Cetti, 1777)

Sparus aurata (Linnaeus, 1758)



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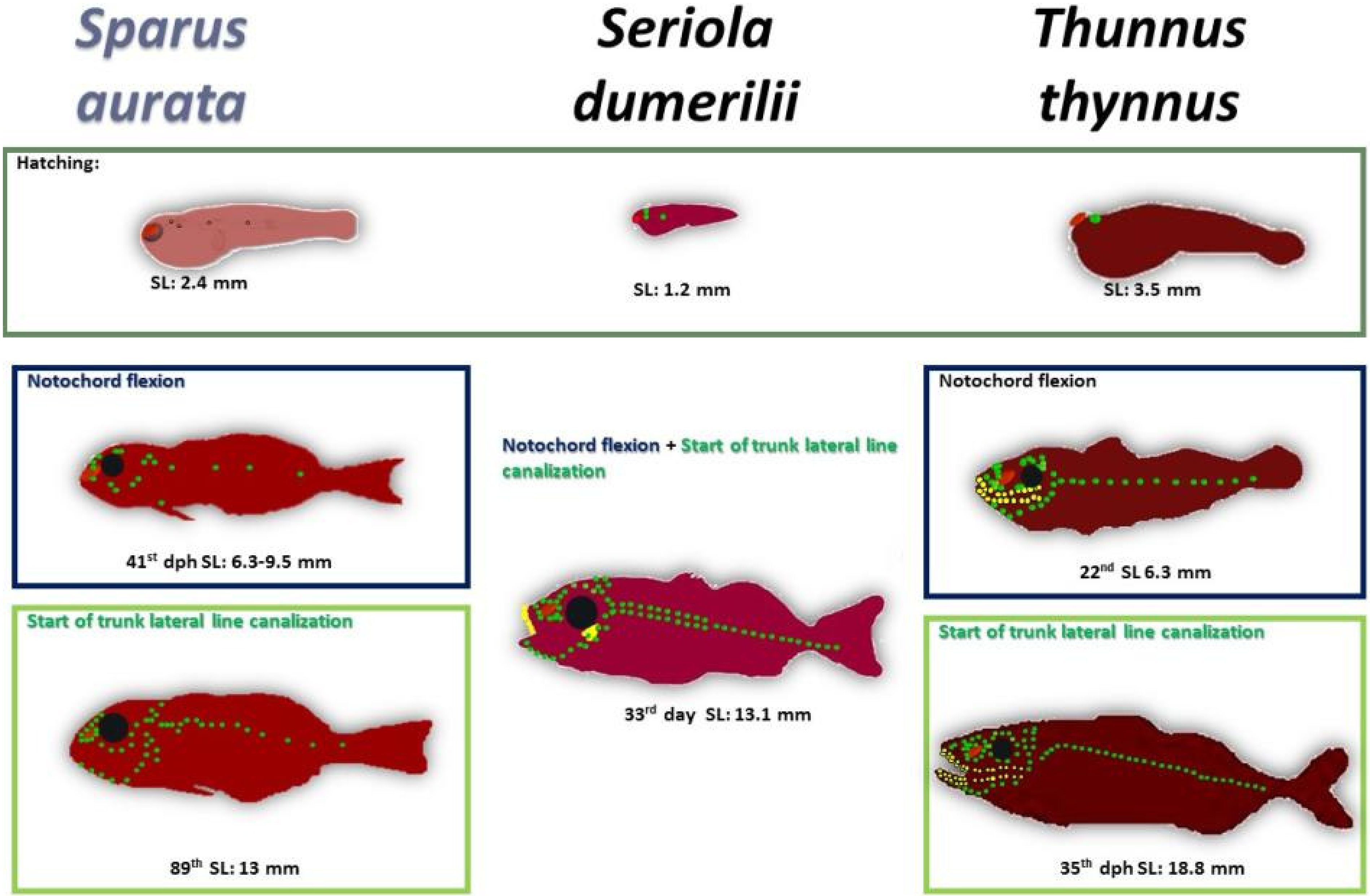
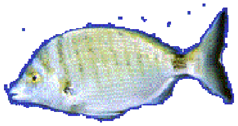
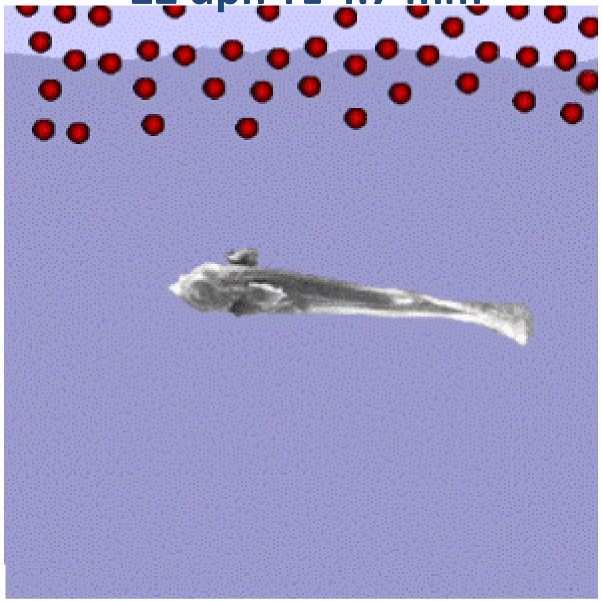

