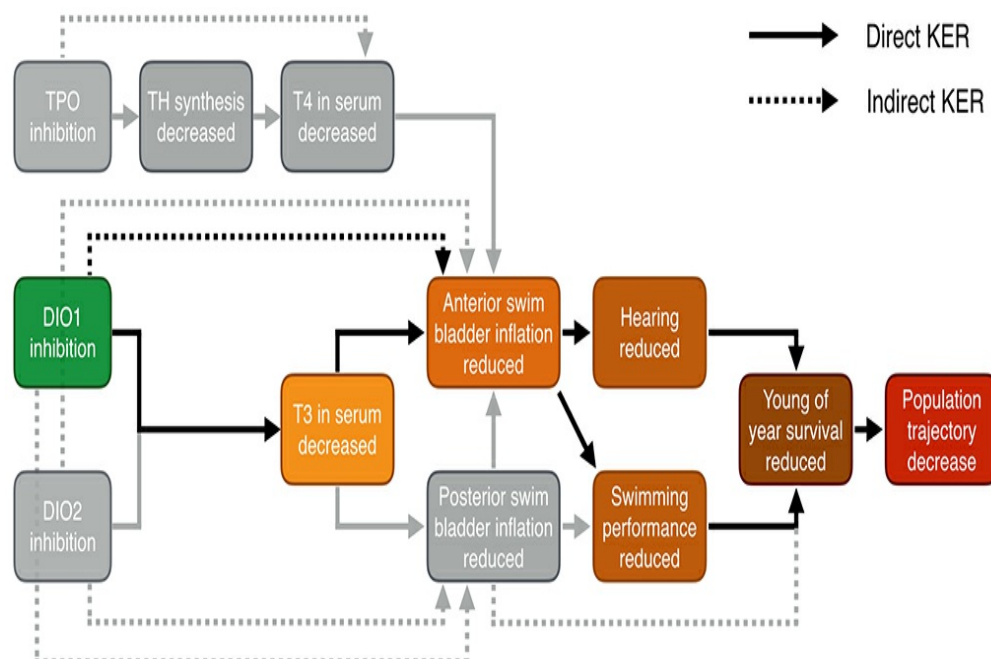


AOP 158: Deiodinase 1 inhibition leading to reduced young of year survival via anterior swim bladder inflation

Short Title: DIO1i anterior swim bladder

Graphical Representation



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Status

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Abstract

The AOP describes the effects of inhibition of deiodinase 1 (DIO1) on anterior swim bladder inflation leading to reduced young of year survival and population trajectory decline. The inhibition of DIO1 is the molecular-initiating event (MIE), which results in decreased circulating concentrations of triiodothyronine (T3) in serum. Disruption of the thyroid hormone (TH) system is increasingly being recognized as an important MoA that can lead to adverse outcomes, especially during embryonic development. In fish, many different adverse effects during early development resulting from

disruption of the TH endocrine system have been reported (e.g., effects on body and eye size, head-to-trunk angle, heartbeat, otolith formation, pigmentation index, swim bladder inflation, hatching time, somite formation, escape response and photoreceptor development). As in amphibians, the transition in fish between the different developmental phases, including maturation and inflation of the swim bladder, have been shown to be mediated by THs. Fish larvae exposed to PTU, a known DIO1 inhibitor, has been known to result in impairment of swim bladder inflation. Chemicals interfering with the conversion of T4 to T3 have the potential to inhibit anterior chamber inflation which may result in reduced auditory capacity and reduced swimming capacity of the fish, a relevant adverse outcome that can affect feeding behaviour and predator avoidance, resulting in lower survival probability and ultimately population trajectory decline (Czesny et al., 2005; Woolley and Qin, 2010).

Summary of the AOP

Events

Molecular Initiating Events (MIE), Key Events (KE), Adverse Outcomes (AO)

Sequence	Type	Event ID	Title	Short name
1	MIE	1009	Inhibition, Deiodinase 1 (https://aopwiki.org/events/1009)	Inhibition, Deiodinase 1
2	KE	1003	Decreased, Triiodothyronine (T3) in serum (https://aopwiki.org/events/1003)	Decreased, Triiodothyronine (T3) in serum
3	KE	1007	Reduced, Anterior swim bladder inflation (https://aopwiki.org/events/1007)	Reduced, Anterior swim bladder inflation
4	KE	1008	Reduced, Hearing (https://aopwiki.org/events/1008)	Reduced, Hearing
5	KE	1005	Reduced, Swimming performance (https://aopwiki.org/events/1005)	Reduced, Swimming performance
6	KE	1006	Reduced, Young of year survival (https://aopwiki.org/events/1006)	Reduced, Young of year survival
7	AO	360	Decrease, Population trajectory (https://aopwiki.org/events/360)	Decrease, Population trajectory

Key Event Relationships

Upstream Event	Relationship Type	Downstream Event	Evidence	Quantitative Understanding
Inhibition, Deiodinase 1 (https://aopwiki.org/relationships/1037)	adjacent	Decreased, Triiodothyronine (T3) in serum		
Decreased, Triiodothyronine (T3) in serum (https://aopwiki.org/relationships/1035)	adjacent	Reduced, Anterior swim bladder inflation		
Reduced, Anterior swim bladder inflation (https://aopwiki.org/relationships/1032)	adjacent	Reduced, Hearing		
Reduced, Hearing (https://aopwiki.org/relationships/1033)	adjacent	Reduced, Young of year survival		
Reduced, Young of year survival (https://aopwiki.org/relationships/1030)	adjacent	Decrease, Population trajectory		
Reduced, Anterior swim bladder inflation (https://aopwiki.org/relationships/1034)	adjacent	Reduced, Swimming performance		

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Upstream Event	Relationship Type	Downstream Event	Evidence	Quantitative Understanding
Reduced, Swimming performance (https://aopwiki.org/relationships/1029)	adjacent	Reduced, Young of year survival		
Inhibition, Deiodinase 1 (https://aopwiki.org/relationships/1045)	non-adjacent	Reduced, Anterior swim bladder inflation		

Stressors

Name	Evidence
Propylthiouracil	

Overall Assessment of the AOP

Domain of Applicability

Life Stage Applicability

Life Stage	Evidence
Development	

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
zebrafish	Danio rerio		NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=7955)
fathead minnow	Pimephales promelas		NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=90988)

Sex Applicability

Sex	Evidence
Unspecific	

References

Appendix 1

List of MIEs in this AOP

Event: 1009: Inhibition, Deiodinase 1 (<https://aopwiki.org/events/1009>)

Short Name: Inhibition, Deiodinase 1

Key Event Component

Process	Object	Action
catalytic activity	type I iodothyronine deiodinase	decreased

AOPs Including This Key Event

AOP158

AOP ID and Name	Event Type
Aop:157 - Deiodinase 1 inhibition leading to reduced young of year survival via posterior swim bladder inflation (https://aopwiki.org/aops/157)	MolecularInitiatingEvent
Aop:158 - Deiodinase 1 inhibition leading to reduced young of year survival via anterior swim bladder inflation (https://aopwiki.org/aops/158)	MolecularInitiatingEvent
Aop:189 - Type I iodothyronine deiodinase (DIO1) inhibition leading to altered amphibian metamorphosis (https://aopwiki.org/aops/189)	MolecularInitiatingEvent

Stressors

Name
iopanoic acid
Propylthiouracil

Biological Context

Level of Biological Organization
Molecular

Domain of Applicability

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
zebrafish	Danio rerio		NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=7955)
fathead minnow	Pimephales promelas		NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=90988)

Deiodination by DIO enzymes is known to exist in a wide range of vertebrates and invertebrates.

Key Event Description

Disruption of the thyroid hormone system is increasingly being recognized as an important toxicity pathway, as it can cause many adverse outcomes. Thyroid hormones do not only play an important role in the adult individual, but they are also critical during embryonic development. Thyroid hormones (THs) play an important role in a wide range of biological processes in vertebrates including growth, development, reproduction, cardiac function, thermoregulation, response to injury, tissue repair and homeostasis. Numerous chemicals are known to disturb thyroid function, for example by inhibiting thyroperoxidase (TPO) or deiodinase (DIO), upregulating excretion pathways or modifying gene expression. The two major thyroid hormones are triiodothyronine (T3) and thyroxine (T4), both iodinated derivatives of tyrosine. The synthesis of the thyroid hormones is a process that involves several steps. Thyroglobulin, the thyroid hormone precursor, is produced by the thyroid epithelial cells and transported to the lumen via exocytosis. Then thyroperoxidase (TPO) plays an essential role in the production of mainly T4. The prohormone T4 is then released in the circulation under the influence of thyroid stimulating hormone (TSH), in order to be transported to the various tissues, including the liver, the kidneys and the heart. Most TH actions depend on the binding of T3 to its nuclear receptors. Active and inactive THs are tightly regulated by enzymes called iodothyronine deiodinases (DIO). The activation occurs via outer ring deiodination (ORD), i.e. removing iodine from the phenolic ring of T4 to form T3, while inactivation occurs via inner ring deiodination (IRD), i.e. removing iodine from the inner tyrosol ring of T4 or T3.

