What can we learn by studying dead fish fry?

Alain Pasquet, Emilie Realis-doyelle, Pascal Fontaine, Fabrice Teletchea

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Keywords

Fresh water fish, malformations, Salmo trutta , temperature.

Abstract

This study investigated the influence of temperature (4, 8 and 12°C) on development and survival of brown trout (Salmo trutta) fry. The three aims of this study were: (a) to propose a typology of malformations; (b) to compare malformation types between live and dead fry and (c) to establish relationships between temperature and malformation occurrences. It was found 20 single malformations and 39 combinations of two or more malformations. Comparison between dead and live fry at different de-velopment stages (hatching, emergence and first food intake) showed that malformations of yolk sac were predominant at hatching and then decreased, while malformations of skeleton or multiple malformations were higher thereafter. All dead fry, and only 14% of live fry were malformed. Dead fry were mainly characterized by yolk sac malformations and multiple malformations whatever the temperature. Live fry showed a higher rate of skeleton malformations at 12°C, and the different types of malformations were equally represented at two other temperatures (4 and 8°C). To conclude, it is suggested that some malformations (yolk sac at hatching, yolk sac associated with skeleton malformations at emergence and skeleton at first food intake or combinations of malformations at all stages)might be lethal as they were founding dead fry and that temperature influences differently the occurrence of malformations.

Introduction

Different terms such as deformity, defect, or malformation are indistinctly used to qualify a structural abnormality of the body (Boglione, Gavaia, et al., 2013; Boglione, Gisbert, et al., 2013). The term malformation is restricted for fish larvae to an abnormality of the whole body (jaw, lordosis, kyphosis, scoliosis, yolk sac oedema) and deformity addressed more precisely bones defects (compression, fusion of vertebral bodies) (Witten, Gil-Martens, Huysseune, Takle, & Hjelde, 2009). Most often, bone defects are identified by X-rays (Aunsmo et al., 2008; Witten, Gil-Martens, Hall, Huysseune, & Obach, 2005; Witten et al., 2009). Because in this study, only visual examination of the fry under a stereomicroscope on photographs was used to identify abnormalities of the body, only the word malformation will be used. Malformations can affect survival. For instance, yolk sac malformations cause significant lower survival rate of Japanese eel fry (Anguilla japonica) (Kurokawa, Shibahara, Gen, Nomura, & Tanaka, 2013). Generally, the high mortality rate due to malformations occurring during early life stages, partly explains why they are so rare in adults. For instance, Cobcroft and Battaglene (2013) showed that the prevalence of malformations of the verte-bral column in several marine species, decreased with age, due to the deadly nature of these malformations. In only 2% or 3% of adult wild salmonids, few skeletal malformations seemed not to be lethal (Gill & Fisk, 1966; Poynton, 1987). Andrades, Becerra, and Fernandez-Llebrez (1996) showed that about 27% of sea bream (Pagrus major) fry at hatching showed spin malformations and 81% of these died soon after hatching.

Early life stages appeared to be the most prone to malforma-tions. Many authors have observed an increase in malformations due to biotic factors, among which stress on brood stock during vitellogenesis (Alix, Zarski, Chardard, Fontaine, & Schaerlinger, 2017; Divanach, Papandroulakis, Anastasiadis, Koumoundouros, & Kentouri, 1997; Haya, 1989), genetic origin (Divanach et al., 1997; Paperna, 1978), or gamete quality (Nowosad & Kucharczyk, 2019; Nowosad, Sikora, & Kucharczyk, 2018; Samarin et al., 2017). Malformations, even when they are not directly lethal, contributed to an increased susceptibility to predation or diseases (Boglione, Gisbert, et al., 2013; Koumoundouros, 2010; Koumoundouros, Oran, Divanach, Stefanakis, & Kentouri, 1997). Abiotic factors may also affect development resulting in malformed individuals, such as salinity (Lee & Menu, 1981), water turbulence (Chatain, 1994), radiations (UV-B) (Blaustein, Kiesecker, Chivers, & Anthony, 1997;