Sparus aurata
Seriola dumerilii
Thunnus thynnus
Hatching:
SL: 2.4 mm
SL: 1.2 mm
SL: 3.5 mm
Notochord flexion
41st dph SL: 6.3-9.5 mm
Start of trunk lateral line canalization
89th SL: 13 mm
Notochord flexion + Start of trunk lateral line canalization
33rd day SL: 13.1 mm
Notochord flexion
22nd SL 6.3 mm
Start of trunk lateral line canalization
35th dph SL: 18.8 mm

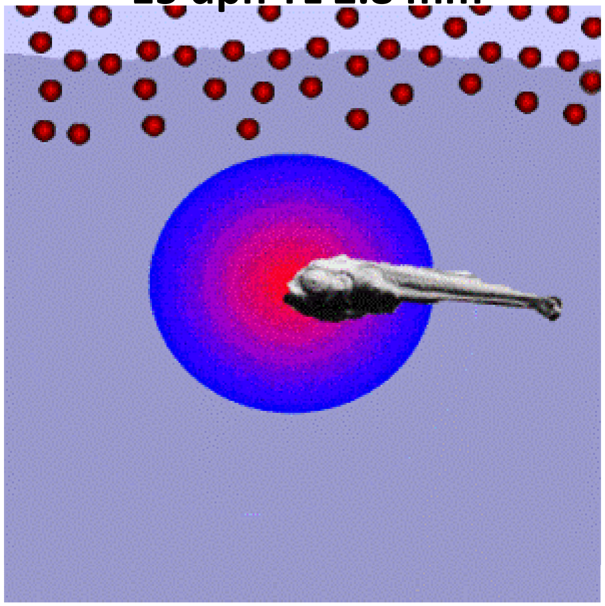


22 dph TL 4.7 mm

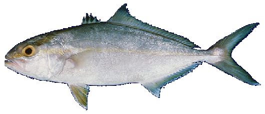


Mechanoreceptive larva mainly reotactic.

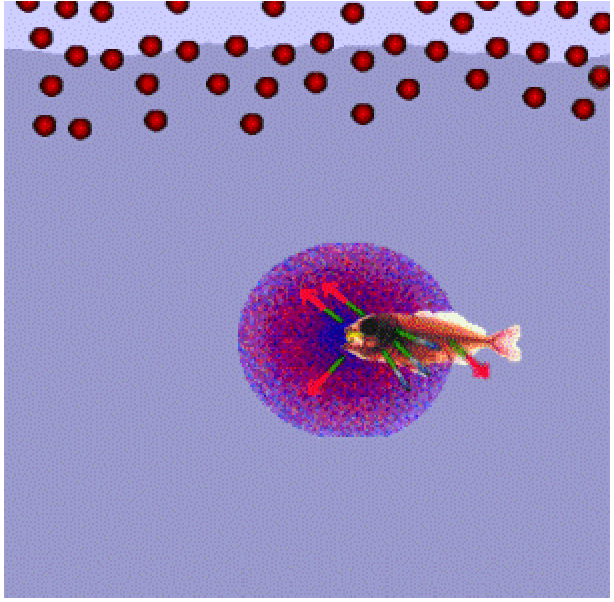
15 dph TL 2.8 mm



Highly chemoreceptive larva



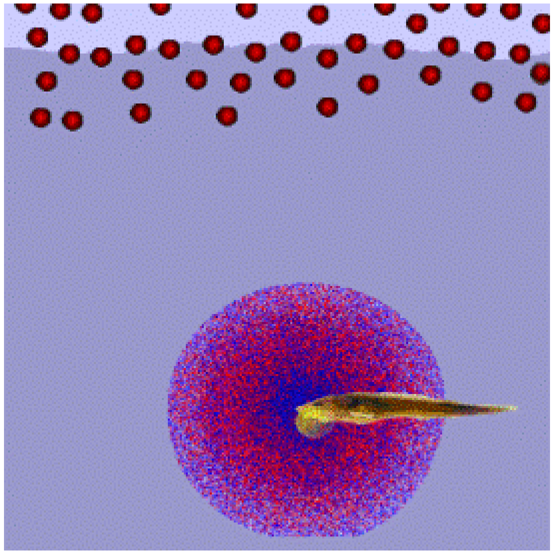
33 dph TL 13.1 mm



Highly and precociously chemorecetive larva

12 dph TL 17 mm

Highly osmatic and electrosensitive larva



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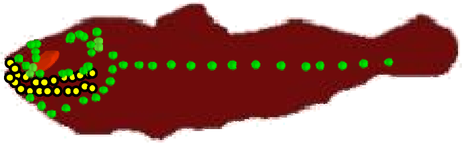
BFT TUNA



10 – 17 dph (TL 4.2-5.6 mm):