Three types of iodothyronine deiodinases (DIO1-3) have been described in vertebrates that activate or inactivate THs and are therefore important mediators of TH action. All deiodinases are integral membrane proteins of the thioredoxin superfamily that contain selenocysteine in their catalytic centre. Type I deiodinase is capable to convert T4 into T3, as well as to convert rT3 to the inactive thyroid hormone 3,3'-T2, through outer ring deiodination. rT3 is the preferred substrate for DIO1 (Hennemann G, Visser TJ 1997). Type II deiodinase (DIO2) is only capable of ORD activity with T4 as a preferred substrate. DIO3 can inner ring deiodinate T4 and T3 to the inactive forms of THs, reverse T3, (rT3) and 3,3'-T2 respectively

How it is Measured or Detected

At this time, there are no approved OECD or EPA guideline protocols for measurement of DIO inhibition. Deiodination is the major pathway regulating T3 bioavailability in mammalian tissues. The objective of this in vitro assay is to examine inhibition of deiodinase 1 (DIO1) activity upon exposure to thyroid disrupting compounds, using unexposed pig liver tissue. There are three types of deiodinase measurements available. A first in vitro assay measures deiodinase activities by quantifying the radioactive iodine release from iodine-labelled substrates, depending on the preferred substrates of the isoforms of deiodinases. A second assay is a chromatography-based method coupled to mass spectroscopy to measure products of thyroxine by deiodinase type-1 activity (Butt et al., 2010). Finally, a colorimetric method was developed (Renko et al., 2012) that measures the release of iodine from T4.

Although the radioactive based assays uses radioactivity to measure deiodinase activity, they provide a good balance between specificity and resources needed. The chromatography-based assay has a high sensitivity and specificity to measure all thyroid hormones metabolites, but a high degree of technical expertise and expensive instrumentation is required. Although the colorimetric method is a promising alternative, the sensitivity of this assay is still limited.

For all the reasons above, we chose to use the radioactive method. Since DIO1 and DIO2 prefer a different substrate to deiodinate, i.e. rT3 and T4 respectively, it is possible to quantify outer-ring deiodination using the specific enzymkinetics of both enzymes. This assay measures the amount of radioactive iodine that is released from 125I-labelled substrates by conversion of one of the substrates by the DIO enzymes. We used a pig liver homogenate preparation and reaction buffers containing DTT as co-substrate. Furthermore, a concentration range of potential thyroid-disrupting chemicals can be added to measure the inhibitory potencies of the chemicals the inhibit DIO enzyme activity. Enzyme activity is expressed as picomoles or femtomoles of released radioactive iodine per minute per mg protein and if inhibition occurs, the half maximal inhibitory concentration (IC50) was determined.

References

- Visser, T.J., Van Overmeeren, E., Fekkes, D., Docter, R., Hennemann, G. 1979. Inhibition of iodothyronine 5'-deiodinase by thioureylenes: structure-activity relationship. *FEBS Letters*, 103, 2.
- Butt, C.M., Wang, D., Stapleton, H.M. 2011. Halogenated phenolic contaminants inhibit the in vitro activity of the thyroid-regulating deiodinases in human liver. *Toxicological sciences* 124: 339-347.
- Renko, K., Hoefie, C.S., Hiller, F., Schomburg, L., Köhrle, J. 2012. Identification of lipoic acid as substrate of type 1 deiodinase by a novel nonradioactive iodide-release assay. *Endocrinology*, 153: 2506-2513.

List of Key Events in the AOP

Event: 1003: Decreased, Triiodothyronine (T3) in serum (<https://aopwiki.org/events/1003>)

Short Name: Decreased, Triiodothyronine (T3) in serum

Key Event Component

Process	Object	Action
abnormal circulating hormone level		decreased

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:155 - Deiodinase 2 inhibition leading to reduced young of year survival via posterior swim bladder inflation (https://aopwiki.org/aops/155)	KeyEvent
Aop:156 - Deiodinase 2 inhibition leading to reduced young of year survival via anterior swim bladder inflation (https://aopwiki.org/aops/156)	KeyEvent
Aop:157 - Deiodinase 1 inhibition leading to reduced young of year survival via posterior swim bladder inflation (https://aopwiki.org/aops/157)	KeyEvent
Aop:158 - Deiodinase 1 inhibition leading to reduced young of year survival via anterior swim bladder inflation (https://aopwiki.org/aops/158)	KeyEvent
Aop:189 - Type I iodothyronine deiodinase (DIO1) inhibition leading to altered amphibian metamorphosis (https://aopwiki.org/aops/189)	KeyEvent

Biological Context

Level of Biological Organization
Tissue

Organ term

Organ term
serum

Domain of Applicability

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
zebrafish	Danio rerio		NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=7955)
fathead minnow	Pimephales promelas		NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=90988)

The overall evidence supporting taxonomic applicability is strong. With few exceptions vertebrate species have circulating T3 and T4 that are bound to transport proteins in blood. Clear species differences exist in transport proteins (Yamauchi and Ishihara, 2009). Specifically, the majority of supporting data for TH decreases in serum come from rat studies, and the predominant iodothyronine binding protein in rat serum is transthyretin (TT4). TT4 demonstrates a reduced binding affinity for T4 when compared with thyroxine binding globulin (TBG), the predominant serum binding protein for T4 in humans. This difference in serum binding protein affinity for THs is thought to modulate serum half-life for T4; the half-life of T4 in rats is 12-24 hr, whereas the half-life in humans is 5-9 days (Capen, 1997). While these species differences impact hormone half-life, possibly regulatory feedback mechanisms, and quantitative dose-response relationships, measurement of serum THs is still regarded as a measurable key event causatively linked to downstream adverse outcomes.

THs are evolutionarily conserved molecules present in all vertebrate species (Hulbert, 2000; Yen, 2001). Moreover, their crucial role in amphibian and larbean metamorphoses is well established (Manzon and Youson, 1997; Yaoita and Brown, 1990). Their existence and importance has been also described in many different animal and plant kingdoms (Eales, 1997; Heyland and Moroz, 2005), while their role as environmental messenger via exogenous routes in echinoderms confirms the hypothesis that these molecules are widely distributed among the living organisms (Heyland and Hodin, 2004). However, the role of TH in the different species may differ depending on the expression or function of specific proteins (e.g. receptors or enzymes) that are related to TH function, and therefore extrapolation between species should be done with cautious.

Key Event Description

There are two biological active thyroid hormones (THs), triiodothyronine (T3) and thyroxine (T4), and a few inactive iodothyronines (rT3, 3,5-T2), which are all derived from the modification of tyrosine molecules (Hulbert, 2000). However, the plasma concentrations of the other iodothyronines are significantly lower than those of T3 and T4. The different iodothyronines are formed by the sequential outer or inner ring monodeiodination of T4 by the deiodinating enzymes, Dio1, Dio2, and Dio3 (Gereben et al., 2008). Deiodinase structure is considered to be unique, as THs are the only molecules in the body that incorporate iodide.

The circulatory system serves as the major transport and delivery system for THs from synthesis in the gland to delivery to tissues. The majority of THs in the blood are bound to transport proteins (Bartalena and Robbins, 1993). In humans, the major transport proteins are TBG (thyroxine binding globulin), TTR (transthyretin) and albumin. The percent bound to these proteins in adult humans is about 75, 15 and 10 percent, respectively (Schussler 2000). Unbound (free) hormones are approximately 0.03 and 0.3 percent for T4 and T3, respectively. In serum, it is the free form of the hormone that is active.

There are major species differences in the predominant binding proteins and their affinities for THs (see section below on Taxonomic applicability). However, there is broad agreement that changes in serum concentrations of THs is diagnostic of thyroid disease or chemical-induced disruption of thyroid homeostasis (Zoeller et al., 2007).

