AOP ID and Title:

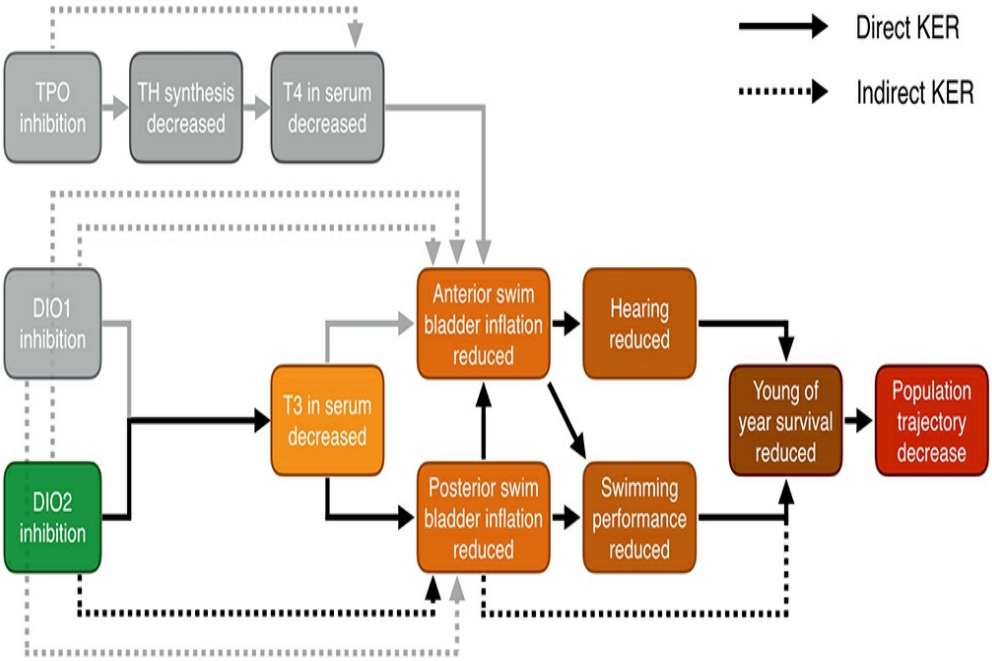
SNAPSHOT

Created at: 2019-08-13 12:26

AOP 155: Deiodinase 2 inhibition leading to reduced young of year survival via posterior swim bladder inflation

Short Title: DIO2i posterior swim bladder

Graphical Representation



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Status

Author status	OECD status	OECD project	SAAOP status
Under development: Not open for comment. Do not cite	Under Development	1.35	Included in OECD Work Plan

Abstract

The AOP describes the effects of inhibition of iodothyronine deiodinase 2 (DIO2) on posterior swim bladder inflation in fish leading to reduced young of year survival and population trajectory decline. DIO1 and DIO2 are thryoid hormone (TH) activating deiodinases that convert thyroxin (T4) to the more biologically active 3,5,3′-triiodothyronine (T3). The inhibition of DIO2 results in decreased circulating concentrations of T3 in serum. Disruption of the TH system is increasingly being recognized as an important mode of action 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. Chemicals interfering with the conversion of T4 to T3 by inhibiting DI21 have the potential to inhibit posterior chamber inflation which may result in 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. The current state of the art suggests that DIO2 is more important than DIO1 in regulating posterior chamber inflation. Therefore the AOP that is described here may be more biologically relevant than the corresponding AOP leading from DIO1 inhibition to reduced young of year survival via posterior swim bladder inflation .

Summary of the AOP

Events

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

Sequence	Type	Event ID	Title	Short name
1	MIE	1002	Inhibition, Deiodinase 2 (https://aopwiki.org/events/1002)	Inhibition, Deiodinase 2
2	KE	1003	Decreased, Triiodothyronine (T3) in serum (https://aopwiki.org/events/1003)	Decreased, Triiodothyronine (T3) in serum
3	KE	1004	Reduced, Posterior swim bladder inflation (https://aopwiki.org/events/1004)	Reduced, Posterior swim bladder inflation
4	KE	1005	Reduced, Swimming performance (https://aopwiki.org/events/1005)	Reduced, Swimming performance
5	KE	1006	Reduced, Young of year survival (https://aopwiki.org/events/1006)	Reduced, Young of year survival
6	KE	1007	Reduced, Anterior swim bladder inflation (https://aopwiki.org/events/1007)	Reduced, Anterior swim bladder inflation
7	KE	1008	Reduced, Hearing (https://aopwiki.org/events/1008)	Reduced, Hearing
8	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 2 (https://aopwiki.org/relationships/1026)	adjacent	Decreased, Triiodothyronine (T3) in serum		
Decreased, Triiodothyronine (T3) in serum (https://aopwiki.org/relationships/1027)	adjacent	Reduced, Posterior swim bladder inflation		
Reduced, Posterior swim bladder inflation (https://aopwiki.org/relationships/1028)	adjacent	Reduced, Swimming performance		
Reduced, Swimming performance (https://aopwiki.org/relationships/1029)	adjacent	Reduced, Young of year survival		

Upstream Event	Relationship Type	Downstream Event	Evidence	Quantitative Understanding
Reduced, Young of year survival (https://aopwiki.org/relationships/1030)	adjacent	Decrease, Population trajectory		
Reduced, Posterior swim bladder inflation (https://aopwiki.org/relationships/1031)	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, Anterior swim bladder inflation (https://aopwiki.org/relationships/1034)	adjacent	Reduced, Swimming performance		
Inhibition, Deiodinase 2 (https://aopwiki.org/relationships/1042)	non-adjacent	Reduced, Posterior swim bladder inflation	Moderate	Low
Reduced, Posterior swim bladder inflation (https://aopwiki.org/relationships/1041)	non-adjacent	Reduced, Young of year survival	High	Low

Overall Assessment of the AOP

Domain of Applicability

Life Stage Applicability

Life Stage	Evidence
Embryo	High

Taxonomic Applicability

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

Sex Applicability

Sex	Evidence
Unspecific	

References

Appendix 1

List of MIEs in this AOP

Event: 1002: Inhibition, Deiodinase 2 (https://aopwiki.org/events/1002)

Short Name: Inhibition, Deiodinase 2

Key Event Component

Process	Object	Action
catalytic activity	type II iodothyronine deiodinase	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)	MolecularInitiatingEvent
Aop:156 - Deiodinase 2 inhibition leading to reduced young of year survival via anterior swim bladder inflation (https://aopwiki.org/aops/156)	MolecularInitiatingEvent
Aop:190 - Type II iodothyronine deiodinase (DIO2) inhibition leading to altered amphibian metamorphosis (https://aopwiki.org/aops/190)	MolecularInitiatingEvent

Stressors

Name
iopanoic acid
PERFLUOROOCTANOIC ACID

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)

Life Stage Applicability

Life Stage	Evidence
All life stages	Moderate

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 2 (DIO2) 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 thyroxin 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. Enzym 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 Iopanoic 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 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)	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 Isihara, 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, wherease 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 differrent 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 (Glinoer, 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 HLPC 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 a 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.
- Glinoer 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 thyroidectomised 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: 1004: Reduced, Posterior swim bladder inflation (https://aopwiki.org/events/1004)

Short Name: Reduced, Posterior swim bladder inflation

Key Event Component

Process	Object	Action
swim bladder inflation	posterior 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:157 - Deiodinase 1 inhibition leading to reduced young of year survival via posterior swim bladder inflation (https://aopwiki.org/aops/157)	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 posterior 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 posterior chamber is formed from a bud originating from the foregut endoderm (Winata et al., 2009). The posterior chamber operates as a hydrostatic organ. The volume of gas in the adult swim bladder is continuously adjusted to regulate body density and buoyancy.

Many amphibians and frogs go through an embryo-larval transition phase marking the switch from endogenous feeding (from the yolk) to exogenous feeding. In zebrafish, embryonic-to-larval transition takes place around 96 hours post fertilization (hpf). As in amphibians, the transition between the different developmental phases includes maturation and inflation of the swim bladder (Liu and Chan, 2002).