Differentiation of all organs involved in FEEDING

Allometric developmental phase (head of juveniles; trunk of a larva)

CSCs + early differentiation of MSCs → high olfactory skill able to chemically individuate preys at large range (> 100 m) (Pavlov and Kasumyan, 1990)

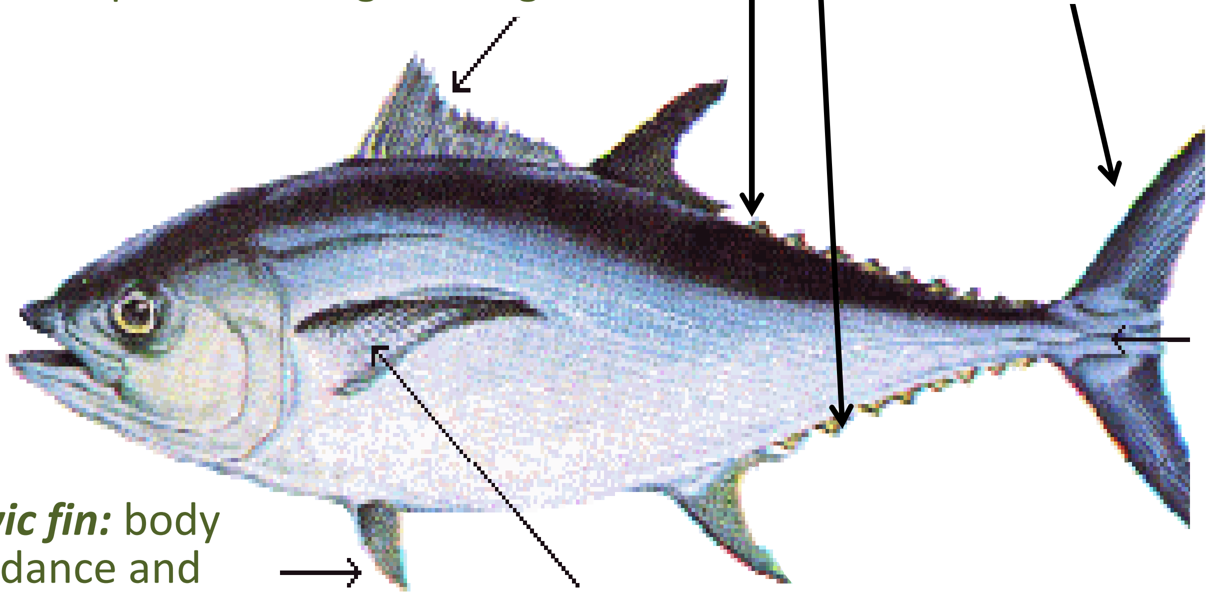
Large number of cephalic NMs, symmetrically arranged, with cupolae → reotaxis + mecchanoreception of live preys (zooplankton) + schooling (Myrberg e Fuiman, 2002) + permanence and feeding in the water column (Iwai, 1980)

Canine-like teeth on buccal rims and in the pharynx → feeding on ichthyoplankton (Harder , 1975)

Early differentiation of inner TBs (readsorbed in adults?) → final ingestion after organoleptic evaluation

Boglione: unpub. data

• Why to study normal skeletogenesis?



Dorsal and ventral finlets: to control the water flux in the caudal peduncle *(Walters, 1962; Collette, 1978; Nauen e Lauder, 2001)*

1st dorsal fin: hosted in a dorsal pocket during cruising

Caudal fin flaps: to augment or to diminish caudal fin curvature *(Westneat and Wainwright, 2001)*

Caudal fin: to sustain high swimming speed *(Westneat and Wainwright, 2001)*

Caudal peduncle keel: *to reduce vortices (Walters, 1962; Collette, 1978; Nauen e Lauder, 2001)*

Pelvic fin: body guidance and balancing *(Osburn, 1906)* / tilting *(Harris, 1983)*

Pectoral fin: *hosted in a lateral cavity during cruising*

Fins ontogenesis marks the acquisition of peculiar, species-specific swimming behaviour that must be considered in evaluating what are the best rearing tanks/conditions