It is notable that the changes measured in the TH concentration reflect mainly the changes in the serum transport proteins rather than changes in the thyroid status. These thyroid-binding proteins serve as hormonal store which ensure their even and constant distribution in the different tissues, while they protect the most sensitive ones in the case of severe changes in thyroid availability, like in thyroidectomies (Obregon et al., 1981). Until recently, it was believed that all of the effects of TH were mediated by the binding of T3 to the thyroid nuclear receptors (TRa and TRb), a notion which is now questionable due to the increasing evidence that support the non-genomic action of TH (Davis et al., 2010; Moeller et al., 2006). Many non-nuclear TH binding sites have been identified to date and they usually lead to rapid cellular response in TH-effects (Bassett et al., 2003), but the specific pathways that are activated in this regard need to be elucidated.

The production of THs in the thyroid gland and the circulation levels in the bloodstream are self-controlled by an efficiently regulated feedback mechanism across the Hypothalamus-Pituitary-Thyroid (HPT) axis. One of the most unique characteristics of TH is their ability to regulate their own concentration, not only in the plasma level, but also in the individual cell level, to maintain their homeostasis. This is succeed by the efficient regulatory mechanism of the thyroid hormone axis which consists of the following: (1) the hypothalamic secretion of the thyrotropin-releasing

hormone (TRH), (2) the thyroid-stimulating hormone (TSH) secretion from the anterior pituitary, (3) hormonal transport by the plasma binding proteins, (4) cellular uptake mechanisms in the cell level, (5) intracellular control of TH concentration by the deiodinating mechanism (6) transcriptional function of the nuclear thyroid hormone receptor and (7) in the fetus, the transplacental passage of T4 and T3 (Cheng et al., 2010).

In regards to the brain, the TH concentration involves also an additional level of regulation, namely the hormonal transport through the Blood Brain Barrier (BBB) (Williams, 2008). The TRH and the TSH are actually regulating the production of pro-hormone T4 and in a lesser extent of T3, which is the biologically active TH. The rest of the required amount of T3 is produced by outer ring deiodination of T4 by the deiodinating enzymes D1 and D2 (Bianco et al., 2006), a process which takes place mainly in liver and kidneys but also in other target organs such as in the brain, the anterior pituitary, brown adipose tissue, thyroid and skeletal muscle (Gereben et al., 2008; Larsen, 2009). Both hormones exert their action in almost all tissues of mammals and they are acting intracellularly, and thus the uptake of T3 and T4 by the target cells is a crucial step of the overall pathway. The trans-membrane transport of TH is performed mainly through transporters that differ depending on the cell type (Hennemann et al., 2001; Friesema et al., 2005; Visser et al., 2008). Many transporter proteins have been identified up to date but the monocarboxylate transporters (Mct8, Mct10) and the anion-transporting polypeptide (OATP1c1) show the highest degree of affinity towards TH (Jansen et al., 2005).

T3 and T4 have significant effects on normal development, neural differentiation, growth rate and metabolism (Yen, 2001; Brent, 2012; Williams, 2008), with the most prominent ones to occur during the fetal development and early childhood. The clinical features of hypothyroidism and hyperthyroidism emphasize the pleiotropic effects of these hormones on many different pathways and target organs. The thyroidal actions though are not only restricted to mammals, as their high significance has been identified also for other vertebrates, with the most well-studied to be the amphibian metamorphosis (Furlow and Neff, 2006). The importance of the thyroid-regulated pathways becomes more apparent in iodine deficient areas of the world, where a higher rate of cretinism and growth retardation has been observed and linked to decreased TH levels (Gilbert et al., 2012). Another very common cause of severe hypothyroidism in human is the congenital hypothyroidism, but the manifestation of these effects is only detectable in the lack of adequate treatment and is mainly related to neurological impairment and growth retardation (Glinioer, 2001), emphasizing the role of TH in neurodevelopment in all above cases. In adults, the thyroid-related effects are mainly linked to metabolic activities, such as deficiencies in oxygen consumption, and in the metabolism of the vitamin, proteins, lipids and carbohydrates, but these defects are subtle and reversible (Oetting and Yen, 2007). Blood tests to detect the amount of thyroid hormone (T4) and thyroid stimulating hormone (TSH) are routinely done for newborn babies for the diagnosis of congenital hypothyroidism at the earliest stage possible.

How it is Measured or Detected

T3 and T4 can be measured as free (unbound) or total (bound + unbound). Free hormone are considered more direct indicators of T4 and T3 activities in the body. The majority of T3 and T4 measurements are made using either RIA or ELISA kits. In animal studies, total T3 and T4 are typically measured as the concentrations of free hormone are very low and difficult to detect. Historically, the most widely used method in toxicology is RIA. The method is routinely used in rodent endocrine and toxicity studies. The ELISA method has become more routine in rodent studies. The ELISA method is a commonly used as a human clinical test method. Least common is analytical determination of iodothyronines (T3, T4, rT3, T2) and their conjugates, though methods employing HPLC and mass spectrometry (DeVito et al., 1999; Miller et al., 2009).

Any of these measurements should be evaluated for fit-for-purpose, relationship to the actual endpoint of interest, repeatability, and reproducibility. All three of the methods summarized above would be fit-for-purpose, depending on the number of samples to be evaluated and the associated costs of each method. Both RIA and ELISA measure THs by an indirect methodology, whereas analytical determination is the most direct measurement available. All of these methods, particularly RIA, are repeatable and reproducible.

References

- Bartalena L, Robbins J. Thyroid hormone transport proteins. *Clin Lab Med*. 1993 Sep;13(3):583-98.
- Bassett JH, Harvey CB, Williams GR. (2003). Mechanisms of thyroid hormone receptor-specific nuclear and extra nuclear actions. *Mol Cell Endocrinol*. 213:1-11.
- Bianco AC, Kim BW. (2006). Deiodinases: implications of the local control of thyroid hormone action. *J Clin Invest*. 116: 2571–2579.
- Brent GA. (2012). Mechanisms of thyroid hormone action. *J Clin Invest*. 122: 3035-3043.
- Cheng SY, Leonard JL, Davis PJ. (2010). Molecular aspects of thyroid hormone actions. *Endocr Rev*. 31:139–170.
- Davis PJ, Zhou M, Davis FB, Lansing L, Mousa SA, Lin HY. (2010). Mini-review: Cell surface receptor for thyroid hormone and nongenomic regulation of ion fluxes in excitable cells. *Physiol Behav*. 99:237–239.
- DeVito M, Biegel L, Brouwer A, Brown S, Brucker-Davis F, Cheek AO, Christensen R, Colborn T, Cooke P, Crissman J, Crofton K, Doerge D, Gray E, Hauser P, Hurley P, Kohn M, Lazar J, McMaster S, McClain M, McConnell E, *Meier C, Miller R, Tietge J, Tyl R. (1999). Screening methods for thyroid hormone disruptors. *Environ Health Perspect*. 107:407-415.
- Eales JG. (1997). Iodine metabolism and thyroid related functions in organisms lacking thyroid follicles: Are thyroid hormones also vitamins? *Proc Soc Exp Biol Med*. 214:302-317.
- Friesema EC, Jansen J, Milici C, Visser TJ. (2005). Thyroid hormone transporters. *Vitam Horm*. 70: 137–167.
- Furlow JD, Neff ES. (2006). A developmental switch induced by thyroid hormone: *Xenopus laevis* metamorphosis. *Trends Endocrinol Metab*. 17:40–47.
- Gereben B, Zavacki AM, Ribich S, Kim BW, Huang SA, Simonides WS, Zeöld A, Bianco AC. (2008). Cellular and molecular basis of deiodinase-regulated thyroid hormone signalling. *Endocr Rev*. 29:898–938.
- Gilbert ME, Rovet J, Chen Z, Koibuchi N. (2012). Developmental thyroid hormone disruption: prevalence, environmental contaminants and neurodevelopmental consequences. *Neurotoxicology*. 33: 842-852.
- Glinioer D. (2001). Potential consequences of maternal hypothyroidism on the offspring: evidence and implications. *Horm Res*. 55:109-114.
- Hennemann G, Docter R, Friesema EC, de Jong M, Krenning EP, Visser TJ. (2001). Plasma membrane transport of thyroid hormones and its role in thyroid hormone metabolism and bioavailability. *Endocr Rev*. 22:451-476.
- Heyland A, Hodin J. (2004). Heterochronic developmental shift caused by thyroid hormone in larval sand dollars and its implications for phenotypic plasticity and the evolution of non-feeding development. *Evolution*. 58: 524-538.
- Heyland A, Moroz LL. (2005). Cross-kingdom hormonal signaling: an insight from thyroid hormone functions in marine larvae. *J Exp Biol*.