Reduced inflation of the posterior chamber may manifest itself as either a complete failure to inflate the chamber or a reduced size of the chamber.

How it is Measured or Detected

In several fish species, inflation of the posterior chamber can easily be observed using a stereomicroscope because the larvae are still transparent during those early developmental stages. This is for example true for zebrafish and fathead minnow. Posterior 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 zebrafishswimbladder and mammalian lung. PLoS One 6, http://dx.doi.org/10.1371/journal.pone.0024019 (http://dx.doi.org/10.1371/journal.pone.0024019).

Winata, C.L., Korzh, S., Kondrychyn, I., Zheng, W., Korzh, V., Gong, Z., 2009.Development of zebrafish swimbladder: the requirement of Hedgehogsignaling in specification and organization of the three tissue layers. Dev. Biol.331, 222–236, http://dx.doi.org/10.1016/j.ydbio.2009.04.035 (http://dx.doi.org/10.1016/j.ydbio.2009.04.035).

Liu, Y.W., Chan, W.K., 2002. Thyroid hormones are important for embryonic tolarval transitory phase in zebrafish. Differentiation 70, 36–45, http://dx.doi.org/10.1046/j.1432-0436.2002.700104.x (http://dx.doi.org/10.1046/j.1432-0436.2002.700104.x).

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. PFOSaffects posterior swim bladder chamber inflation and swimming performanceof 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., Villenueve, 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; Stickens, 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

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

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 and 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)
- 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.

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
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
Aop:290 - DNA methyltransferase inhibition leading to reduced fecundity associated population decline (https://aopwiki.org/aops/290)	AdverseOutcome
Aop:291 - DNA methyltransferase inhibition leading to transgenerational DNA methylation associated population decline (https://aopwiki.org/aops/291)	AdverseOutcome
Aop:292 - Inhibition of tyrosinase leads to decreased population in fish (https://aopwiki.org/aops/292)	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 17ß-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: 1026: Inhibition, Deiodinase 2 leads to Decreased, Triiodothyronine (T3) in serum (https://aopwiki.org/relationships/1026)

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**		

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)

Mol et al. (1998) concluded that deiodinases in teleosts were more similar to mammalian deiodinases than had been generally accepted, based on the similarities in susceptibility to inhibition and the agreement of the Km values.

Key Event Relationship Description

Iodothyronine deiodinase or DIO is a peroxidase enzyme that is involved in the activation or deactivition of thyroid hormones. Currently, three types of iodothyronine deiodinases (DIO1-3) have been described in vertebrates that locally activate or inactivate THs and are therefore important mediators of TH action. All deiodinases are integral membrane proteins of the thioredoxin superfamily that contain the amino acid selenocysteine in their catalytic centre. DIO1 and DIO2 are capable of converting T4 into the more biologically active T3. DIO3 on the other hand converts T4 and T3 to the inactive forms of THs. The inhibition of DIO 1 and 2 enzymes results in decreased serum T3 levels and decreased T3 levels at the site of action.

Evidence Supporting this KER

Biological Plausibility
Inhibition of DIO2 activity is widely accepted to directly decrease the T3 levels in serum, since the conversion of T4 to T3 is inhibited.

Empirical Evidence

- Houbrechts et al. (2016) developed a Dio2 knockout and confirmed both the absence of the full length Dio2 protein in the liver and the dramatical decrease of T4 activating enzyme activity in liver, brain and eyes. Finally, they found decreased levels of T3 in liver, brain and eyes.
- Winata et al. (2009, 2010) reported reduced pigmentation, otic vesicle length and head-trunk angle in DIO1+2 and DIO2 knockdown fish. These effects were rescued after T3 supplementation but not by T4 supplementation, confirming that decreased T3 levels were at the basis of the observed effects.
- In the study of Cavallin et al. (2017) fathead minnow larvae were exposed to IOP, a model iodothyronine deiodinase inhibitor that is assumed to inhibit all three deiodinase enzymes (DIO1,2,3). Transcriptional analysis showed that especially DIO2, but also DIO3 mRNA levels (in some treatments), were increased in 10 to 21 day old larvae exposed to IOP as of the age of 6 days. This suggests that IOP effectively inhibited DIO2 and DIO3 in the larvae and that mRNA levels increased as a compensatory response. The authors also observed pronounced decreases of whole body T3 concentrations and increases of whole body T4 concentrations.

Uncertainties and Inconsistencies
In the study of Cavallin et al. (2017) fathead minnow embryos were exposed to IOP, a model iodothyronine deiodinase inhibitor that is assumed to inhibit all three deiodinase enzymes (DIO1,2,3). The authors observed increased whole body T3 concentrations in 4 and 6 day old embryos, while they observed decreased T3 concentrations in 10 to 21 day old larvae exposed to IOP as of the age of 6 days. One possible explanation for the elevated T3 concentrations may be the potential impact of IOP exposure on DIO3. DIO3 is an inactivating enzyme that removes iodine from the inner ring of both T4 and T3, resulting in reverse T3 (rT3) and 3,5-diiodo-L-thyronine (T2), respectively (Bianco and Kim, 2006). Maternal sources of thyroid hormones are known to include both T4 and T3 (Power et al., 2001; Walpita et al., 2007). Consequently, reduced conversion of maternal T3 to inactive forms may be one plausible explanation for the
increase.

References

Bianco, A.C., Kim, B.W., 2006. Deiodinases: implications of the local control of thyroid hormone action. Journal of Clinical Investigation 116, 2571-2579.

Cavallin, J.E., Ankley, G.T., Blackwell, B.R., Blanksma, C.A., Fay, K.A., Jensen, K.M., Kahl, M.D., Knapen, D., Kosian, P.A., Poole, S.T., Randolph, E.C., Schroeder, A.L., Vergauwen, L., Villeneuve, D.L., 2017. Impaired swim bladder inflation in early life stage fathead minnows exposed to a deiodinase inhibitor, iopanoic acid. Environmental Toxicology and Chemistry 36, 2942-2952.

Houbrechts, A.M., Delarue, J., Gabriels, I.J., Sourbron, J., Darras, V.M., 2016. Permanent Deiodinase Type 2 Deficiency Strongly Perturbs Zebrafish Development, Growth, and Fertility. Endocrinology 157, 3668-3681.

Mol, K.A., Van der Geyten, S., Burel, C., Kuhn, E.R., Boujard, T., Darras, V.M., 1998. Comparative study of iodothyronine outer ring and inner ring deiodinase activities in five teleostean fishes. Fish Physiology and Biochemistry 18, 253-266.

Power, D.M., Llewellyn, L., Faustino, M., Nowell, M.A., Bjornsson, B.T., Einarsdottir, I.E., Canario, A.V., Sweeney, G.E., 2001. Thyroid hormones in growth and development of fish. Comp Biochem Physiol C Toxicol Pharmacol 130, 447-459.

Walpita, C.N., Van der Geyten, S., Rurangwa, E., Darras, V.M., 2007. The effect of 3,5,3'-triiodothyronine supplementation on zebrafish (Danio rerio) embryonic development and expression of iodothyronine deiodinases and thyroid hormone receptors. Gen Comp Endocrinol 152, 206-214.

Winata, C.L., Korzh, S., Kondrychyn, I., Korzh, V., Gong, Z. 2010. The role of vasulature and blood circulation in zebrafish swim bladder development. Dev. Biol. 10:3.

Winata, C.L., Korzh, S., Kondrychyn, I., Zheng, W., Korzh, V., Gong, Z. 2009. Development of zebrafish swimbladder: the requirement of Hedgehog signaling in specification and organization of the three tissue layers. Dev. Biol.331, 222–236, http://dx.doi.org/10.1016/j.ydbio.2009.04.035.