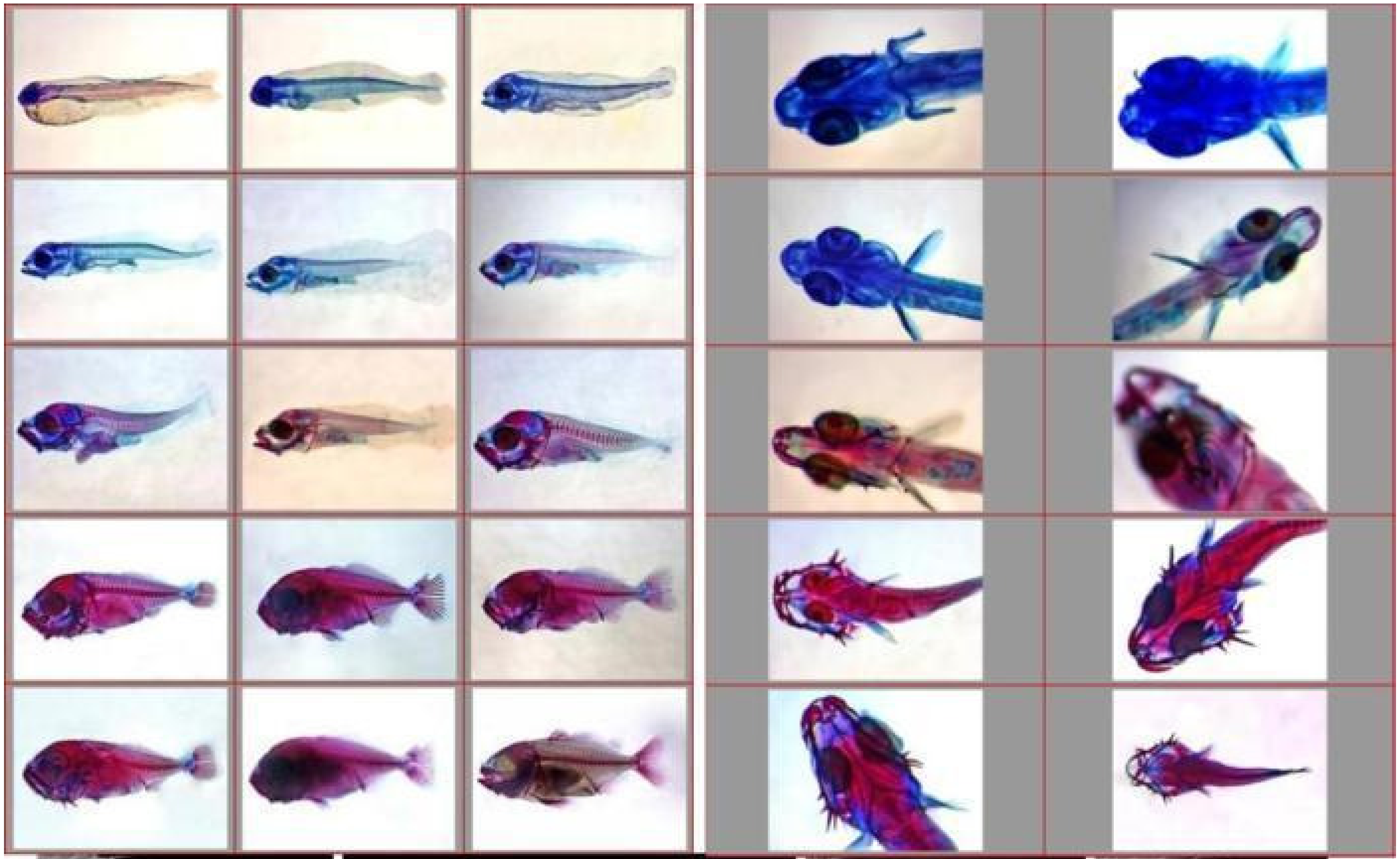
Why to study anomalous skeletogenesis?

✓The presence of anomalous developmental processes is one of the consequences

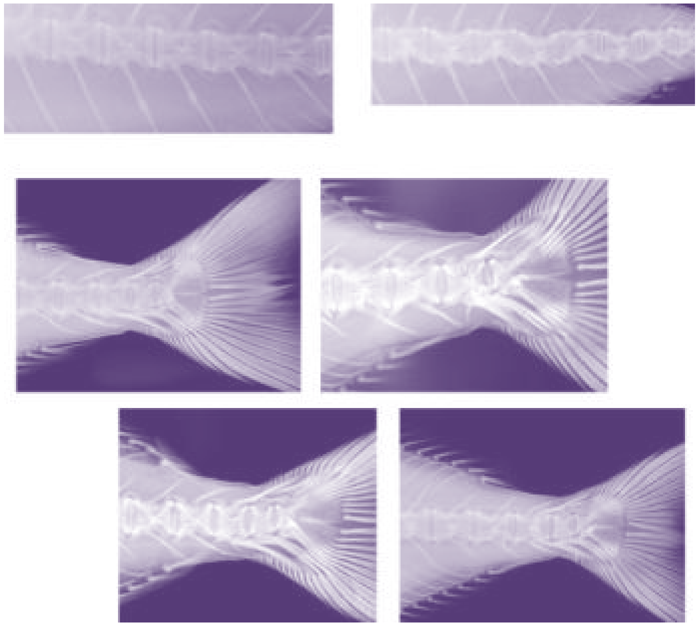
- of inappropriate rearing conditions
- of altered (temperature, salinity, chemical pollution) environment

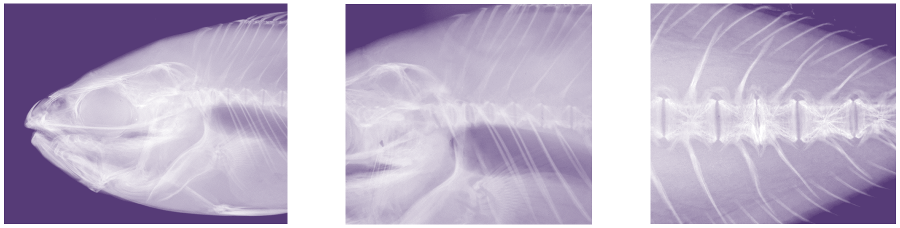
✓The ontogenetic pattern (onset timing and affected skeletal elements) can help the individuation of species-specific critical periods for aquaculture or the exposition time to altered conditions