Romansic, Waggener, Bancroft, & Blaustein, 2009), presence of toxic waterborne metals, and pesticides (Fernandez, Garvaia, Laize, & Cancela, 2018), even though temperature seems to be one of the most important (Abdele et al., 2004; Lahnsteiner & Mansour, 2012; Loffler, Ott, Ahnelt, & Keckeis, 2008; Meeuwig, Bayer, & Seelye, 2013; Schultz & Bonar, 2009; Treasurer, 1983). Some malformations were more numerous at high incubation temperatures than the optimal temperature in numerous fish species (Kurokawa et al., 2013; Lahnsteiner, 2012; Sfakianakis et al., 2006; Wang & Tsai, 2000; Wiegand, Hataley, Kitchen, & Buchanan, 1989). In freshwa-ter fish species, temperature above the thermal tolerance has often resulted in larval malformations (Camus & Koutsikopoulos, 1984; Herzig & Winkler, 1986; Rana, 1990; Steinarsson & Björnsson, 1999; Wang & Tsai, 2000; Wiegand et al., 1989). The main malformations observed during the early life stages were located on the skeleton, especially lordosis (Kihara, Ogata, Kawano, Kubota, & Yamaguchi, 2002; Kurokawa et al., 2013; Lahnsteiner, 2012; Linares-Casenave, Werner, Van Eenennaam, & Doroshov, 2013; Sfakianakis et al., 2006).

Brown trout (Salmo trutta) is a cold stenothermal species (Teletchea, Fostier, et al., 2009; Teletchea, Gardeur, Kamler, & Fontaine, 2009) and its fry are very sensitive to temperature changes (Lahnsteiner, 2012; Ojanguren & Braña, 2003; Realis-Doyelle, Pasquet, Charleroy, Fontaine, & Teletchea, 2016). Previous studies showed that there was an optimal temperature for survival rate between 6 and 8°C (Lahnsteiner, 2012; Ojanguren & Braña, 2003; Realis-Doyelle et al., 2016) at hatching, emergence and first food intake (Realis-Doyelle et al., 2016), and the main observed malformations were on the skeleton (Lahnsteiner, 2012), but there is a lack of knowledge on the possible link between the presence of mal-formations and fry survival.

This study aimed at (a) realizing a typology of malformations of brown trout fry, (b) comparing malformations between live and dead fry at different development stages, and (c) studying the influence of the temperatures on the occurrence of these malformations during the early development.

Material and Methods

2.1. Animal acclimation and rearing

Brown trout fertilized eggs were obtained from the Institute for Nature and Forest (INBO), Bosonderzoek (Belgium). Breeders (seven females and seven males) were caught in earthen ponds, on 19 December 2013. The water temperature of the ponds was 8°C. The eggs were obtained by stripping of the females and fertilization was done. Fertilized eggs were transferred on the same day to the University of Lorraine (Nancy-France). The temperature during transport was maintained to 8° C. Eggs were put into three incubators, each containing eight individual racks (44 × 11 × 7 cm) (around 650 eggs per rack). In each rack, the eggs of all the females were mixed. At their arrival, eggs were acclimated to 8° C water and acclimated to the other temperatures (4 and 12° C) by a kinetic of 1°C H−1 (Peterson & Martin-Robichaud, 1989). Three temperatures were therefore tested: 4, 8 and 12° C. The photoperiod was 16L/8D. Both temperature and dissolved oxygen were checked daily (range of probe was 0.1° C for temperature and dissolved oxygen remained at 9 mg/L). Water quality (ammonia, nitrite, and pH) was checked each week. Total ammonia and nitrite concentrations in each tank were kept below 0.05 and 0.010 mg/L respectively and pH remained at 8 (SD = 0.5) (Realis-Doyelle et al., 2016).

2.2. Samplings

Three periods were considered: from fertilization to hatching time (P1), from hatching to emergence (P2), defined when 50% of fry moved from the bottom of each rack into the water column, and from emergence to first food intake (P3) defined as 50% of the fry got their first food intake (Figure 1). Dead fry were removed every day using a pipette to produce weak water movement. The number of dead fry at the end of each period (P1, P2 and P3) was the sum of the number of dead fry per day during the duration of the corresponding period. We used this sampling strategy for dead fry in order to minimize the number of lost individuals because dead fry disappeared rapidly. For each temperature, 128 (16 per rack) live fry were sampled at the end of each period, except at the end of P3 for 12° C, where we removed only 77 fry, because the mortality rate was too high. The comparison between dead and live fry was done at the end of each period. The study was conducted in accordance with the Animal Ethics Committee of the University of Lorraine (no. C54-547-18).