208:4355-4361.

- Hulbert A J. (2000). Thyroid hormones and their effects: A new perspective. *Biol Rev.* 75: 519-631.
- Jansen J, Friesema EC, Milici C, Visser TJ. (2005). Thyroid hormone transporters in health and disease. *Thyroid.* 15: 757-768.
- Larsen PR. (2009). Type 2 iodothyronine deiodinase in human skeletal muscle: new insights into its physiological role and regulation. *J Clin Endocrinol Metab.* 94:1893-1895.
- Manzon RG, Youson JH. (1997). The effects of exogenous thyroxine (T4) or triiodothyronine (T3), in the presence and absence of potassium perchlorate, on the incidence of metamorphosis and on serum T4 and T3 concentrations in larval sea lampreys (*Petromyzon marinus* L.). *Gen Comp Endocrinol.* 106:211-220.
- Miller MD, Crofton KM, Rice DC, Zoeller RT. (2009). Thyroid-disrupting chemicals: interpreting upstream biomarkers of adverse outcomes. *Environ Health Perspect.* 117:1033-1041.
- Moeller LC, Dumitrescu AM, Seo H, Refetoff S. (2006). Thyroid hormone mediated changes in gene expression can be initiated by cytosolic action of the thyroid hormone receptor β through the phosphatidylinositol 3-kinase pathway. *NRS.* 4:1-4.
- Obregon MJ, Mallol J, Escobar del Rey F, Morreale de Escobar G. (1981). Presence of L-thyroxine and 3,5,3-triiodo-L-thyronine in tissues from thyroidectomized rats. *Endocrinology* 109:908-913.
- Oetting A, Yen PM. (2007). New insights into thyroid hormone action. *Best Pract Res Clin Endocrinol Metab.* 21:193-208.
- Schussler, G.C. (2000). The thyroxine-binding proteins. *Thyroid* 10:141-149.
- Visser WE, Friesema EC, Jansen J, Visser TJ. (2008). Thyroid hormone transport in and out of cells. *Trends Endocrinol Metab.* 19:50-56.
- Williams GR. (2008). Neurodevelopmental and neurophysiological actions of thyroid hormone. *J Neuroendocrinol.* 20:784-794.
- Yamauchi K1, Ishihara A. Evolutionary changes to transthyretin: developmentally regulated and tissue-specific gene expression. *FEBS J.* 2009 Oct;276(19):5357-66.
- Yaoita Y, Brown DD. (1990). A correlation of thyroid hormone receptor gene expression with amphibian metamorphosis. *Genes Dev.* 4:1917-1924.
- Yen PM. (2001). Physiological and molecular basis of thyroid hormone action. *Physiol Rev.* 81:1097-1142.
- Zoeller RT, Tan SW, Tyl RW. General background on the hypothalamic-pituitary-thyroid (HPT) axis. *Crit Rev Toxicol.* 2007 Jan-Feb;37(1-2):11-53

Event: 1007: Reduced, Anterior swim bladder inflation (<https://aopwiki.org/events/1007>)

Short Name: Reduced, Anterior swim bladder inflation

Key Event Component

Process	Object	Action
swim bladder inflation	anterior chamber swim bladder	decreased

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:155 - Deiodinase 2 inhibition leading to reduced young of year survival via posterior swim bladder inflation (https://aopwiki.org/aops/155)	KeyEvent
Aop:156 - Deiodinase 2 inhibition leading to reduced young of year survival via anterior swim bladder inflation (https://aopwiki.org/aops/156)	KeyEvent
Aop:157 - Deiodinase 1 inhibition leading to reduced young of year survival via posterior swim bladder inflation (https://aopwiki.org/aops/157)	KeyEvent
Aop:158 - Deiodinase 1 inhibition leading to reduced young of year survival via anterior swim bladder inflation (https://aopwiki.org/aops/158)	KeyEvent
Aop:159 - Thyroperoxidase inhibition leading to reduced young of year survival via anterior swim bladder inflation (https://aopwiki.org/aops/159)	KeyEvent

Biological Context

Level of Biological Organization
Organ

Organ term

Organ term
swim bladder

Domain of Applicability

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
zebrafish	Danio rerio		NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=7955)
fathead minnow	Pimephales promelas		NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=90988)

The evidence for impaired inflation of the anterior chamber of the swim bladder currently comes from work on zebrafish and fathead minnow.

Key Event Description

The swim bladder of bony fish is evolutionary homologous to the lung (Zheng et al., 2011). The teleost swim bladder is a gas-filled structure that consists of two chambers, the posterior and anterior chamber. In zebrafish, the posterior chamber inflates around 96 h post fertilization (hpf) which is 2 days post hatch, and the anterior chamber inflates around 21 dpf. In fathead minnow, the posterior and anterior chamber inflate around 6 and 14 dpf respectively.

The anterior chamber is formed by evagination from the cranial end of the posterior chamber (Robertson et al., 2007). Dumbarton et al. (2010) showed that the anterior chamber of zebrafish has particularly closely packed and highly organized bundles of muscle fibres, suggesting that contraction of these muscles would reduce swim bladder volume. While it had previously been suggested that the posterior chamber had a more important role as a hydrostatic organ, this implies high importance of the anterior chamber for buoyancy. The anterior chamber has an additional role in hearing (Bang et al., 2002). Weberian ossicles (the Weberian apparatus) connect the anterior chamber to the inner ear resulting in an amplification of sound waves. Reduced inflation of the anterior chamber may manifest itself as either a complete failure to inflate the chamber or reduced size of the chamber. Reduced size is often associated with a deviating morphology.

How it is Measured or Detected

In several fish species, inflation of the anterior chamber can be observed using a stereomicroscope because the larvae are still transparent during the larval stage. This is for example true for zebrafish and fathead minnow. Anterior chamber size can then be measured based on photographs with a calibrator.

References

- Zheng, W., Wang, Z., Collins, J.E., Andrews, R.M., Stemple, D., Gong, Z. 2011. Comparative transcriptome analyses indicate molecular homology of zebrafish swim bladder and mammalian lung. PLoS One 6, <http://dx.doi.org/10.1371/> (<http://dx.doi.org/10.1371/>)
- Roberston, G.N., McGee, C.A.S., Dumbarton, T.C., Croll, R.P., Smith, F.M., 2007. Development of the swim bladder and its innervation in the zebrafish, *Danio rerio*. J. Morphol. 268, 967–985, <http://dx.doi.org/10.1002/jmor> (<http://dx.doi.org/10.1002/jmor>).
- Dumbarton, T.C., Stoyek, M., Croll, R.P., Smith, F.M., 2010. Adrenergic control of swimbladder deflation in the zebrafish (*Danio rerio*). J. Exp. Biol. 213,2536–2546, <http://dx.doi.org/10.1242/jeb.039792> (<http://dx.doi.org/10.1242/jeb.039792>).
- Bang, P.I., Yelick, P.C., Malicko, J.J., Sewell, W.F. 2002. High-throughput behavioral screening method for detecting auditory response defects in zebrafish. Journal of Neuroscience Methods. 118, 177-187.

Event: 1008: Reduced, Hearing (<https://aopwiki.org/events/1008>)

Short Name: Reduced, Hearing

Key Event Component

Process	Object	Action
sensory perception of sound		decreased

AOPs Including This Key Event

AOP158

AOP ID and Name	Event Type
Aop:155 - Deiodinase 2 inhibition leading to reduced young of year survival via posterior swim bladder inflation (https://aopwiki.org/aops/155)	KeyEvent
Aop:156 - Deiodinase 2 inhibition leading to reduced young of year survival via anterior swim bladder inflation (https://aopwiki.org/aops/156)	KeyEvent
Aop:157 - Deiodinase 1 inhibition leading to reduced young of year survival via posterior swim bladder inflation (https://aopwiki.org/aops/157)	KeyEvent
Aop:158 - Deiodinase 1 inhibition leading to reduced young of year survival via anterior swim bladder inflation (https://aopwiki.org/aops/158)	KeyEvent
Aop:159 - Thyroperoxidase inhibition leading to reduced young of year survival via anterior swim bladder inflation (https://aopwiki.org/aops/159)	KeyEvent

Biological Context

Level of Biological Organization
Organ

Organ term

Organ term
ear

Domain of Applicability

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
Vertebrates	Vertebrates		NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=0)
Invertebrates	Invertebrates		NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=0)

- A sense of hearing is known to exist in a wide range of vertebrates and invertebrates, although the organs and structures involved vary widely.