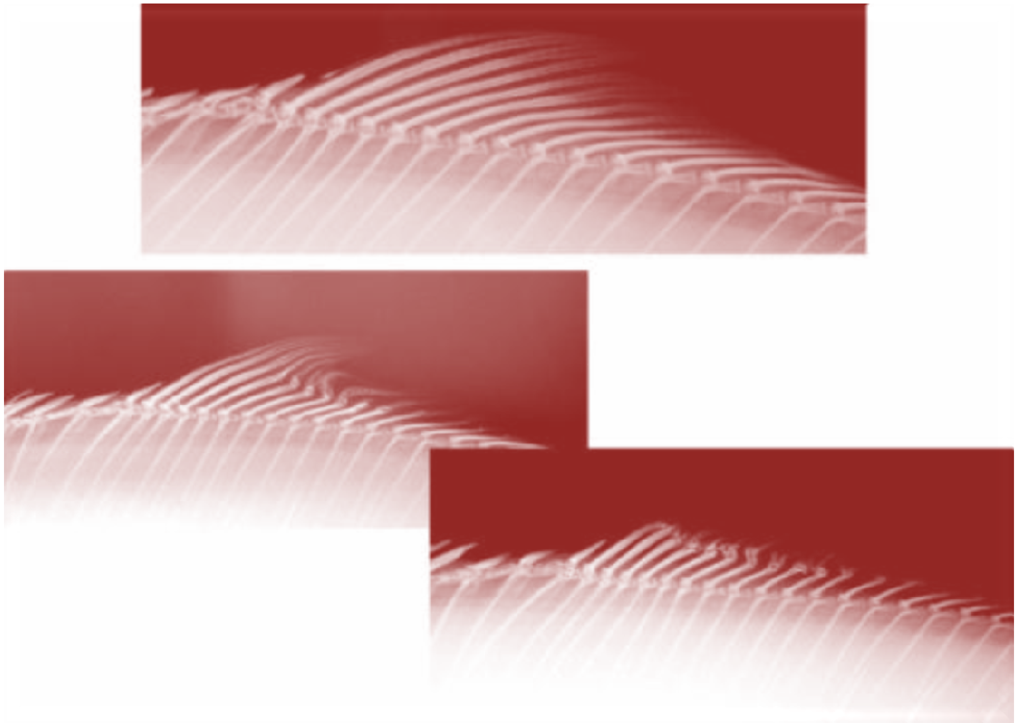
Greater amberjack (*Seriola dumerili*)



- the **anomalies incidence tend to augment with age**, with 322 dph lots showing the highest incidences of anomalies
- severe anomalies tend to diminish with age, probably indicating a **selective death** for those individuals affected by such anomalies







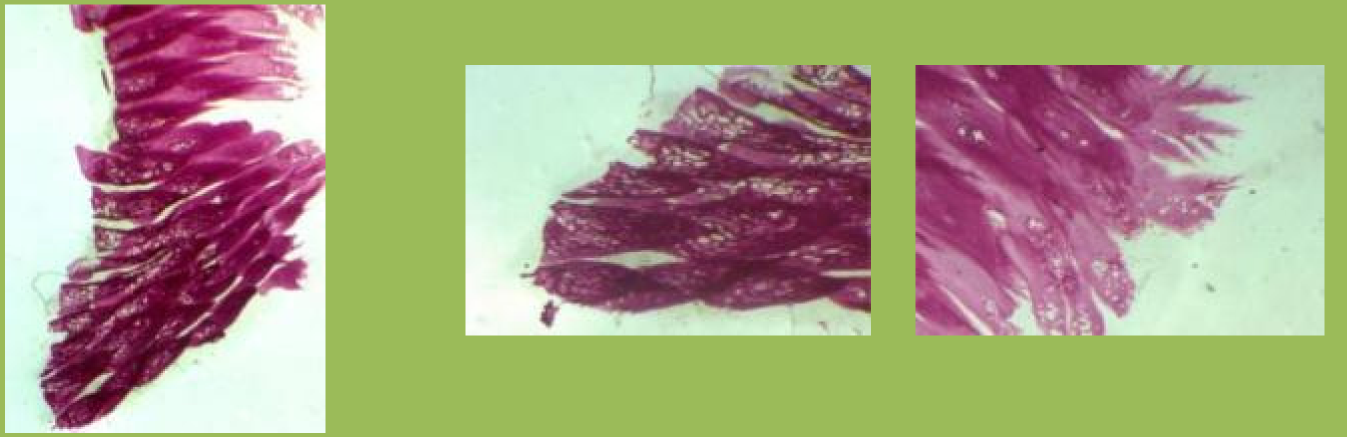
- Some anomalies could be ascribed to limited space availability !!!!