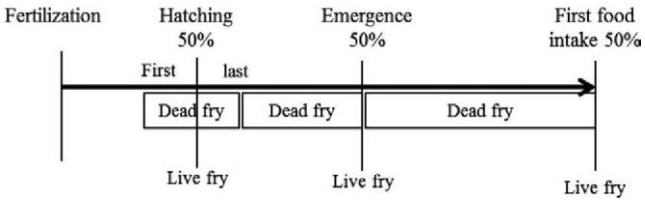

FIGURE 1 Schematic representation of sampling timing on live and dead fry

2.3. Malformations

All sampled fry were conserved on paraformaldehyde at 4% (pH = 6.9) and after in freezer (4°C). Malformations were identified under X10 objective microscope (OPTIKA microscope, SZP-10 with camera MICROVISION Instruments, Lw1235C-GT1 coupled with the software Archimed MICROVISION Instruments 6.0.14). It was identified 20 single malformations (Figure 2) and 39 combinations (multiple malformations).

The typology of malformations (Figure 2) was based on the works of Afonso et al. (2000), Holm et al. (2005), Jezierska, Ługowska, and Witeska (2009) and Lahnsteiner (2012). Skeleton malformations included scoliosis, lordosis and kyphosis. Scoliosis corresponds to a lateral malformed, zig-zag shape (Afonso et al., 2000) or lateral concave curvature of the lumbar region of the skeleton (Holm et al., 2005). Lordosis

is a dorsal malformation, forming a V shape (Afonso et al., 2000) or a concave curvature of the lumbar region of the skeleton (Holm et al., 2005). Kyphosis is a ventral malformation, forming a '3' horizontal shape (Afonso et al., 2000) or a convex curvature of the thoracic region of the skeleton (Holm et al., 2005). The yolk sac malformation is here only oedema defined as an accumulation of body fluid in the region separating the yolk sac from the digestive tube (Holm et al., 2005) or between yolk and the yolk syncytium layer (Alix, Chardard, Ledoré, Fontaine, & Schaerlinger, 2015). The other malformations considered were: on the head, it concerned the jaws, with prognathism (the lower jaw was longer than the upper one) or retro-prognathism, and the eye, with no eye, or only one, or multiple eyes more than two. The fins may be also affected with no fin or atrophied caudal or dorsal fins. Haemorrhages (on the yolk sac or between yolk sac and fry body) and twin forms (two or three fry for one yolk sac or two, three or four heads for one body) could appear (Figure 2). Fry were classified as single or multi-malformed; that is, when fry showed at least two malformations simultaneously (e.g. lordosis and yolk sac malformation or kyphosis and no eye or yolk sac malformation). Live or dead fry that had no visible malformation were considered as well formed.

2.4. Parameters

The dead or live malformed fry were distributed among four categories: those on the skeleton, on the yolk sac, others, and multiple malformations. For each temperature, the total number of dead fry at the end of each period was calculated as the sum of dead fry per day during the length of the period. For dead fry, the percentage of malformations at the end of each period of development (P1, P2 and P3) and for each temperature was calculated by dividing the number of malformed fry of each categories by the number of dead fry. For live fry, the percentage of malformed fry of each category was obtained by dividing the number of malformed fry by the number of sampled fry (n = 128 for each temperature and at the end of each period, except for 12° C at first food intake (P3) where n = 77). We also calculated the percentage of fish with single (or multiple) malformations, for each period at each temperature.

2.5. Statistical analysis

The use of different sampling methods: every day (for dead fry) or just at the end of the period (for live fry) did not allow us to compare directly the percentages of malformations at the end of each period and for each temperature. So the distributions of the different types of malformations were analysed separately for live and dead fry, but with the same statistical procedure. A chi-squared test was use to analyse occurrence of malformations for each status (dead or live) and periods. It was analysed the effect of temperature, period and the interaction between the two with a two-way table for contingency with a log linear test (Sokal & Rolhf, 1995). When the interaction temperature-period was significant, we did piece-wise comparisons on temperatures with a $\chi 2$ test. All statistical analyses were realized with the software Statistica (version 10). All the results were considered significant at the level of $p < 0.05$.