Key Event Description

Hearing refers to the ability to perceive sound vibrations propagated as pressure changes through a medium such as air or water. Reduced hearing in the context of this key event can refer to reduction in the perceived volume of a sound relative to the amplitude of sound waves. Reduced hearing may also refer to a reduced range of frequencies that can be perceived.

How it is Measured or Detected

Hearing is generally measured behaviorally or electrophysiologically.

- Common behavioral tests involve transmission of pure tones of defined amplitude and frequency using an audiometer or PC and using a behavioral response (e.g., clicking a button; startle response) to determine whether the tone is perceived.

Electrophysiological tests:

- Auditory brainstem response (ABR): Uses electrodes placed on the head to detect auditory evoked potentials from background electrical activity in the brain.

Hearing tests in Fish:

- Through the mid-late 1980s conditioning and behavioral tests were most commonly employed in testing fish hearing. Methods reviewed by Fay (1988)

AOP158

- A high throughput behavioral test for detecting auditory response in fish has been described (Bang et al. 2002).
- Invasive electrophysiological methods involving surgical insertion of electrodes into the auditory nerves have been employed.
- Non-invasive recording of Auditory Evoked Potentials (AEPs; synonymous with ABRs) are now the most common approach for measuring hearing in fish. AEPs can be recorded via electrodes attached cutaneously to the head (see review by Ladich and Fay, 2013).

References

- Fay RR (1988) Hearing in vertebrates: a psychophysics databook. Hill-Fay Associates, Winnetka, Ill
- Ladich F, Fay RR. Auditory evoked potential audiometry in fish. Reviews in Fish Biology and Fisheries. 2013;23(3):317-364. doi:10.1007/s11160-012-9297-z.
- Bang PI, Yelick PC, Malicki JJ, Sewell WF. High-throughput behavioral screening method for detecting auditory response defects in zebrafish. J Neurosci Methods. 2002 Aug 30;118(2):177-87. PubMed PMID: 12204308.

Event: 1005: Reduced, Swimming performance (<https://aopwiki.org/events/1005>)

Short Name: Reduced, Swimming performance

Key Event Component

Process	Object	Action
aquatic locomotion		decreased

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:155 - Deiodinase 2 inhibition leading to reduced young of year survival via posterior swim bladder inflation (https://aopwiki.org/aops/155)	KeyEvent
Aop:156 - Deiodinase 2 inhibition leading to reduced young of year survival via anterior swim bladder inflation (https://aopwiki.org/aops/156)	KeyEvent
Aop:157 - Deiodinase 1 inhibition leading to reduced young of year survival via posterior swim bladder inflation (https://aopwiki.org/aops/157)	KeyEvent
Aop:158 - Deiodinase 1 inhibition leading to reduced young of year survival via anterior swim bladder inflation (https://aopwiki.org/aops/158)	KeyEvent
Aop:159 - Thyroperoxidase inhibition leading to reduced young of year survival via anterior swim bladder inflation (https://aopwiki.org/aops/159)	KeyEvent
Aop:242 - Inhibition of lysyl oxidase leading to enhanced chronic fish toxicity (https://aopwiki.org/aops/242)	KeyEvent

Biological Context

Level of Biological Organization
Individual

Domain of Applicability

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
zebrafish	Danio rerio		NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=7955)

Importance of swimming performance for natural behaviour is generally applicable to fish.

Key Event Description

Adequate swimming performance in fish is essential for behaviour such as foraging, predator avoidance and reproduction.

How it is Measured or Detected

For fish larvae, automated observation and tracking systems are commercially available and increasingly used for measuring swimming performance including distance travelled, duration of movements, swimming speed, etc. This kind of measurements is often included in publications describing effects of chemicals in zebrafish larvae (Hagenaars et al., 2014; Stinckens et al., 2016; Vergauwen et al., 2015).

References

Hagenaars, A., Stinckens, E., Vergauwen, L., Bervoets, L., Knapen, D., 2014. PFOS affects posterior swim bladder chamber inflation and swimming performance of zebrafish larvae. *Aquat. Toxicol.* 157, 225–235.

Stinckens, E., Vergauwen, L., Schroeder, A.L., Maho, W., Blackwell, B., Witter, H., Blust, R., Ankley, G.T., Covaci, A., Villeneuve, D.L., Knapen, D., 2016. Disruption of thyroid hormone balance after 2-mercaptobenzothiazole exposure causes swim bladder inflation impairment—part II: zebrafish. *Aquat. Toxicol.* 173:204-17.

Vergauwen, Lucia; Nørgaard Schmidt, Stine; Maho, Walid; Stinckens, Evelyn; Hagenaars, An; Blust, Ronny; Mayer, Philipp; Covaci, Adrian; Knapen, Dries. 2014. A high throughput passive dosing format for the Fish Embryo Acute Toxicity test. *Chemosphere.* 139: 9-17.

Event: 1006: Reduced, Young of year survival (<https://aopwiki.org/events/1006>)

Short Name: Reduced, Young of year survival

Key Event Component

Process	Object	Action
survival		decreased

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:155 - Deiodinase 2 inhibition leading to reduced young of year survival via posterior swim bladder inflation (https://aopwiki.org/aops/155)	KeyEvent
Aop:156 - Deiodinase 2 inhibition leading to reduced young of year survival via anterior swim bladder inflation (https://aopwiki.org/aops/156)	KeyEvent
Aop:157 - Deiodinase 1 inhibition leading to reduced young of year survival via posterior swim bladder inflation (https://aopwiki.org/aops/157)	KeyEvent
Aop:158 - Deiodinase 1 inhibition leading to reduced young of year survival via anterior swim bladder inflation (https://aopwiki.org/aops/158)	KeyEvent
Aop:159 - Thyroperoxidase inhibition leading to reduced young of year survival via anterior swim bladder inflation (https://aopwiki.org/aops/159)	KeyEvent

Biological Context

Level of Biological Organization
Individual

Domain of Applicability

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
zebrafish	Danio rerio		NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=7955)

Survival is important for all species.

Key Event Description

Young of year refers to young animals (usually fish) produced in one reproductive year, which have not yet reached one year of age. Small fish, hatched from eggs spawned in the current year, are considered young of year.

Young of year survival directly impacts population structure, growth and fitness. Maintenance of sustainable fish and wildlife populations is an accepted regulatory goal upon which risk assessments and risk management decisions are based.

How it is Measured or Detected

Young of year survival can be measured:

- in the lab by recording survival during prolonged exposure experiments
- in dedicated mesocosms, or in drainable ponds
- in the field, for example by determining age structure after one capture, or by capture-tag-recapture efforts

List of Adverse Outcomes in this AOP

Event: 360: Decrease, Population trajectory (<https://aopwiki.org/events/360>)

Short Name: Decrease, Population trajectory

Key Event Component

Process	Object	Action
population growth rate		decreased

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:23 - Androgen receptor agonism leading to reproductive dysfunction (in repeat-spawning fish) (https://aopwiki.org/aops/23)	AdverseOutcome
Aop:25 - Aromatase inhibition leading to reproductive dysfunction (https://aopwiki.org/aops/25)	AdverseOutcome
Aop:29 - Estrogen receptor agonism leading to reproductive dysfunction (https://aopwiki.org/aops/29)	AdverseOutcome
Aop:30 - Estrogen receptor antagonism leading to reproductive dysfunction (https://aopwiki.org/aops/30)	AdverseOutcome
Aop:100 - Cyclooxygenase inhibition leading to reproductive dysfunction via inhibition of female spawning behavior (https://aopwiki.org/aops/100)	AdverseOutcome
Aop:122 - Prolyl hydroxylase inhibition leading to reproductive dysfunction via increased HIF1 heterodimer formation (https://aopwiki.org/aops/122)	AdverseOutcome
Aop:123 - Unknown MIE leading to reproductive dysfunction via increased HIF-1alpha transcription (https://aopwiki.org/aops/123)	AdverseOutcome
Aop:155 - Deiodinase 2 inhibition leading to reduced young of year survival via posterior swim bladder inflation (https://aopwiki.org/aops/155)	AdverseOutcome
Aop:156 - Deiodinase 2 inhibition leading to reduced young of year survival via anterior swim bladder inflation (https://aopwiki.org/aops/156)	AdverseOutcome
Aop:157 - Deiodinase 1 inhibition leading to reduced young of year survival via posterior swim bladder inflation (https://aopwiki.org/aops/157)	AdverseOutcome
Aop:158 - Deiodinase 1 inhibition leading to reduced young of year survival via anterior swim bladder inflation (https://aopwiki.org/aops/158)	AdverseOutcome
Aop:159 - Thyroperoxidase inhibition leading to reduced young of year survival via anterior swim bladder inflation (https://aopwiki.org/aops/159)	AdverseOutcome
Aop:101 - Cyclooxygenase inhibition leading to reproductive dysfunction via inhibition of pheromone release (https://aopwiki.org/aops/101)	AdverseOutcome