Relationship: 1027: Decreased, Triiodothyronine (T3) in serum leads to Reduced, Posterior swim bladder inflation (https://aopwiki.org/relationships/1027)

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 1 inhibition leading to reduced young of year survival via posterior swim bladder inflation (https://aopwiki.org/aops/157)	**adjacent**	**Moderate**	**Low**

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

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

Life Stage Applicability

Life Stage	Evidence
Embryo	High

Sex Applicability

Sex	Evidence
Unspecific	High

Key Event Relationship Description

Reduced T3 levels in serum prohibit local TH action in the target tissues. Since swim bladder development and/or inflation is regulated by thyroid hormones, this results in impaired posterior chamber inflation.

Evidence Supporting this KER

There is convincing evidence that decreased T3 levels result in impaired posterior chamber inflation, but the underlying mechanisms are not completely understood. The quantitative understanding is currently very limited because T3 levels and posterior inflation are seldom measured in the same study. Therefore the evidence supporting this KER can be considered moderate.

Biological Plausibility

Thyroid hormones are known to be involved in development, especially in metamorphosis in amphibians and in embryonic-to-larval transition (Liu and Chan, 2002) and larval-to-juvenile transition (Brown et al., 1997) in fish. Inflation of the posterior chamber is part of the embryonic-to-larval transition in fish, together with structural and functional maturation of the mouth and gastrointestinal tract, and resorption of the yolk sac (Liu and Chan, 2002). Marelli et al. (2016) showed that thyroid hormone receptor alpha and beta are both expressed in swim bladder tissue of zebrafish at 5 days post fertilization, corresponding to the timing of posterior inflation. this time point has additionally been shown to coincide with increased T3 and T4 levels (Chang et al., 2012), suggesting that posterior inflation is under thyroid hormone regulation.

Empirical Evidence

- 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).
- Dong et al. (2013) and Thisse et al. (2003) showed localized expression of DIO1 and DIO2 in the swim bladder tissue of 96 and 120 hpf zebrafish larvae, suggesting that local activation of thryoid hormones (i.e. conversion of T4 to T3) is requried in swim bladder tissue around that time period.
- Marelli et al. (2016) used morpholinos to block translation of thryoid hormone receptor alpha or beta in zebrafish. They found that thryoid

hormone receptor alpha and beta failed to inflate the posterior chamber of the swim bladder by 120 hpf, suggesting that the action of T3 is needed for proper inflation of the posterior chamber.

- Stinckens et al. (2018) showed that effects on posterior chamber inflation in zebrafish could be predicted based on in chemico DIO2 inhibition potential with only few false positives and false negatives. While T3 levels were not determined in this study, DIO2 inhibition is expected to result in decreased T3 levels.
- Bagci et al. (2015) and Heijlen et al. (2013, 2014) reported that knockdown of DIO1+2 in zebrafish resulted in impairment of the inflation of the posterior chamber of the swim bladder. DIO1 and 2 knockdown is expected to result in reduced T3 levels. Indeed, Walpita et al. (2009, 2010) showed that T3 supplementation effectively rescued the effects of DIO1 and 2 knockdown, while T4 supplementation did not.
- de Vrieze et al. (2014) found that knockdown of monocarboxylate transporter 8 (mct8) in zebrafish resulted in a dose-dependent impairment of posterior chamber inflation. Since this transporter is known to transport thyroid hormones across cell membranes, this supports the importance of thyroid hormones in regulating posterior chamber inflation.
- Shi et al. (2019) found that exposure of adult zebrafish to 6:2 chlorinated polyfluorinated ether sulfonate (F-53B), an alternative to perfluorooctanesulfonate (PFOS), decreased T3 levels in both male and female zebrafish. Additionally, F-53B was maternally transferred to the offspring. Decreased T3 levels together with impaired posterior chamber inflation was observed in the F1 offspring.

Evidence of dose-concordance:

- Rehberger et al. (2018) observed decreased T3 levels in the thyroid follicles (which is expected to result in decreased T3 levels in serum) of 120 hpf zebrafish embryos after exposure to PTU starting from 10 mg/L PTU. Stinckens et al.(2018) showed that the downstream KE, impaired posterior chamber inflation, occured at much higher concentrations (EC10: 184 mg/L)

Uncertainties and Inconsistencies

The mechanism through which altered TH levels result in impaired posterior chamber inflation still needs to be elucidated. It is currently unclear which aspect of swim bladder development and inflation is affected by TH disruption. Based on the developmental stages of the posterior chamber, several hypotheses could explain effects on posterior chamber inflation due to disrupted TH levels. A first hypothesis includes effects on the budding of the posterior chamber inflation. Secondly, the effect on posterior chamber inflation could also be caused by disturbing the formation and growth of the three tissue layers of this organ. It has been reported that the Hedgehog signalling pathway plays an essential role in swim bladder development and is required for growth and differentiation of cells of the swim bladder. The Wnt/β-catenin signalling pathway is required for the organization and growth of all three tissue layers (Yin et al., 2011, 2012, Winata 2009, Kress et al., 2009). Both signalling pathways have been related to THs in amphibian and rodent species (Kress et al., 2009; Plateroti et al., 2006; Stolow and Shi, 1995). Several other hypotheses include effects on the successful initial inflation of the posterior chamber, effects on lactic acid production that is required for the maintenance of the swim bladder volume, or effects on the production of surfactant that is crucial to maintain the surface tension necessary for swim bladder inflation.

Another uncertainty lies in the relative importance of the different T4 actvating iodothyronine deiodinases (DIO1, DIO2) in regulating swim bladder inflation. Stinckens et al. (2018) showed that exposure of zebrafish embryos to seven strong DIO1 inhibitors (measured using in chemico enzyme inhibition assays), six out of seven compounds impaired posterior chamber inflation. Exposure to strong DIO2 inhibitors on the other hand affected posterior chamber inflation and/or surface area in all cases. These results suggest that DIO2 enzymes may play a more important role in swim bladder inflation compared to DIO1 enzymes. it has been previously suggested that DIO2 is the major contributor to TH activation in developing zebrafish embryos (Darras et al., 2015; Walpita et al., 2010). It has been shown that a morpholino knockdown targeting DIO1 mRNA alone did not affect embryonic development in zebrafish, while knockdown of DIO2 delayed progression of otic vesicle length, head-trunk angle and pigmentation index (Houbrechts et al., 2016; Walpita et al., 2010, 2009). DIO1 inhibition may only become essential in hypothyroidal circumstances, for example when DIO2 is inhibited or in case of iodine deficiency, in zebrafish (Walpita et al., 2010) and mice (Galton et al., 2009; Schneider et al., 2006).

As reported by Bagci et al. (2015) and Heijlen et al. (2014), posterior chamber inflation was impaired in DIO3 knockdown zebrafish. Heijlen et al. (2014) additionally reported histologically abnormal tissue layers in the swim bladder of DIO3 knockdown zebrafish. DIO3 is a thyroid hormone inactivating enzyme, which would result in higher levels of T3 in serum. Wei et al. (2018) showed that exposure to bisphenol S in adult zebrafish decreased T4 levels and increased T3 levels, and these changes in thyroid hormone levels were transferred to the offspring, in which impaired swim bladder inflation was observed. This indicates that not only too low, but also too high T3 levels, impact posterior chamber inflation. The underlying mechanism is currently unknown. In the study of Cavallin et al. (2017) fathead minnow embryos were exposed to IOP, a model iodothyronine deiodinase inhibitor that is assumed to inhibit all three deiodinase enzymes (DIO1,2,3). The authors observed increased whole body T3 concentrations in 4 and 6 day old embryos, together with impaired posterior chamber inflation. Transcript levels of DIO1, 2 and 3 remained unaltered and thus offered no proof of a compensatory mechanism that could explain these results.

References

Bagci, E., Heijlen, M., Vergauwen, L., Hagenaars, A., Houbrechts, A.M., Esguerra, C.V., Blust, R., Darras, V.M., Knapen, D., 2015. Deiodinase knockdown during early zebrafish development affects growth, development, energy metabolism, motility and phototransduction. PLOS One 10, e0123285.