Identification of anomalous mineralization in wild adult tuna?

WORK IN PROGRESS

SUBOPERCULAR

PREOPERCULAR



INTEROPERCULAR

Results

Boglione: unpub. data

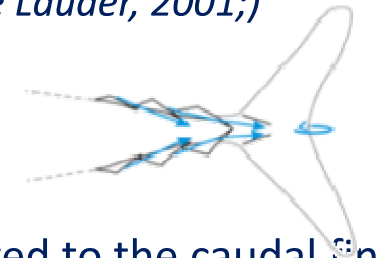
BFT differentiate all the elements involved in *cruising* in a TL range = 6.3 – 48.8 mm

- ✓1st ,2nd ,3rd and 4th hypuralia fuse to form hypural plate, LT = 14,5 mm → *to increase the stiffness of caudal fin* (Collette, 1978)
- ✓caudal fin flaps, LT = 8,6 mm, connected with *flexors ventralis* and *dorsalis* muscles → *to augment or to diminuish caudal fin curvature* (Westneat e Wainwright, 2001)
- ✓ principal caudal rays inserted medially to the hypural plate by robust tendons (*subdermal sheath*) (LT 13,3 mm) → *to sustain high swimming speed* (Westneat e Wainwright, 2001)
- ✓ hypural plate is connected to the 6 posteriormost myosepta by big tendons → *for stiffening the caudal peduncle and/or for supporting lateral keels* (Fierstine e Walters, 1968)

- ✓ two lateral and 1 medial keels
- ✓ 8 dorsal and 7 anal finlets

➡

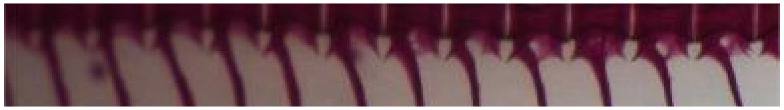
to control the water flux in the caudal peduncle, to reduce vortices (Walters, 1962; Collette, 1978; Nauen e Lauder, 2001;)



In Tuna, the 100% of force generated by axial muscles is transmitted to the caudal fin (Gibb et al., 1999)

Boglione: unpub. data

38.1 mm

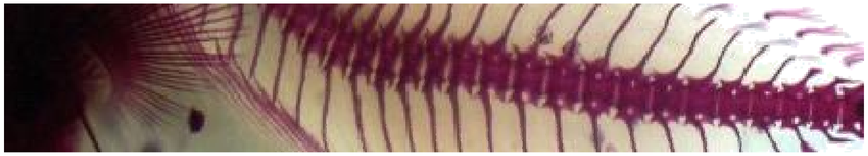


Skeletal anomalies analysis

	15-30 dph	35-41 dph	56-84 dph	Wild
N of observed individuals	24	206	78	22
Rate of malformed individuals	100%	100%	100%	0%
Malformation charge	5.7	11.8	12.4	0
Observed anomalies typologies	15	49	30	0
Rate of severely deformed individuals	100%	100%	96.2%	0%
Ratio severe/light anomalies	32.1	31.1	20.0	0
Severe anomalies charge	1.8	3.6	2.6	0

All reared tunas has at least one anomaly; malformation charge augment with age;
35-41 dph seems to be the more 'deformed' stage;