Results

3.1. Typology of the malformations

Overall, 348 dead fry were analysed, which represented 3% of the population (n = 25) at 4° C, 8% (n = 108) at 8° C and 30% (n = 214) at 12° C from hatching to first food intake (P1 to P3) (Table 1). All dead fry had malformations: 192 single malformations (55%) and 155 multiple malformations (45%). The malformations from hatching to first food intake were represented by 55% of single malformations (21% on skeleton, 30% on yolk sac, and 4% of other malformations, Figure 3a). Among the 999 live fry sampled, 7% (n = 73) displayed single malformation; no multiple malformations were observed. From hatching to first food intake, the number of live fry malformed represented 9% (n = 32) of the population at 4° C, 3% (n = 11) at 8° C and 26% (n = 78) at 12° C (Table 1). Malformations of the skeleton represented 54% of the total followed by, others malformations 27%, and yolk sac malformations 19% (Figure 3b).

3.2. Influence of temperature and stages on malformations of live fry

The interaction between temperature and period was significant ($\chi 2 = 63.5$, df = 8, $p < 0.001$) (Table 2). At hatching, the majority of malformations was on yolk sac (59%) at 4° C, belonged to other groups (40% of twin forms and 20% of head malformations) at 8°C, and were found on skeleton (75% only by scoliosis) at 12° C. At emergence for all temperatures, the majority of malformations was on skeleton, and represented by scoliosis (80% of the malformations at 4° C, 100% at 8° C, and 83% at 12°C). At first food intake, the malformations were more numerous on skeleton and represented by scoliosis (57% at 4° C, 44% at 8° C and 89% at 12° C) (Table 2).

At hatching, most malformations concerned the yolk sac (47%). At emergence and first food intake, malformations were mainly found on skeleton (Figure 4a).The percentage of these types of malformations was different between temperatures ($\chi 2 = 52.0$, df = 2, $p < 0.001$). At 4 and 8°C, the malformations were equally distributed (30%–40 %) between the three main types (skeleton, yolk sac and others). At 12°C most malformations (82%) were on skeleton (Figure 4b) and not on yolk sac.

3.3. Influence of temperature and stages on malformations of dead fry

The interaction between temperature and periods was significant ($\chi 2 = 63.5$, df = 8, $p < 0.001$). At hatching, the majority of malforma-tions were single malformations on the yolk sac for all temperatures (55% at 4° C, 76% at 8° C and 79% at 12° C) (Table 3). At emergence, the majority of malformations were multiple malformations (62% at 4° C, 52% at 8° C and 67% at 12° C) (Table 3). In this case, multiple malformations were mainly on skeleton and yolk sac malformations except at 8° C, where the association between yolk sac and head malformations (27%) or lordosis (27%) were dominant. At first food intake, the majority of the malformations were on the skeleton (53% including 30% lordosis and 23%

kyphosis) at 4°C. At 8° C, the skeleton malformations were also more numerous (36% including 27% lordosis and 9% kyphosis). At 12° C, multiple malformations predominated with an association between skeleton and yolk sac oedema (60%) or between three malformations (9%) (Table 3).

The percentage of malformation was different between the periods. At hatching, malformations (70%) were mainly found on the yolk sac, but at emergence and first food intake, multiple malformations including lordosis and yolk sac malformations were much higher (62% and 51% respectively; Figure 5a). The percentage of malformations was different between temperatures ($\chi 2 = 73.3$, df = 2, $p < 0.001$). At 4 and 8°C, the majority of malformations was represented by multiple malformations (40% and 36% respectively) and yolk sac malformations (35% and 31% respectively). At 12°C, the majority of malformations were represented mainly by multiple malformations (55%) (Figure 5b).

Discussion

This study highlights a higher percentage of malformations on dead (100%) compared to live fry (7%). For the dead fry, multiple malformations were the most common whatever the temperature and periods of life, mainly a combination of skeleton and yolk sac malformations. For live fry, the scoliosis was the most common malformation whatever the temperature and periods. The comparison of the distribution of the different types of malformations between each period showed that malformations of the yolk sac were predominant at hatching and then decreased followed by multiple malformations or malformations of the skeleton at emergence and first food intake. This fact could be explained by the consumption of yolk sac during early development. Malformations on dead fry at 4 and 8°C followed the same pattern of distribution with higher yolk sac oedema and multiple malformations; at 12°C multiple malformations were more numerous. For live fry, skeleton malformations were the more numerous at 12°C, and for the other temperatures (4 and 8°C) the malformations were equally distributed.