Brown, C.L., Doroshov, S.I., Nunez, J.M., Hadley, C., Vaneenennaam, J., Nishioka, R.S., Bern, H.A., 1988. MATERNAL TRIIODOTHYRONINE INJECTIONS CAUSE INCREASES IN SWIMBLADDER INFLATION AND SURVIVAL RATES IN LARVAL STRIPED BASS, MORONE-SAXATILIS. Journal of Experimental Zoology 248, 168-176.

Brown, D.D., 1997. The role of thyroid hormone in zebrafish and axolotl development. Proceedings of the National Academy of Sciences of the United States of America 94, 13011-13016.

Cavallin, J.E., Ankley, G.T., Blackwell, B.R., Blanksma, C.A., Fay, K.A., Jensen, K.M., Kahl, M.D., Knapen, D., Kosian, P.A., Poole, S.T., Randolph, E.C., Schroeder, A.L., Vergauwen, L., Villeneuve, D.L., 2017. Impaired swim bladder inflation in early life stage fathead minnows exposed to a deiodinase inhibitor, iopanoic acid. Environmental Toxicology and Chemistry 36, 2942-2952.

Chang, J., Wang, M., Gui, W., Zhao, Y., Yu, L., Zhu, G., 2012. Changes in Thyroid Hormone Levels during Zebrafish Development. Zoological Science 29, 181-184.

Darras, V.M., Houbrechts, A.M., Van Herck, S.L.J., 2015. Intracellular thyroid hormone metabolism as a local regulator of nuclear thyroid hormone receptor-mediated impact on vertebrate development. Biochimica Et Biophysica Acta-Gene Regulatory Mechanisms 1849, 130-141.

de Vrieze, E., van de Wiel, S.M.W., Zethof, J., Flik, G., Klaren, P.H.M., Arjona, F.J., 2014. Knockdown of Monocarboxylate Transporter 8 (mct8) Disturbs Brain Development and Locomotion in Zebrafish. Endocrinology 155, 2320-2330.

Dong, W., Macaulay, L.J., Kwok, K.W., Hinton, D.E., Stapleton, H.M., 2013. Using whole mount in situ hybridization to examine thyroid hormone deiodinase expression in embryonic and larval zebrafish: a tool for examining OH-BDE toxicity to early life stages. Aquat Toxicol 132-133, 190-199.

Galton, V.A., Schneider, M.J., Clark, A.S., St Germain, D.L., 2009. Life without thyroxine to 3,5,3'-triiodothyronine conversion: studies in mice devoid of the 5'-deiodinases. Endocrinology 150, 2957-2963.

Heijlen, M., Houbrechts, A., Bagci, E., Van Herck, S., Kersseboom, S., Esguerra, C., Blust, R., Visser, T., Knapen, D., Darras, V., 2014. Knockdown of type 3 iodothyronine deiodinase severely perturbs both embryonic and early larval development in zebrafish. Endocrinology 155, 1547-1559.

Heijlen, M., Houbrechts, A.M., Darras, V.M., 2013. Zebrafish as a model to study peripheral thyroid hormone metabolism in vertebrate development. Gen.Comp. Endocrinol. 188, 289–296, http://dx.doi.org/10.1016/j.ygcen.2013.04.004 (http://dx.doi.org/10.1016/j.ygcen.2013.04.004).

Kress, E., Rezza, A., Nadjar, J., Samarut, J., Plateroti, M., 2009. The frizzled-related sFRP2 gene is a target of thyroid hormone receptor alpha1 and activates beta-catenin signaling in mouse intestine. J Biol Chem 284, 1234-1241.

Liu, Y.W., Chan, W.K., 2002. Thyroid hormones are important for embryonic to larval transitory phase in zebrafish. Differentiation 70, 36-45.

Marelli, F., Carra, S., Agostini, M., Cotelli, F., Peeters, R., Chatterjee, K., Persani, L., 2016. Patterns of thyroid hormone receptor expression in zebrafish and generation of a novel model of resistance to thyroid hormone action. Molecular and Cellular Endocrinology 424, 102-117.

Plateroti, M., Kress, E., Mori, J.I., Samarut, J., 2006. Thyroid hormone receptor alpha1 directly controls transcription of the beta-catenin gene in intestinal epithelial cells. Mol Cell Biol 26, 3204-3214.

Rehberger, K., Baumann, L., Hecker, M., Braunbeck, T., 2018. Intrafollicular thyroid hormone staining in whole-mount zebrafish (Danio rerio) embryos for the detection of thyroid hormone synthesis disruption. Fish Physiology and Biochemistry 44, 997-1010.

Schneider, M.J., Fiering, S.N., Thai, B., Wu, S.Y., St Germain, E., Parlow, A.F., St Germain, D.L., Galton, V.A., 2006. Targeted disruption of the type 1 selenodeiodinase gene (Dio1) results in marked changes in thyroid hormone economy in mice. Endocrinology 147, 580-589.

Shi, G., Wang, J., Guoa, H., Shenga, N., Cui, Q., Pan, Y., Guob, Y., Sun, Y., Dai, J., 2019. Parental exposure to 6:2 chlorinated polyfluorinated ether sulfonate (F-53B) induced transgenerational thyroid hormone disruption in zebrafish. Science of the Total Environment 665, 855-863.

Stinckens, E., Vergauwen, L., Ankley, G.T., Blust, R., Darras, V.M., Villeneuve, D.L., Witters, H., Volz, D.C., Knapen, D., 2018. An AOP-based alternative testing strategy to predict the impact of thyroid hormone disruption on swim bladder inflation in zebrafish. Aquatic Toxicology 200, 1-12.

Stolow, M.A., Shi, Y.B., 1995. XENOPUS SONIC HEDGEHOG AS A POTENTIAL MORPHOGEN DURING EMBRYOGENESIS AND THYROID HORMONE-DEPENDENT METAMORPHOSIS. Nucleic Acids Research 23, 2555-2562.

Thisse, C., Degrave, A., Kryukov, G.V., Gladyshev, V.N., Obrecht-Pflumio, S., Krol, A., Thisse, B., Lescure, A., 2003. Spatial and temporal expression patterns of selenoprotein genes during embryogenesis in zebrafish. Gene Expr Patterns 3, 525-532.

Walpita, C.N., Crawford, A.D., Darras, V.M., 2010. Combined antisense knockdown of type 1 and type 2 iodothyronine deiodinases disrupts embryonic development in zebrafish (Danio rerio). General and Comparative Endocrinology 166, 134-141.

Walpita, C.N., Crawford, A.D., Janssens, E.D., Van der Geyten, S., Darras, V.M., 2009. Type 2 iodothyronine deiodinase is essential for thyroid hormone-dependent embryonic development and pigmentation in zebrafish. Endocrinology 150, 530-539.

Wei, P.H., Zhao, F., Zhang, X.N., Liu, W.M., Jiang, G.B., Wang, H.F., Ru, S.G., 2018. Transgenerational thyroid endocrine disruption induced by bisphenol S affects the early development of zebrafish offspring. Environmental Pollution 243, 800-808.

Winata, C.L., Korzh, S., Kondrychyn, I., Zheng, W., Korzh, V., Gong, Z., 2009. Development of zebrafish swimbladder: The requirement of Hedgehog signaling in specification and organization of the three tissue layers. Developmental Biology 331, 222-236.

Yin, A., Korzh, S., Winata, C.L., Korzh, V., Gong, Z., 2011. Wnt signaling is required for early development of zebrafish swimbladder. PLoS One 6, e18431.

Yin, A., Korzh, V., Gong, Z., 2012. Perturbation of zebrafish swimbladder development by enhancing Wnt signaling in Wif1 morphants. Biochim Biophys Acta 1823, 236-244.