<u>100% of 15-41 dph tunas had abortive swim bladder</u>



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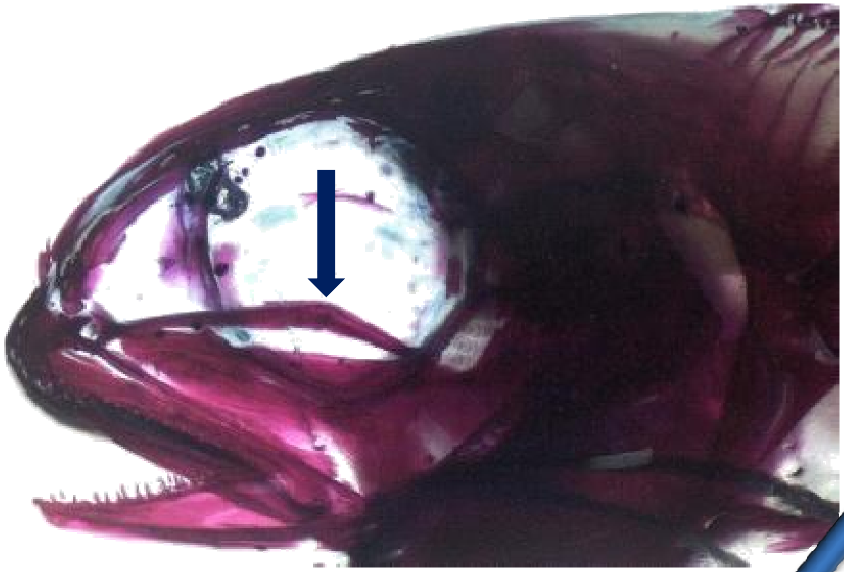
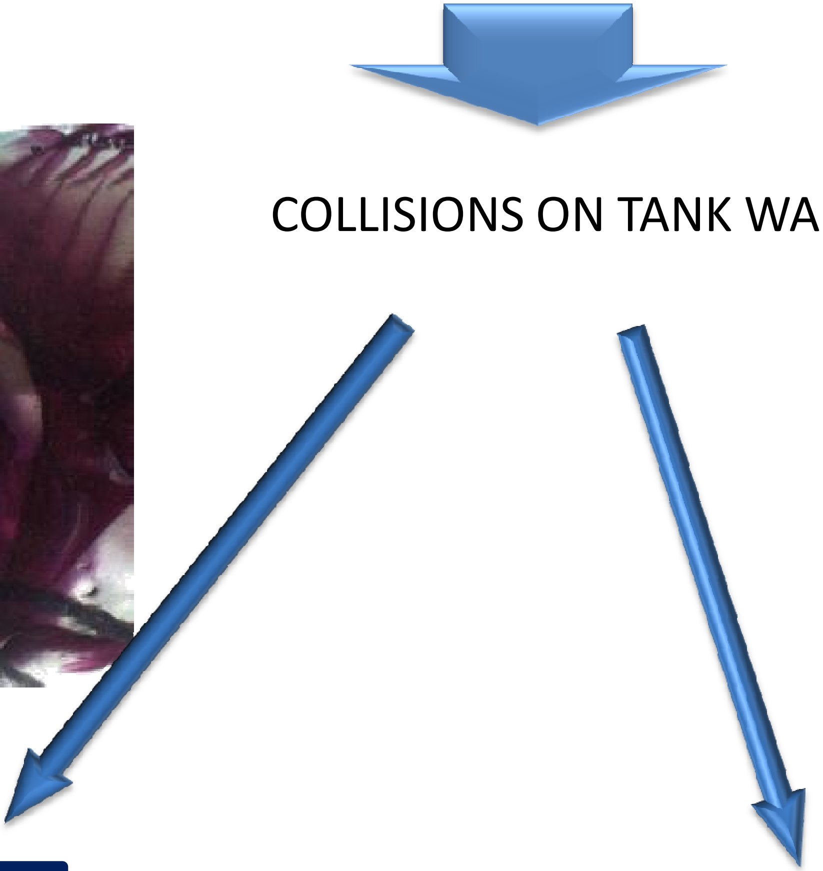




Some tunas with SL ranging 10 - 37 mm exhibited a broken palatine

COLLISIONS ON TANK WALLS

Underdeveloped scotopic vision in tuna juveniles than in other marine juveniles (Ishibashi *et al*., 2009).