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AOP ID and Name	Event Type
Aop:102 - Cyclooxygenase inhibition leading to reproductive dysfunction via interference with meiotic prophase I/metaphase I transition (https://aopwiki.org/aops/102)	AdverseOutcome
Aop:63 - Cyclooxygenase inhibition leading to reproductive dysfunction (https://aopwiki.org/aops/63)	AdverseOutcome
Aop:103 - Cyclooxygenase inhibition leading to reproductive dysfunction via interference with spindle assembly checkpoint (https://aopwiki.org/aops/103)	AdverseOutcome

Biological Context

Level of Biological Organization
Population

Domain of Applicability

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
all species	all species		NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=0)

Life Stage Applicability

Life Stage	Evidence
All life stages	Not Specified

Sex Applicability

Sex	Evidence
Unspecific	Not Specified

Consideration of population size and changes in population size over time is potentially relevant to all living organisms.

Key Event Description

Maintenance of sustainable fish and wildlife populations (i.e., adequate to ensure long-term delivery of valued ecosystem services) is an accepted regulatory goal upon which risk assessments and risk management decisions are based.

How it is Measured or Detected

Population trajectories, either hypothetical or site specific, can be estimated via population modeling based on measurements of vital rates or reasonable surrogates measured in laboratory studies. As an example, Miller and Ankley 2004 used measures of cumulative fecundity from laboratory studies with repeat spawning fish species to predict population-level consequences of continuous exposure.

Regulatory Significance of the AO

Maintenance of sustainable fish and wildlife populations (i.e., adequate to ensure long-term delivery of valued ecosystem services) is a widely accepted regulatory goal upon which risk assessments and risk management decisions are based.

References

- Miller DH, Ankley GT. 2004. Modeling impacts on populations: fathead minnow (*Pimephales promelas*) exposure to the endocrine disruptor 17β-trenbolone as a case study. *Ecotoxicology and Environmental Safety* 59: 1-9.

Appendix 2

List of Key Event Relationships in the AOP

List of Adjacent Key Event Relationships

Relationship: 1037: Inhibition, Deiodinase 1 leads to Decreased, Triiodothyronine (T3) in serum (<https://aopwiki.org/relationships/1037>)

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Deiodinase 1 inhibition leading to reduced young of year survival via posterior swim bladder inflation (https://aopwiki.org/aops/157)	adjacent		
Deiodinase 1 inhibition leading to reduced young of year survival via anterior swim bladder inflation (https://aopwiki.org/aops/158)	adjacent		

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
zebrafish	Danio rerio		NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=7955)
fathead minnow	Pimephales promelas		NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=90988)

Evidence Supporting this KER

Biological Plausibility

Inhibition of DIO activity is widely accepted to directly impact the T3 levels in serum, since the conversion of T4 to T3 is inhibited.

Relationship: 1035: Decreased, Triiodothyronine (T3) in serum leads to Reduced, Anterior swim bladder inflation (<https://aopwiki.org/relationships/1035>)

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Deiodinase 2 inhibition leading to reduced young of year survival via anterior swim bladder inflation (https://aopwiki.org/aops/156)	adjacent		
Deiodinase 1 inhibition leading to reduced young of year survival via anterior swim bladder inflation (https://aopwiki.org/aops/158)	adjacent		

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
zebrafish	Danio rerio		NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=7955)
fathead minnow	Pimephales promelas		NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=90988)

Evidence Supporting this KER

Empirical Evidence

- In a study in which embryo-larval fathead minnows (*Pimephales promelas*) were exposed to the thyroid peroxidase inhibitor 2-mercaptobenzothiazole (MBT), T3 concentrations measured at 14dpf were reduced at the same concentration (1 mg/L) that significantly reduced anterior swim bladder inflation at the same time-point (Nelson et al. 2016).
- Maternal injection of T3, resulting in increased T3 concentrations in the eggs of striped bass (*Morone saxatilis*) lead to significant increases in both swim bladder inflation and survival (Brown et al., 1988).

Uncertainties and Inconsistencies

- Li et al. (2011) reported impairment of swim bladder inflation in Chinese rare minnows (*Gobiocypris rarus*) exposed to exogenous T3.

References

- Nelson KR, Schroeder AL, Ankley GT, Blackwell BR, Blanksma C, Degitz SJ, Flynn KM, Jensen KM, Johnson RD, Kahl MD, Knapen D, Kosian PA, Milsik RY, Randolph EC, Saari T, Stinckens E, Vergauwen L, Villeneuve DL. Impaired anterior swim bladder inflation following exposure to the thyroid peroxidase inhibitor 2-mercaptobenzothiazole part I: Fathead minnow. *Aquat Toxicol.* 2016 Apr;173:192-203. doi: 10.1016/j.aquatox.2015.12.024.
- Brown, C. L., Doroshov, S. I., Nunez, J. M., Hadley, C., Vaneenennaam, J., Nishioka, R. S. and Bern, H. A. (1988), Maternal triiodothyronine injections cause increases in swimbladder inflation and survival rates in larval striped bass, *Morone saxatilis*. *J. Exp. Zool.*, 248: 168–176. doi: 10.1002/jez.1402480207
- Li W, Zha J, Yang L, Li Z, Wang Z. Regulation of thyroid hormone related genes mRNA expression by exogenous T₃ in larvae and adult Chinese rare minnow (*Gobiocypris rarus*). *Environ Toxicol Pharmacol.* 2011 Jan;31(1):189-97. doi: 10.1016/j.etap.2010.10.007.

Relationship: 1032: Reduced, Anterior swim bladder inflation leads to Reduced, Hearing
(<https://aopwiki.org/relationships/1032>)

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Deiodinase 2 inhibition leading to reduced young of year survival via posterior swim bladder inflation (https://aopwiki.org/aops/155)	adjacent		
Deiodinase 2 inhibition leading to reduced young of year survival via anterior swim bladder inflation (https://aopwiki.org/aops/156)	adjacent		
Deiodinase 1 inhibition leading to reduced young of year survival via posterior swim bladder inflation (https://aopwiki.org/aops/157)	adjacent		
Deiodinase 1 inhibition leading to reduced young of year survival via anterior swim bladder inflation (https://aopwiki.org/aops/158)	adjacent		
Thyropoxidase inhibition leading to reduced young of year survival via anterior swim bladder inflation (https://aopwiki.org/aops/159)	adjacent		

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
zebrafish	Danio rerio		NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=7955)
fathead minnow	Pimephales promelas		NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=90988)

Within fish we can distinguish between hearing generalists (non-specialists) such as cichlids, salmonids, sunfishes and toadfishes and hearing specialists which have accessory hearing structures (specializations) such as the Weberian apparatus in otophysines, suprabranchial chambers in labyrinth fish and auditory bullae in mormyrids (Ladich and Wysocki, 2003; Ladich and Fay, 2013). In fish that do not possess an anterior chamber with a function in hearing this KER is not applicable.

Key Event Relationship Description

Apart from a role in buoyancy that is not completely understood with regard to the relation to the function of the posterior chamber, the anterior chamber of the swim bladder of many fish species has an additional role in the production and/or detection of sound (Popper et al., 1974; Bang et al., 2002). Several fish families have Weberian ossicles (tiny bones, also called the Weberian apparatus), connecting the anterior chamber to the

inner ear resulting in an amplification of sound waves. Therefore it is plausible to assume that if the anterior chamber does not inflate or inflates to a reduced size, the connection to the Weberian ossicles is lost and hearing is impaired.

Evidence Supporting this KER

Biological Plausibility

It is plausible to assume that if the anterior chamber does not inflate or inflates to a reduced size, the connection to the Weberian ossicles is lost and hearing is impaired.