Relationship: 1028: Reduced, Posterior swim bladder inflation leads to Reduced, Swimming performance (https://aopwiki.org/relationships/1028)

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 1 inhibition leading to reduced young of year survival via posterior swim bladder inflation (https://aopwiki.org/aops/157)	**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 posterior swim bladder inflation and reduced swimming performance, is intermediate.

Biological Plausibility
The posterior chamber of the swim bladder has a function in regulating the buoyancy of fish, by altering the volume of the swim bladder (Roberston 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, as has been suggested previously (Bagci et al., 2015; Hagenaars et al., 2014). Fish without a 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.

Lindsey et al., 2010 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, they reported that the range of swimming depth varies with stages of swim bladder development.

Czesny et al., 2005 reported that yellow perch larvae without inflated swim bladders capture free-swimming prey poorly and expend more energy on feeding and maintaining their position within the water column, due to impacted swimming behaviour.

Kurata et al., 2014 observed that Bluefin tuna larvae present at the bottom of a tank, incapable of swimming upwards, had significantly lower swim bladder inflation.

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. 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).

Several chemical exposures to thyroid disrupting compounds resulted in an effect on posterior chamber inflation and following a direct effect on the swimming distance of the zebrafish larvae (Stinckens et al., unpublished).

Uncertainties and Inconsistencies
Robertson et al., (2007) reported that the swim bladder only becomes functional as a buoyancy regulator when it is fully developed into a double-chambered swim bladder. This would implicate that effects on posterior chamber inflation would not directly result in effects on swimming capacity. However, it was also reported that gases in the swim bladder increase the buoyancy of zebrafish larvae just after initial inflation, but active control only after 28–30 d post hatch. Therefore, an effect on swimming capacity is still likely.

PTU exposure resulted in an effect on posterior chamber inflation, but did not result in a direct effect on the swimming distance of the zebrafish larvae (Stinckens et al., unpublished). Furthermore, the swimming activity of zebrafish larvae was reduced after 5 days MBT exposure in zebrafish, which had normal inflated posterior chambers, indicating the effects on swimming behaviour via other modes of action.

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.

Bagci, E., Heijlen, M., Vergauwen, L., Hagenaars, A., Houbrechts, A.M., Esguerra, C.V.,Blust, R., Darras, V.M., Knapen, D., 2015. Deiodinase knockdown during earlyzebrafish development affects growth, development, energy metabolism,motility and phototransduction. PLoS One 10, e0123285, http://dx.doi.org/10.1371/journal.pone.0123285 (http://dx.doi.org/10.1371/journal.pone.0123285).

Hagenaars, A., Stinckens, E., Vergauwen, L., Bervoets, L., Knapen, D., 2014. PFOSaffects posterior swim bladder chamber inflation and swimming performanceof zebrafish larvae. Aquat. Toxicol. 157, 225–235, http://dx.doi.org/10.1016/j.aquatox.2014.10.017 (http://dx.doi.org/10.1016/j.aquatox.2014.10.017).

Lindsey, B.W., Smith, F.M., Croll, R.P., 2010. From inflation to flotation: contributionof the swimbladder to whole-body density and swimming depth duringdevelopment 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 fleixble ligands. Applications of Autodock. J. Mol. Recogonition, 9:1-5.

Czesny, S.J., Graeb, B.D.S., Dettmersn, J.M., 2005. Ecological consequences of swimbladder 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).

Kurata, M., Ishibashi, Y., Takii, K., Kumai, H., Miyashita, S., Sawada, Y., 2014.Influence of initial swimbladder inflation failure on survival of Pacific bluefintuna, Thuunus orientalis (Temminck and Schlegl) larvae. Aquacult. Res. 45,882–892.

Chatain, B., 1994. Abnormal swimbladder 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.

Stinckens, E., Vergauwen, L., Schroeder, A.L., Maho, W., Blackwell, B., Witter, H.,Blust, R., Ankley, G.T., Covaci, A., Villenueve, 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.

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**		
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**		
Thyroperoxidase 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 biologicaly plausible to affect young-of-year survival, especially in a non-laboratory environment where food is scarce and predators are abundant.

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**		
Thyroperoxidase 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: 1031: Reduced, Posterior swim bladder inflation leads to Reduced, Anterior swim bladder inflation (https://aopwiki.org/relationships/1031)

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 1 inhibition leading to reduced young of year survival via posterior swim bladder inflation (https://aopwiki.org/aops/157)	**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)

Life Stage Applicability

Life Stage	Evidence
Juvenile	High

Sex Applicability

Sex	Evidence
Unspecific	High

There is no evidence of sex-dependent processes involved in swim bladder chamber develoment and inflation. Additionally, zebrafish are undifferentiated gonochorists, and gonad differentiation starts only around 23-25 dpf (Uchida et al., 2002), after the time point of anterior chamber inflation (around 21 dpf).

Evidence Supporting this KER

Biological Plausibility

The anterior chamber is formed by evagination from the cranial end of the posterior chamber (Robertson et al., 2007, Winata et al., 2009). Therefore it is plausible to assume that the anterior chamber cannot inflate in cases where the posterior chamber is not inflated.

References

Robertson, G.N., McGee, C.A.S., Dumbarton, T.C., Croll, R.P., Smith, F.M., 2007. Development of the swimbladder and its innervation in the zebrafish, Danio rerio. Journal of Morphology 268, 967-985.

Uchida, D., Yamashita, M., Kitano, T., Iguchi, T., 2002. Oocyte apoptosis during the transition from ovary-like tissue to testes during sex differentiation of juvenile zebrafish. Journal of Experimental Biology 205, 711-718.

Winata, C.L., Korzh, S., Kondrychyn, I., Zheng, W., Korzh, V., Gong, Z., 2009. Development of zebrafish swimbladder: The requirement of Hedgehog signaling in specification and organization of the three tissue layers. Developmental Biology 331, 222-236.

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**		

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 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**		
Thyroperoxidase 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, supbrabranchial 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**		
Thyroperoxidase 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 (Slabbekoorn 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: 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**		
Thyroperoxidase 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 (Roberston 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. The swim bladder only becomes capable of regulating buoyancy when it has fully developed into a double-chambered swim bladder. 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 only during late development. Exposure to MBT, a TPO inhibitor, from 0 to 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 severly 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 bouncy 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., Villenueve, 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: contributionof the swimbladder to whole-body density and swimming depth duringdevelopment 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 fleixble ligands. Applications of Autodock. J. Mol. Recogonition, 9:1-5.

Chatain, B., 1994. Abnormal swimbladder 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., Dettmersn, J.M., 2005. Ecological consequences of swimbladder 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. Swimbladder inflation and its implication to theculture 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 swimbladder inflation failure on survival of Pacific bluefintuna, Thuunus orientalis (Temminck and Schlegl) larvae. Aquacult. Res. 45,882–892.

Lechner, W., Ladich, F., 2008. Size matters: diversity in swimbladders andWeberian 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 novelauditory stimuli by fathead minnows (Pimephales promelas) and glowlighttetras (Hemigrammus erythrozonus) after single simultaneous pairings withconspecific 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.

List of Non Adjacent Key Event Relationships

Relationship: 1042: Inhibition, Deiodinase 2 leads to Reduced, Posterior swim bladder inflation (https://aopwiki.org/relationships/1042)

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)	**non-adjacent**	**Moderate**	**Low**

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

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

Life Stage Applicability

Life Stage	Evidence
Embryo	High

Sex Applicability

Sex	Evidence
Unspecific	High

The evidence for a relationship between DIO2 inhibition and inflation of the posterior chamber of the swim bladder is currently based on work in zebrafish and fathead minnow.

Mol et al. (1998) concluded that deiodinases in teleosts were more similar to mammalian deiodinases than had been generally accepted, based on the similarities in susceptibility to inhibition and the agreement of the Km values.

This KER is probably not sex-dependent since both females and males rely on activation of THs by deiodinase for regulation of vital processes. Additionally, zebrafish are undifferentiated gonochorists, and gonad differentiation starts only around 23-25 dpf (Uchida et al., 2002), well after the time point of posterior chamber inflation (around 5 dpf).