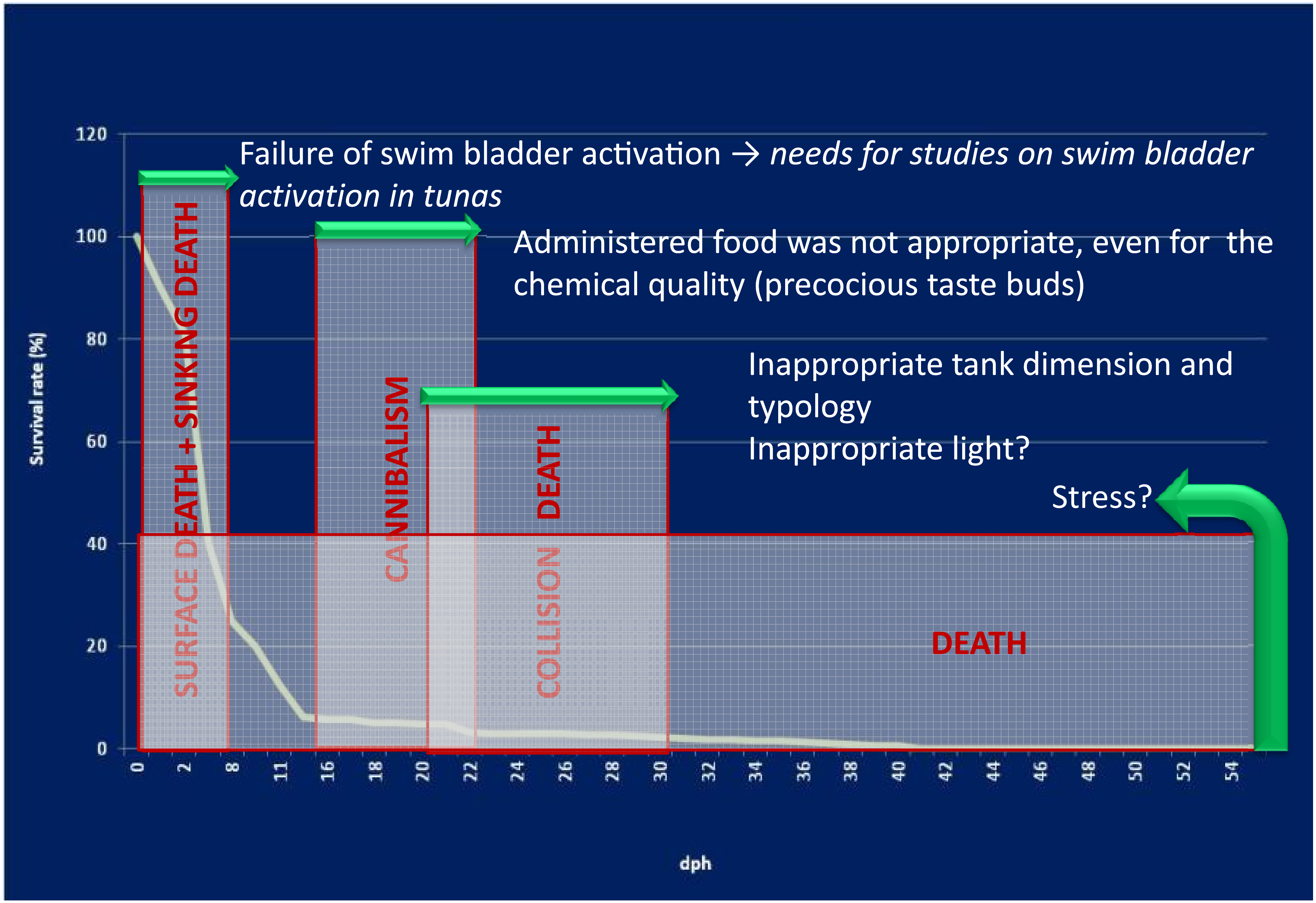
+

Cruising ability of larvae

Boglione: unpub. data

Differently from other reared marine finfish larvae, BFT larvae seem ....

- to be affected by developmental asynchronies;
- to be unable to activate swim bladder in rearing conditions, even in presence of superficial air skimmers;
- to be characterized by very precocious faster and continuous swimming (*cruising*), so demanding for larger rearing volumes;
- …………………………..



Failure of swim bladder activation → *needs for studies on swim bladder activation in tunas*

Administered food was not appropriate, even for the chemical quality (precocious taste buds)

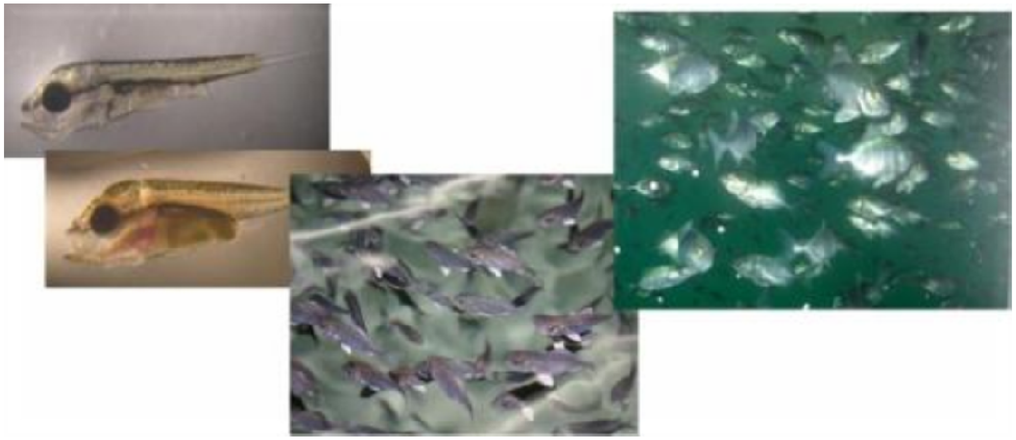
Inappropriate tank dimension and typology
Inappropriate light?

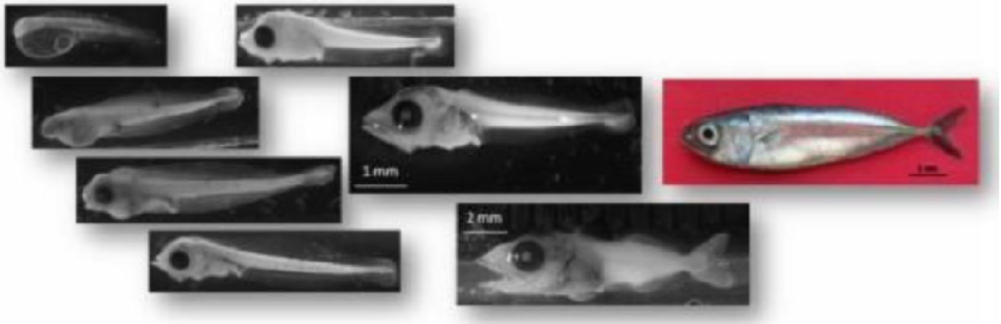
Stress?

Boglione: unpub. data

I would like to thank Prof. Cataudella S and all my «students» of Tor Vergata

- Gianluca Amoroso
- Matilde de Francesco
- Maurizio Giganti
- Martina Marroncini
- Laura Orzali
- Marco Padroni
- Ylenia Pennacchi
- Loredana Prestinicola
- Domitilla Pulcini
- Simone Serra
- Claudio Selmo





Thanks for your attention