Empirical Evidence

- Bang et al. (2002) developed a behavioural screening method for detecting hearing defects in zebrafish. In this method they measure a rapid escape reflex in response to a loud sound. They tested 6500 wildtype fish and found that 1% of them had a hearing deficit. When investigating the morphology of the auditory system of these non-responders, they found that nearly all of them showed abnormalities in the swim bladder or Weberian ossicles. Specifically, in 36% of the cases there was only one swim bladder chamber and it was clear that the swim bladder did not touch the first Weberian ossicle (the tripus). Another 36% showed abnormalities in the vertebrae associated with the Weberian ossicles. Fish with normal acoustically mediated startle responses showed no obvious malformations of the swim bladder or Weberian ossicles.
- Ladich and Wysocki (2003) removed the Weberian ossicle directly associated with the anterior chamber (the tripod) in goldfish and showed a frequency-dependent increase of the threshold for perceiving sound.
- Different families of catfish have large variation in the morphology of the swim bladder as well as in the number and size of Weberian ossicles. Lechner and Ladich (2008) showed that over a large range of catfish families larger swim bladders and larger as well as higher numbers of ossicles were related to better hearing abilities.
- Yan et al. (2000) experimentally deflated the swim bladder of goldfish and found that this resulted in a frequency-dependent increase of the threshold for perceiving sound.

Include consideration of temporal concordance here

References

- Bang, P.I., Yelick, P.C., Malicki, J.J., Sewell, W.F., 2002. High-throughput behavioral screening method for detecting auditory response defects in zebrafish. *Journal of Neuroscience Methods* 118, 177-187.
- Ladich, F., Fay, R.R., 2013. Auditory evoked potential audiometry in fish. *Reviews in Fish Biology and Fisheries* 23, 317-364.
- Ladich, F., Wysocki, L.E., 2003. How does tripus extirpation affect auditory sensitivity in goldfish? *Hearing Research* 182, 119-129.
- Lechner, W., Ladich, F., 2008. Size matters: Diversity in swimbladders and Weberian ossicles affects hearing in catfishes. *Journal of Experimental Biology* 211, 1681-1689.
- Popper, A.N., 1974. Response of swim bladder of goldfish (*Carassius auratus*) to acoustic stimuli. *Journal of Experimental Biology* 60, 295-304.
- Yan, H.Y., Fine, M.L., Horn, N.S., Colon, W.E., 2000. Variability in the role of the gasbladder in fish audition. *Journal of Comparative Physiology a-Sensory Neural and Behavioral Physiology* 186, 435-445.

Relationship: 1033: Reduced, Hearing leads to Reduced, Young of year survival (<https://aopwiki.org/relationships/1033>)

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Deiodinase 2 inhibition leading to reduced young of year survival via posterior swim bladder inflation (https://aopwiki.org/aops/155)	adjacent		
Deiodinase 2 inhibition leading to reduced young of year survival via anterior swim bladder inflation (https://aopwiki.org/aops/156)	adjacent		
Deiodinase 1 inhibition leading to reduced young of year survival via posterior swim bladder inflation (https://aopwiki.org/aops/157)	adjacent		
Deiodinase 1 inhibition leading to reduced young of year survival via anterior swim bladder inflation (https://aopwiki.org/aops/158)	adjacent		
Thyropoxidase inhibition leading to reduced young of year survival via anterior swim bladder inflation (https://aopwiki.org/aops/159)	adjacent		

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
zebrafish	Danio rerio		NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=7955)

Key Event Relationship Description

Impaired hearing could result in an impact on ecologically relevant endpoint, such as predator avoidance and prey capture. Therefore, it can be assumed that an affect on hearing could reduce young of year survival.

Evidence Supporting this KER

Biological Plausibility

- In birds, acoustic signals play key roles in territory defense and mate attraction (Slabbekoom and Ripmeester, 2008).

Roles of Acoustic signaling in fish (reviewed by Kasumayan 2009):

- Reproductive isolation - among fish capable of generating sound, sound emission during spawning is the most prominent life stage during which acoustic signaling occurs. Includes mate attraction, courtship, establishment of territory.
- Defensive sounds - fright and stress, alert conspecifics to potential threats.
- Organization of group/aggregative behaviors
- Feeding behaviors - in many fish conditioned reflex to the sounds of conspecifics feeding can be formed and cause orientation or attraction of fish toward their source, particularly in combination with corresponding visual stimuli and odors.

References

- Kasumayan AO. 2009. Acoustic signaling in fish. J. Ichthyology. 49:963-1020.
- SLABBEKOORN, H. and RIPMEESTER, E. A. P. (2008), Birdsong and anthropogenic noise: implications and applications for conservation. Molecular Ecology, 17: 72–83. doi:10.1111/j.1365-294X.2007.03487.x

Relationship: 1030: Reduced, Young of year survival leads to Decrease, Population trajectory
(<https://aopwiki.org/relationships/1030>)

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Deiodinase 2 inhibition leading to reduced young of year survival via posterior swim bladder inflation (https://aopwiki.org/aops/155)	adjacent		
Deiodinase 2 inhibition leading to reduced young of year survival via anterior swim bladder inflation (https://aopwiki.org/aops/156)	adjacent		
Deiodinase 1 inhibition leading to reduced young of year survival via posterior swim bladder inflation (https://aopwiki.org/aops/157)	adjacent		
Deiodinase 1 inhibition leading to reduced young of year survival via anterior swim bladder inflation (https://aopwiki.org/aops/158)	adjacent		
Thyropoxidase inhibition leading to reduced young of year survival via anterior swim bladder inflation (https://aopwiki.org/aops/159)	adjacent		

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
all species	all species		NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=0)

Key Event Relationship Description

If young of year survival is reduced, ultimately the population trajectory will decrease.

Evidence Supporting this KER

Biological Plausibility

It is widely accepted that if young of year survival is reduced, the population trajectory will eventually decrease.

Relationship: 1034: Reduced, Anterior swim bladder inflation leads to Reduced, Swimming performance
(<https://aopwiki.org/relationships/1034>)

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Deiodinase 2 inhibition leading to reduced young of year survival via posterior swim bladder inflation (https://aopwiki.org/aops/155)	adjacent		
Deiodinase 2 inhibition leading to reduced young of year survival via anterior swim bladder inflation (https://aopwiki.org/aops/156)	adjacent		
Deiodinase 1 inhibition leading to reduced young of year survival via posterior swim bladder inflation (https://aopwiki.org/aops/157)	adjacent		
Deiodinase 1 inhibition leading to reduced young of year survival via anterior swim bladder inflation (https://aopwiki.org/aops/158)	adjacent		
Thyropoxidase inhibition leading to reduced young of year survival via anterior swim bladder inflation (https://aopwiki.org/aops/159)	adjacent		

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
zebrafish	Danio rerio		NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=7955)
fathead minnow	Pimephales promelas		NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=90988)

Importance of swimming performance for natural behaviour is generally applicable to fish.

Key Event Relationship Description

Effects on swim bladder inflation can alter swimming performance and buoyancy of fish, which is essential for predator avoidance, energy sparing, migration, reproduction and feeding behaviour, resulting in lower young-of-year survival.

Evidence Supporting this KER

The weight of evidence supporting a direct linkage between these two KEs, i.e. reduced anterior swim bladder inflation and reduced swimming performance, is weak.

Biological Plausibility

The anterior chamber of the swim bladder has a function in regulating the buoyancy of fish, by altering the volume of the swim bladder (Robertson et al., 2007). Fish rely on the lipid and gas content in their body to regulate their position within the water column, with the latter being more efficient at increasing body buoyancy. Therefore, fish with functional swim bladders have no problem supporting their body (Brix 2002), while it is highly likely that impaired inflation severely impacts swimming performance. Fish with no functional swim bladder can survive, but are severely disadvantaged, making the likelihood of surviving smaller.

Empirical Evidence

Buoyancy is one of the primary mechanisms of fish to regulate behaviour, swimming performance and energy expenditure. Robertson et al., (2007) suggested that the swim bladder only starts regulating buoyancy actively from 32 dpf onward in zebrafish, indicating that impaired swim bladder inflation possibly affects swimming activity during late development. The swim bladder only becomes capable of regulating buoyancy when it has fully developed into a double-chambered. However, during an MBT exposure, a TPO inhibitor, for 32 days post fertilization (dpf) in zebrafish, the swimming activity of fish was impacted starting at 26 dpf if the inflation of the anterior chamber of the swim bladder was impaired or had no normal structure/size (Stinckens et al., 2016). This effect was also observed after a 32 dpf exposure to MMI, however only for the highest tested concentration (Stinckens et al., unpublished data).