Key Event Relationship Description

Iodothyronine deiodinase or DIO is a peroxidase enzyme that is involved in the activation or deactivition of thyroid hormones. Currently, three types of iodothyronine deiodinases (DIO1-3) have been described in vertebrates that locally activate or inactivate THs and are therefore important mediators of TH action. All deiodinases are integral membrane proteins of the thioredoxin superfamily that contain the amino acid selenocysteine in their catalytic centre. DIO1 and DIO2 are capable of converting T4 into the more biologically active T3. DIO3 on the other hand converts T4 and T3 to the inactive forms of THs. The inhibition of DIO 1 and 2 enzymes results in decreased serum T3 levels and decreased T3 levels at the site of action. Since swim bladder development and/or inflation is regulated by thyroid hormones, this results in impaired posterior chamber inflation.

Evidence Supporting this KER

There is convincing evidence that inhibition of DIO activity, either through specific knockdown or through chemical exposure, results in impaired posterior chamber inflation, but the underlying mechanisms are not completely understood, including the relative importance of DIO1 and DIO2. Based on current evidence, it seems that DIO2 is more important in regulating posterior chamber inflation. Due to the difficulty of measuring DIO activity in small fish embryos, quantitative linkages and temporal concordance have been difficult to establish. The quantitative understanding is currently based on a relationship between the classification of chemicals according to their in chemico DIO inhibitory potential (using a threshold and uncertainty zone) on the one hand, and occurence of in vivo effects on posterior chamber inflation on the other hand. Predictions based on this relationship have been proven highly successful. Therefore the evidence supporting this KER can be considered moderate.

Biological Plausibility
Inhibition of DIO activity is widely accepted to reduce the T3 levels in serum and is expected to reduce local T3 levels in target tissues, since the conversion of T4 to T3 is inhibited. In fish, many different adverse effects during early development resulting from disruption of the TH endocrine system have been reported, including effects on swim bladder inflation. 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.

Empirical Evidence
Deiodinases are criticial for normal development. Several defects have already been reported in cases where the TH hormone balance is disturbed. Winata et al. (2009, 2010) reported reduced pigmentation, otic vesicle length and head-trunk angle in DIO1+2 and DIO2 knockdown fish. These effects were rescued after T3 supplementation, indicating the importance of T4 to T3 conversion by deiodinases.

Substantial evidence for the link between deiodinase inhibition and impaired posterior chamber inflation is available:

- Chang et al., (2012) established a base-line for TH levels during zebrafish development and observed peaks in whole-body T3 content at 5 dpf when the posterior chamber of the swim bladder inflates.
- Bagci et al. (2015) and Heijlen et al. (2013, 2014) reported that knockdown of DIO1+2 in zebrafish resulted in impairment of the inflation of the posterior chamber of the swim bladder.
- DIO1 and DIO2 mRNA has also been shown to be present in zebrafish swim bladder tissue at 96 hpf using whole mount in situ hybridization (Heijlen et al., 2013; Dong et al., 2013), suggesting a tissue-specific role of T3 in the inflation process of the posterior chamber.
- Exposure to PTU, a very potent DIO1 inhibitor, caused thyroid hypertrophy in X. leavis because of the inhibition of the peripheral conversion of T4 to T3 (Degitz et al., 2005). PTU also decreased serum T3 levels in the rat (Frumess and Larsen, 1975) and resulted in effects on posterior chamber inflation in zebrafish (Jomaa et al., 2014; Stinckens et al., 2018). It should be noted that there are some uncertainties related to the species-specific susceptibility of DIO1 to inhibition by PTU (Kuiper et al., 2006; Orozco et al., 2012).

- Stinckens et al. (2018) showed that effects on posterior chamber inflation in zebrafish could be predicted based on in chemico DIO2 inhibition potential with only few false positives and false negatives.
- After exposure of fathead minnows (Pimephales promelas) to the non-specific deiodinase inhibitor IOP from 1-6 dpf, Incidence and length of inflated posterior swim bladders were significantly reduced (Cavallin et al., 2017).

Uncertainties and Inconsistencies
The mechanism through which altered TH levels result in impaired posterior chamber inflation still needs to be elucidated.

It is currently unclear which aspect of swim bladder development and inflation is affected by TH disruption. Based on the developmental stages of the posterior chamber, several hypotheses could explain effects on posterior chamber inflation due to disrupted TH levels. A first hypothesis includes effects on the budding of the posterior chamber inflation. Secondly, the effect on posterior chamber inflation could also be caused by disturbing the formation and growth of the three tissue layers of this organ. It has been reported that the Hedgehog signalling pathway plays an essential role in swim bladder development and is required for growth and differentiation of cells of the swim bladder. The Wnt/β-catenin signalling pathway is required for the organization and growth of all three tissue layers (Yin et al., 2011, 2012, Winata 2009, Kress et al., 2009). Both signalling pathways have been related to THs in amphibian and rodent species (Kress et al., 2009; Plateroti et al., 2006; Stolow and Shi, 1995). Several other hypotheses include effects on the successful initial inflation of the posterior chamber, effects on lactic acid production that is required for the maintenance of the swim bladder volume, or effects on the production of surfactant that is crucial to maintain the surface tension necessary for swim bladder inflation.

Another uncertainty lies in the relative importance of the different T4 activating iodothyronine deiodinases (DIO1, DIO2) in regulating swim bladder inflation. Stinckens et al. (2018) showed that when exposing zebrafish embryos to seven strong DIO1 inhibitors (measured using in chemico enzyme inhibition assays), six out of seven compounds impaired posterior chamber inflation. Exposure to strong DIO2 inhibitors on the other hand affected posterior chamber inflation and/or surface area in all cases. These results suggest that DIO2 enzymes may play a more important role in swim bladder inflation compared to DIO1 enzymes. it has been previously suggested that DIO2 is the major contributor to TH activation in developing zebrafish embryos (Darras et al., 2015; Walpita et al., 2010). It has been shown that a morpholino knockdown targeting DIO1 mRNA alone did not affect embryonic development in zebrafish, while knockdown of DIO2 delayed progression of otic vesicle length, head-trunk angle and pigmentation index (Houbrechts et al., 2016; Walpita et al., 2010, 2009). DIO1 inhibition may only become essential in hypothyroidal circumstances, for example when DIO2 is inhibited or in case of iodine deficiency, in zebrafish (Walpita et al., 2010) and mice (Galton et al., 2009; Schneider et al., 2006).

Heijlen et al. (2015) reported histologically abnormal tissue layers in the swim bladder of DIO3 knockdown zebrafish. As reported in Bagci et al. (2015) and Heijlen et al. (2014), posterior chamber inflation was impaired in DIO3 knockdown zebrafish. DIO3 is a thyroid hormone inactivating enzyme, which would result in higher levels of T3 in serum. This indicates that not only too low, but also too high T3 levels, impact posterior chamber inflation. The underlying mechanism is currently unknown.

References

Bagci, E., Heijlen, M., Vergauwen, L., Hagenaars, A., Houbrechts, A.M., Esguerra, C.V.,Blust, R., Darras, V.M., Knapen, D., 2015. Deiodinase knockdown during early zebrafish development affects growth, development, energy metabolism,motility and phototransduction. PLoS One 10, e0123285, http://dx.doi.org/10.1371/journal.pone.0123285 (http://dx.doi.org/10.1371/journal.pone.0123285).

Cavallin, J.E., Ankley, G.T., Blackwell, B.R., Blanksma, C.A., Fay, K.A., Jensen, K.M., Kahl, M.D., Knapen, D., Kosian, P.A., Poole, S.T., Randolph, E.C., Schroeder, A.L., Vergauwen, L., Villeneuve, D.L., 2017. Impaired swim bladder inflation in early life stage fathead minnows exposed to a deiodinase inhibitor, iopanoic acid. Environmental Toxicology and Chemistry 36, 2942-2952.