The first periods of life are the most sensitive to temperature variations because their thermal tolerance range is reduced compared to juveniles and adults (Pörtner & Peck, 2010). Yet, we have no information on the types of lethal malformations in the early stages of life. Indeed, only two studies were carried out on malformations on dead fry according to the temperature during the endogenous phase. Pittman, Skiftesvik, and Harboe (1989) reared halibut (Hippoglossus hippoglossus L.) larvae at three temperatures (4, 6 and 9°C). Only 16.8% of dead fry were analysed because of their decomposition and about 50% of these dead fry had malformations. The difference with present results could be explained by the important effort of sampling during the experimentation (100% of dead fry between hatching and first food intake were analysed). Moreover, the main malformations observed during the experiment of Pittman et al., 1989 were on jaw (18%), and on yolk sac at the highest temperature (9°C). In this study, malformations of the jaws on dead fry accounted for only 0.8%. Comparing with our study, it was hypothesized that some malformations may affect the general development characteristics for many species (the yolk sac is common) but also particular malformations in some species (i.e. flat fish: malformations of the head). Koumoundouros, Maingot, Divanach and Kentouri(2002) had studied only kyphosis on sea bass (Dicentrarchus labrax L.) and the impact of these malformations on fish mortality. In their study, they sampled dead fish randomly at 45 days post hatching and compared with live fry of the same size (TL = 17mm). They found that 52.6% of kyphosis on dead and 26.4% on live fry: the skeleton was dorsally interrupted, with the corresponding neural processes twisted, resulting in a significant compression of the neural tube. They concluded that the incidence of this deformity decreased exponentially over time due to the high mortality of affected fish by early kyphosis. In this study it was found kyphosis malformations principally on dead fry (at 4°C), which is consistent with the conclusions of Koumoundouros, Maingot, Divanach, and Kentouri (2002). In conclusion, kyphosis seems to be a lethal deformity, and a decrease in temperature increases its presence in the population. Indeed, we showed an increase in multiple malformations on dead fry at 12°C. Moreover, we can make the assumption that yolk sac oedema malformation could be lethal at hatching for all temperatures because the percentage decreases strongly after this stage and we could assume that fry died before, because they were not able to use their yolk.

Many authors observed on live fry an increase in skeleton malfor-mations due to temperature variations during the endogenous feeding period on different fish species (Kihara et al., 2002; Kurokawa et al., 2013; Lahnsteiner, 2012; Linares-Casenave et al., 2013; Sfakianakis et al., 2006). For brown trout, Lahnsteiner (2012) showed that the fry reared at 8.5°C (temperature closed to the optimal) had more malformations of the skeleton than at extreme temperatures (3 and 13°C). Embody (1934) observed that with a temperature lower than 3°C than the normal one (between 8 and 10°C), malformations of the skeleton appeared more quickly at 10°C. In this study, results showed that at 12°C, the percentage of skeleton malformations of fry (live or dead) was higher than at the other temperatures. Many authors pointed out the hypothesis that during hatching, fry are not able to modify the orientation of their column, which had been malformed by temperature (Blaxter, 1992; Cook, Guthrie, Rust, & Plesha, 2005; Lahnsteiner, 2011, 2012; Linares-Casenave et al., 2013; Yamagami, 1988). Malformations would also appear later during the training phase of swimming (Kihara et al., 2002; Sfakianakis et al., 2006), which in Salmonidae is linked to the emergence. Moreover, most of the time, swimming difficulties could be a consequence of the non-inflation of the gas bladder (Kitajima, Tsukashima, Fujita, Watanabe, & Yone, 1981). This could imply some difficulties in the swimming course and the fry tried to compensate the lack of stabilization and dynamism during swimming with pectoral fins. In conclusion, they swam in an oblique way that leads to malformations of the column (Koumoundouros et al., 2002). This could be explained by the overload of increase muscle at the midposterior region of the trunk exerted on vertebrae during these first stages of life; this could cause a progressive inflection of the axis of the notochord resulting in the malformed and fusion of the vertebrates (Boglione et al., 1994; Kitajima et al., 1981; Kranenbarg, Waarsing, Muller, Weinans, & Van Leeuwen, 2005). Nevertheless, this deformity of the notochord would not involve a kyphosis but more probably a lordosis (Koumoundouros et al., 2002) expressed in the notochord in the embryo and is implied in the formation of axial skeleton. If the malformations on the skeleton seemed the more numerous whatever the period or the temperature, partly due to our

method of observation, twin forms ranged from 0% to 18% of the malformations at a given stage and temperature. It is known that this type of malformation is rare (0.5% to 4% in the salmon Keta (Oncorhynchus keta) (Yamamoto, Kobayashi, & Kuramoto, 1996), or less than 0.01% in the whitefish (Coregonus maraena) (Nowosad & Kucharczyk, 2019). In the study of Yamamoto et al. (1996) on salmon Keta, a large increase in the temperature (from 8 to 18°C) lead to an increase in twin forms. It was found also that it was especially a decrease in the temperature (from 8 to 4°C) that showed a higher percentage of twin forms. As suggested by Yamamoto et al. (1996), the presence of elements preventing the onset of the axial symmetry of the embryos could be responsible of this early malformation whatever the temperature.