It has also been reported that larvae that fail to inflate their swim bladder use additional energy to maintain buoyancy (Lindsey et al., 2010, Goodsell et al. 1996), possibly contributing to reduced swimming activity. Furthermore, Chatain (1994) associated larvae with non-inflated swim bladders with numerous complications, such as spinal deformities and lordosis and reduced growth rates, adding to the impact on swimming behaviour.

An increasing incidence of swim bladder non-inflation has also been reported in Atlantic salmon (Poppe et al. 1997). Affected fish had severely altered balance and buoyancy, observed through a specific swimming behaviour, as the affected fish were swimming upside down in an almost vertical position (Poppe et al. 1997).

Uncertainties and Inconsistencies

During an MMI exposure, a TPO inhibitor, for 32 dpf in zebrafish, the swimming activity of fish was impacted starting at 26 dpf if the inflation of the anterior chamber of the swim bladder was impaired (Stinckens et al., unpublished). However, this effect was only observed for the highest tested concentration. For the lowest tested concentration, during which the anterior swim bladder was severely impacted as well, no effect on swimming capacity could be observed. As Robertson et al., (2007) reported, the swim bladder only starts regulating buoyancy actively from 32 dpf onward in zebrafish, possibly explaining the lack of effect on swimming capacity for lower MMI concentrations.

The function of the posterior chamber has been clearly linked to buoyancy control and survival (Czesny et al., 2005; Woolley and Qin, 2010; Kurata et al., 2014). The link between anterior chamber inflation and impaired swimming capacity however is less clear. The most important function of the anterior chamber is producing and transducing sound through the Weberian Apparatus (Popper, 1974; Lechner and Ladich, 2008), with only a slight contribution in buoyancy control. It is highly plausible that impaired inflation or size of the anterior swim bladder could lead to a reduction in young-of-year survival as hearing loss would affect their ability to respond to their surrounding environment, thus impacting ecological relevant endpoints such as predator avoidance or prey seeking (Wisenden et al., 2008; Fay2009).

References

- Roberston, G.N., McGee, C.A.S., Dumbarton, T.C., Croll, R.P., Smith, F.M., 2007. Development of the swim bladder and its innervation in the zebrafish, *Danio rerio*. J. Morphol. 268, 967–985, <http://dx.doi.org/10.1002/jmor> (<http://dx.doi.org/10.1002/jmor>).
- Brix O (2002) The physiology of living in water. In: Hart PJ, Reynolds J (eds) Handbook of Fish Biology and Fisheries, Vol. 1, pp. 70–96. Blackwell Publishing, Malden, USA.
- Stinckens, E., Vergauwen, L., Schroeder, A.L., Maho, W., Blackwell, B., Witter, H., Blust, R., Ankley, G.T., Covaci, A., Villeneuve, D.L., Knapen, D., 2016. Disruption of thyroid hormone balance after 2-mercaptobenzothiazole exposure causes swim bladder inflation impairment—part II: zebrafish. Aquat. Toxicol. 173:204-17.
- Lindsey, B.W., Smith, F.M., Croll, R.P., 2010. From inflation to flotation: contribution of the swim bladder to whole-body density and swimming depth during development of the zebrafish (*Danio rerio*). Zebrafish 7, 85–96, <http://dx.doi.org/10.1089/zeb.2009.0616> (<http://dx.doi.org/10.1089/zeb.2009.0616>).
- Goodsell, D.S., Morris, G.M., Olsen, A.J. 1996. Automated docking of flexible ligands. Applications of Autodock. J. Mol. Recognition, 9:1-5.
- Chatain, B., 1994. Abnormal swim bladder development and lordosis in sea bass (*Dicentrarchus labrax*) and sea bream (*Sparus auratus*). Aquaculture 119:371–379.
- Poppe, T.T., Hellberg, H., Griffiths, D., Mendal, H. 1977. Swim bladder abnormality in farmed Atlantic salmon, *Salmo salar*. Diseases of aquatic organisms 30:73-76.
- Czesny, S.J., Graeb, B.D.S., Dettmers, J.M., 2005. Ecological consequences of swim bladder noninflation for larval yellow perch. Trans. Am. Fish. Soc. 134, 1011–1020, <http://dx.doi.org/10.1577/T04-016.1> (<http://dx.doi.org/10.1577/T04-016.1>).
- Woolley, L.D., Qin, J.G., 2010. Swim bladder inflation and its implication to the culture of marine finfish larvae. Rev. Aquac. 2, 181–190, <http://dx.doi.org/10.1111/j.1753-5131.2010.01035.x> (<http://dx.doi.org/10.1111/j.1753-5131.2010.01035.x>).
- Kurata, M., Ishibashi, Y., Takii, K., Kumai, H., Miyashita, S., Sawada, Y., 2014. Influence of initial swim bladder inflation failure on survival of Pacific bluefin tuna, *Thunnus orientalis* (Temminck and Schlegel) larvae. Aquacult. Res. 45, 882–892.
- Lechner, W., Ladich, F., 2008. Size matters: diversity in swim bladders and Weberian ossicles affects hearing in catfishes. J. Exp. Biol. 211, 1681–1689.
- Wisenden, B.D., Pogatschnik, J., Gibson, D., Bonacci, L., Schumacher, A., Willet, A., 2008. Sound the alarm: learned association of predation risk with novel auditory stimuli by fathead minnows (*Pimephales promelas*) and glowlight tetras (*Hemigrammus erythrozonus*) after single simultaneous pairings with conspecific chemical alarm cues. Environ. Biol. Fish 81, 141–147.
- Fay, R., 2009. Soundscapes and the sense of hearing of fishes. Integrative Zool. 4, 26–32.

Relationship: 1029: Reduced, Swimming performance leads to Reduced, Young of year survival (<https://aopwiki.org/relationships/1029>)

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Deiodinase 2 inhibition leading to reduced young of year survival via posterior swim bladder inflation (https://aopwiki.org/aops/155)	adjacent		
Deiodinase 2 inhibition leading to reduced young of year survival via anterior swim bladder inflation (https://aopwiki.org/aops/156)	adjacent		

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Deiodinase 1 inhibition leading to reduced young of year survival via posterior swim bladder inflation (https://aopwiki.org/aops/157)	adjacent		
Deiodinase 1 inhibition leading to reduced young of year survival via anterior swim bladder inflation (https://aopwiki.org/aops/158)	adjacent		
Thyropoxidase inhibition leading to reduced young of year survival via anterior swim bladder inflation (https://aopwiki.org/aops/159)	adjacent		

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
zebrafish	Danio rerio		NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=7955)
fathead minnow	Pimephales promelas		NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=90988)

Importance of swimming performance on young of year survival is generally applicable to fish.

Evidence Supporting this KER

Biological Plausibility

Reduced swimming performance is likely to affect essential endpoints such as predator avoidance, feeding behaviour and reproduction. These parameters are biologically plausible to affect young-of-year survival, especially in a non-laboratory environment where food is scarce and predators are abundant.

List of Non Adjacent Key Event Relationships

Relationship: 1045: Inhibition, Deiodinase 1 leads to Reduced, Anterior swim bladder inflation (<https://aopwiki.org/relationships/1045>)

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Deiodinase 1 inhibition leading to reduced young of year survival via anterior swim bladder inflation (https://aopwiki.org/aops/158)	non-adjacent		

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
zebrafish	Danio rerio		NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=7955)
fathead minnow	Pimephales promelas		NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=90988)

The evidence for a relationship between DIO1 inhibition and inflation of the anterior chamber of the swim bladder currently comes from work on zebrafish and fathead minnow.

Evidence Supporting this KER

Empirical Evidence

Exposure of fathead minnows (*Pimephales promelas*) to the non-specific deiodinase inhibitor iopanoic acid from 6-14 dpf resulted in impaired

inflation and reduced size of the anterior swim bladder. Exposure from 6-18 dpf impacted anterior chamber size, but not inflation rates. Exposure from 6-21 dpf had no effect on anterior swim bladder inflation. Together the results suggest the inhibition of DIO1 can delay, although not necessarily prevent, anterior inflation in fathead minnows (Cavallin et al., unpublished).

Uncertainties and Inconsistencies

Only few studies have been performed, of which none have been published yet.