Chang, J., Wang, M., Gui, W., Zhao, Y., Yu, L., Zhu, G., 2012. Changes in thyroidhormone levels during zebrafish development. Zool. Sci. 29, 181–184, http://dx.doi.org/10.2108/zsj.29.181 (http://dx.doi.org/10.2108/zsj.29.181).

Darras, V.M., Houbrechts, A.M., Van Herck, S.L.J., 2015. Intracellular thyroid hormone metabolism as a local regulator of nuclear thyroid hormone receptor-mediated impact on vertebrate development. Biochimica Et Biophysica Acta-Gene Regulatory Mechanisms 1849, 130-141.

Degitz, S.J., Holcombe, G.W., Flynn, K.M., Kosian, P.A., Korte, J.J., Tietge, J.E., 2005.Progress towards development of an amphibian-based screening assay usinXenopus laevis. Organismal and thyroidal responses to the model compounds6-propylthiouracil, methimazole, and thyroxine. Toxicol. Sci. 87, 353–364.

Dong, W., Macaulay, L., Kwok, K.W.H., Hinton, D.E., Stapleton, H.M., 2013. Using whole mount in situ hydridization to examine thyroid hormone deiodinase expression in embryonic and larval zebrafish: a tool for examining OH-BDE toxicity to early life stages. Aquat. Toxicol. 132–133, 190–199, http://dx.doi.org/10.1016/j.biotechadv.2011.08.021.Secreted (http://dx.doi.org/10.1016/j.biotechadv.2011.08.021.Secreted).

Frumess, R.D., Larsen, P.R. 1975. Correlation of serum triiodothyronine (T3) and thyroxine (T4) with biological effects of thyroid hormone replacement in propylthiouracil-treated rats. Metabolism 24:4.

Galton, V.A., Schneider, M.J., Clark, A.S., St Germain, D.L., 2009. Life without thyroxine to 3,5,3'-triiodothyronine conversion: studies in mice devoid of the 5'-deiodinases. Endocrinology 150, 2957-2963.

Heijlen, M., Houbrechts, A.M., Bagci, E., Van Herck, S.L.J., Kersseboom, S., Esguerra,C.V., Blust, R., Visser, T.J., Knapen, D., Darras, V.M., 2014. Knockdown of type 3iodothyronine deiodinase severely perturbs both embryonic and early larval development in zebrafish. Endocrinology 155, 1547–1559, http://dx.doi.org/10.1210/en.2013-1660 (http://dx.doi.org/10.1210/en.2013-1660).

Heijlen, M., Houbrechts, A.M., Darras, V.M., 2013. Zebrafish as a model to study peripheral thyroid hormone metabolism in vertebrate development. Gen.Comp. Endocrinol. 188, 289–296, http://dx.doi.org/10.1016/j.ygcen.2013.04.004 (http://dx.doi.org/10.1016/j.ygcen.2013.04.004).

Houbrechts, A.M., Delarue, J., Gabriels, I.J., Sourbron, J., Darras, V.M., 2016. Permanent Deiodinase Type 2 Deficiency Strongly Perturbs Zebrafish Development, Growth, and Fertility. Endocrinology 157, 3668-3681.

Jomaa, B., Hermsen, S.A.B., Kessels, M.Y., Van Den Berg, J.H.J., Peijnenburg, A.A.C.M.,Aarts, J.M.M.J.G., Piersma, A.H., Rietjens, I.M.C.M., 2014. Developmental toxicity of thyroid-active compounds in a zebrafish embryotoxicity test. ALTEX 31,303–317, http://dx.doi.org/10.14573/altex.1402011 (http://dx.doi.org/10.14573/altex.1402011).

Kress, E., Rezza, A., Nadjar, J., Samarut, J., Plateroti, M., 2009. The frizzled-relatedsFRP2 gene is a target of thyroid hormone receptor alfa1 and activates beta-catenin signaling in mouse intestine. J. Biol. Chem. 284, 1234–1241, http://dx.doi.org/10.1074/jbc.M806548200 (http://dx.doi.org/10.1074/jbc.M806548200).

Kuiper, G., Klootwijk, W., Dubois, G.M., Destree, O., Darras, V.M., Van der Geyten, S., Demeneix, B., Visser, T.J., 2006. Characterization of recombinant Xenopus laevis type I iodothyronine deiodinase: substitution of a proline residue in the catalytic center by serine (Pro132Ser) restores sensitivity to 6-propyl-2-thiouracil. Endocrinology 147, 3519-3529.

Mol, K.A., Van der Geyten, S., Burel, C., Kuhn, E.R., Boujard, T., Darras, V.M., 1998. Comparative study of iodothyronine outer ring and inner ring deiodinase activities in five teleostean fishes. Fish Physiology and Biochemistry 18, 253-266.

Orozco, A., Valverde, C., Olvera, A., Garcia, C., 2012. Iodothyronine deiodinases: a functional and evolutionary perspective. Journal of Endocrinology 215, 207-219.

Plateroti, M., Kress, E., Mori, J.I., Samarut, J., 2006. Thyroid hormone receptor alpha1 directly controls transcription of the beta-catenin gene in intestinal epithelial cells. Mol. Cell. Biol. 26, 3204–3214, http://dx.doi.org/10.1128/MCB.26.8.3204 (http://dx.doi.org/10.1128/MCB.26.8.3204).

Schneider, M.J., Fiering, S.N., Thai, B., Wu, S.Y., St Germain, E., Parlow, A.F., St Germain, D.L., Galton, V.A., 2006. Targeted disruption of the type 1 selenodeiodinase gene (Dio1) results in marked changes in thyroid hormone economy in mice. Endocrinology 147, 580-589.

Stinckens, E., Vergauwen, L., Ankley, G.T., Blust, R., Darras, V.M., Villeneuve, D.L., Witters, H., Volz, D.C., Knapen, D., 2018. An AOP-based alternative testing strategy to predict the impact of thyroid hormone disruption on swim bladder inflation in zebrafish. Aquatic Toxicology 200, 1-12. 10.1016/j.aquatox.2018.04.009 (https://doi.org/10.1016/j.aquatox.2018.04.009).

Stolow, M.A., Shi, Y.B., 1995. Xenopus sonic hedgehog as a potential morphogen during embryogenesis and thyroid hormone-dependent metamorphosis.Nucleic Acids Res. 23, 2555–2562, http://dx.doi.org/10.1093/nar/23.13.2555 (http://dx.doi.org/10.1093/nar/23.13.2555).

Uchida, D., Yamashita, M., Kitano, T., Iguchi, T., 2002. Oocyte apoptosis during the transition from ovary-like tissue to testes during sex differentiation of juvenile zebrafish. Journal of Experimental Biology 205, 711-718.

Walpita, C.N., Crawford, A.D., Darras, V.M., 2010. Combined antisense knockdown of type 1 and type 2 iodothyronine deiodinases disrupts embryonic development in zebrafish (Danio rerio). General and Comparative Endocrinology 166, 134-141.

Walpita, C.N., Crawford, A.D., Janssens, E.D., Van der Geyten, S., Darras, V.M., 2009. Type 2 iodothyronine deiodinase is essential for thyroid hormone-dependent embryonic development and pigmentation in zebrafish. Endocrinology 150, 530-539.

Winata, C.L., Korzh, S., Kondrychyn, I., Korzh, V., Gong, Z. 2010. The role of vasulature and blood circulation in zebrafish swim bladder development. Dev. Biol. 10:3.

Winata, C.L., Korzh, S., Kondrychyn, I., Zheng, W., Korzh, V., Gong, Z. 2009. Development of zebrafish swimbladder: the requirement of Hedgehog signaling in specification and organization of the three tissue layers. Dev. Biol.331, 222–236, http://dx.doi.org/10.1016/j.ydbio.2009.04.035 (http://dx.doi.org/10.1016/j.ydbio.2009.04.035).

Yin, A., Korzh, S., Winata, C.L., Korzh, V., Gong, Z., 2011. Wnt signaling is required for early development of zebrafish swimbladder. PLoS One 6, http://dx.doi.org/10.1371/journal.pone.0018431 (http://dx.doi.org/10.1371/journal.pone.0018431).

Yin, A., Korzh, V., Gong, Z., 2012. Perturbation of zebrafish swim bladder development by enhancing Wnt signaling in Wif1 morphants. Biochim.Biophys. Acta—Mol. Cell Res. 1823, 236–244, http://dx.doi.org/10.1016/j.bbamcr.2011.09.018 (http://dx.doi.org/10.1016/j.bbamcr.2011.09.018)

Relationship: 1041: Reduced, Posterior swim bladder inflation leads to Reduced, Young of year survival (https://aopwiki.org/relationships/1041)

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)	**non-adjacent**	**High**	**Low**
Deiodinase 1 inhibition leading to reduced young of year survival via posterior swim bladder inflation (https://aopwiki.org/aops/157)	**non-adjacent**	**High**	**Low**

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)

Life Stage Applicability

Life Stage	Evidence
Juvenile	High

Sex Applicability

Sex	Evidence
Unspecific	Moderate

The literature provides strong support for the relevance of this KER for physoclistous fish whose inflation occurs at a critical time in development when the fish must gulp air to inflate its swim bladder before the pneumatic duct closes. The relevance to physostomes that maintain an open pneumatic duct into adulthood is less apparent. This likely reflects greater potential to inflate at some point in development, even if early larval inflation is impaired. However, it is plausible that structural damage that prevented inflation of the organ in a phystostome would be expected to cause similar effects.

This KER is probably not sex-dependent since both females and males rely on the posterior swim bladder chamber to regulate buyoancy.

Key Event Relationship Description

See biological plausibility below.

Evidence Supporting this KER

Biological Plausibility

The fish swim bladder, particularly the posterior chamber is species with a two-chambered organ, plays a critical role in buoyancy control in fish. Modulation of the volume of air in the chamber allows for maintenance of neutral buoyancy at different depths in the water column. Neutral buoyancy is energy sparing and allows for fish to expend less energy in maintaining and changing positions in the water column. Because of its roles in energy sparing and swimming performance, it is expected that failure to inflate the swim bladder (particularly the posterior chamber thought to be involved in buoyancy control in most species) would create increased oxygen and energy demands leading to decreased growth, which in turn leads to decreased probability of young of year survival. In particular, these impacts would be expected in non-laboratory environments where fish much expend energy to capture food and avoid predators and where available food is limited.

Empirical Evidence

- Czesny et al. (2005) demonstrated that swim bladder non-inflation was associated with multiple phenotypic and behavioral outcomes that would be expected to adversely impact young of year survival.
- Yellow perch with non-inflated swim bladders grew more slowly than those with inflated swim bladders. Specifically, mean daily growth rate of fish with inflated swim bladders was 0.50 +/- 0.02 mm/d versus 0.32 +/- 0.01 mm/d for fish without inflated swim bladders.
- Yellow perch with non-inflated swim bladders always captured prey less efficiently than those with inflated swim bladders of the same size class.
- Yellow perch with non-inflated swim bladders experienced significantly increased mortality and lower time to mortality in a foodless environment compared to those with inflated swim bladders.
- Yellow perch with non-inflated swim bladders had significantly greater oxygen consumption than fish of the same size class with inflated swim bladders.
- In Lake Michigan, no yellow perch with a total length >20 mm were collected. Around 15 mm the number of fish collected with non-inflated swim bladders dropped off dramatically. These results reflect both the approximate size at which swim bladder inflation normally occurs within the species and inability to survive and grow to sizes exceeding 20 mm if the swim bladder fails to inflate.
- Note: yellow perch are a physoclistous species in which initial inflation can only occur during a narrow window of development in which the pneumatic duct is still connected to the gut, allowing the fish to gulp air and inflate its swim bladder. Once the pneumatic duct closes, normal inflation is no longer possible.
- In aquaculture systems, failure to inflate the swim bladder has been shown to reduce growth rates and cause high mortalities in a wide range of species (reviewed by Woolley and Qin, 2010).
- Pond-cultured walleye with non-inflated swim bladders were found to be smaller (weight and length) than fish with inflated swim bladders. There was also association with deformities (e.g., lordosis) that were expected to impair survival (Kindschi and Barrows, 1993).
- Review of failed swim bladder inflation in wild perch and 26 other physoclistous species showed that fish whose swim bladders failed to inflate had higher mortality, reduced growth, and increased incidence of spinal malformations stereotypical of persistent upward swimming (Egloff, 1996).
- Chatain reported that sea bream (*Sparus auratus*) and sea bass (*Dicentrarchus labrax*) with non-inflated swim bladders were 20-30% less in weight than those with inflated swim bladders and more susceptible to stress-induced mortality (e.g., associated with handling, hypoxia,

etc.). It was suggested this was due to both increased energetic demands and decreased feeding efficiency.

- Marty et al. 1995 measured increased oxygen consumption in Japanese medaka (*Oryzias latipes*) with non-inflated swim bladders compared to those whose swim bladders had inflated.
- In zebrafish (*Danio rerio*) whose smim bladder inflation was prevented by holding in a closed chamber (preventing air gulping to inflate the swim bladder), larval survival was significantly less than that of fish held in open chambers whose swim bladders could inflate. There was also increased incidence of spinal curvature in the closed chamber fish whose swim bladders were prevented from inflating (Goolish and Oukutake, 1999).
- 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).
- In striped bass, (Morone saxatilis) failure to inflate the swimbladder was reported to results in dysfunctional buoyancy control, deformities, and poor larval survival and growth (Martin-Robichaud and Peterson, 2008).

References

- Sergiusz J. Czesny, Brian D. S. Graeb & John M. Dettmers (2005): Ecological Consequences of Swim Bladder Noninflation for Larval Yellow Perch, Transactions of the American Fisheries Society, 134:4, 1011-1020. http://dx.doi.org/10.1577/T04-016.1 (http://dx.doi.org/10.1577/T04-016.1)
- Woolley, L. D. and Qin, J. G. (2010), Swimbladder inflation and its implication to the culture of marine finfish larvae. Reviews in Aquaculture, 2: 181–190. doi: 10.1111/j.1753-5131.2010.01035.x
- Greg A. Kindschi & Frederic T. Barrows (1993) Survey of Swim Bladder Inflation in Walleyes Reared in Hatchery Production Ponds, The Progressive Fish-Culturist, 55:4,219-223, DOI: 10.1577/1548-8640(1993)055<0219:SOSBII>2.3.CO;2
- Egloff, M. 1996. Failure of swim bladder inflation of perch, Perca fluviatilis, L. found in natural populations. Aquat. Sci. 58(1):15-23.
- Chatain, Beatrice. "Problems related to the lack of functional swimbladder in intensive rearing of the seabass Dicentrarchus labrax and the sea bream Sparus auratus." Advances in Tropical Aquaculture, Workshop at Tahiti, French Polynesia, 20 Feb-4 Mar 1989. 1989.
- Gary D. Marty , David E. Hinton & Joseph J. Cech Jr. (1995) Notes: Oxygen Consumption by Larval Japanese Medaka with Inflated or Uninflated Swim Bladders, Transactions of the American Fisheries Society, 124:4, 623-627, DOI: 10.1577/1548-8659(1995).
- Goolish, E. M. and Okutake, K. (1999), Lack of gas bladder inflation by the larvae of zebrafish in the absence of an air-water interface. Journal of Fish Biology, 55: 1054–1063. doi:10.1111/j.1095-8649.1999.tb00740.x
- 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
- Martin-Robichaud, D. J. and Peterson, R. H. (1998), Effects of light intensity, tank colour and photoperiod on swimbladder inflation success in larval striped bass, Morone saxatilis (Walbaum). Aquaculture Research, 29: 539–547. doi: 10.1046/j.1365-2109.1998.00234.x