In summary, the comparison of the malformations observed between dead and live fry allows highlighting that: (a) dead fry were almost all malformed contrary to the live individuals, strongly suggesting the possible lethality of some malformations; (b) multiple malformations, malformations of the yolk sac as well as lordosis and kyphosis seemed to be lethal during the endogenous phase in common trout. The higher temperature increased possible lethal multiple malformations (at 12°C) during early life stages.

4.1. Part 1

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Conclusion

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Table 1. Titre de la Table

Compound	TEF (WHO05)	Chemical characteristics			Transfer to milk		Transfer to hen eggs		Transfer to duck eggs
		Cln	Log Kow1	MW	TR2, % (n=8)	Level3	TR, % (n=4)	Level	TR4, % (n=1)
2,3,7,8-TCDD	1	4	6.6	322	34.0 ± 6.3	High	39.1 ± 12.6	High	2.0
1,2,3,7,8 -PeCDD	1	5	7.2	340	26.7 ± 7.1	High	35.8 ± 12.2	High	3.4
1,2,3,4,7,8-HxCDD	0.1	6	7.6	391	17.8 ± 8.0	Medium	43.3 ± 16.5	High	2.3
1,2,3,6,7,8-HxCDD	0.1	6	7.6	391	22.7 ± 7.1	Medium	40.6 ± 14.4	High	3.0
1,2,3,7,8,9-HxCDD	0.1	6	7.6	391	13.2 ± 3.4	Medium	29.1 ± 12.4	High	1.3
1,2,3,4,6,7,8-HpCDD	0.01	7	8.0	425	4.1 ± 1.3	Low	16.2 ± 6.2	Medium	1.1
OCDD	0.0003	8	8.4	460	1.2 ± 0.8	Low	6.8 ± 4.8	Low	1.0
2,3,7,8-TCDF	0.1	4	6.5	306	3.4 ± 2.9	Low	39.1 ± 16.8	High	6.4
1,2,3,7,8-PeCDF	0.03	5	7.0	340	4.9 ± 4.5	Low	38.0 ± 7.4	High	4.5
2,3,4,7,8-PeCDF	0.3	5	7.1	340	35.6 ± 14.8	High	40.0 ± 10.1	High	4.8
1,2,3,4,7,8-HxCDF	0.1	6	7.5	375	19.3 ± 8.9	Medium	39.8 ± 13.0	High	2.5
1,2,3,6,7,8-HxCDF	0.1	6	7.6	375	17.7 ± 6.0	Medium	37.3 ± 16.1	High	2.4
1,2,3,7,8,9-HxCDF	0.1	6	7.7	375	10.7 ± 7.0	Medium	25.6 ± 13.0	High	1.9
2,3,4,6,7,8-HxCDF	0.1	6	7.6	375	11.6 ± 8.7	Medium	23.0 ± 16.5	Medium	0.8
1,2,3,4,6,7,8-HpCDF	0.01	7	8.0	409	3.1 ± 1.1	Low	16.6 ± 10.7	Medium	0.7
1,2,3,4,7,8,9-HpCDF	0.01	7	8.2	409	4.6 ± 1.3	Low	17.8 ± 8.7	Medium	1.1
OCDF	0.0003	8	8.6	443	1.0 ± 1.3	Low	4.0 ± 2.5	Low	0.1

Bold mean integrated article in our dataset

1 Statement of steady state (SS) given by the authors or in brackets when statement was made by us.

2 TR- transfer rate, BCF – bioconcentration factor, BA- bioavailability, BTF- biotransfer factor. MISS – missing data. Values of the parameters taken from the authors or in brackets when re-calculated by us.

3 C – concentrations, Q-quantities where ✓ = given in the article; (✓) = Recalculated; ✗= not available

4 Compounds that below limit of quantification (LQ)

5 Decision of integration (✓) or not (✗) of the given study in our dataset.

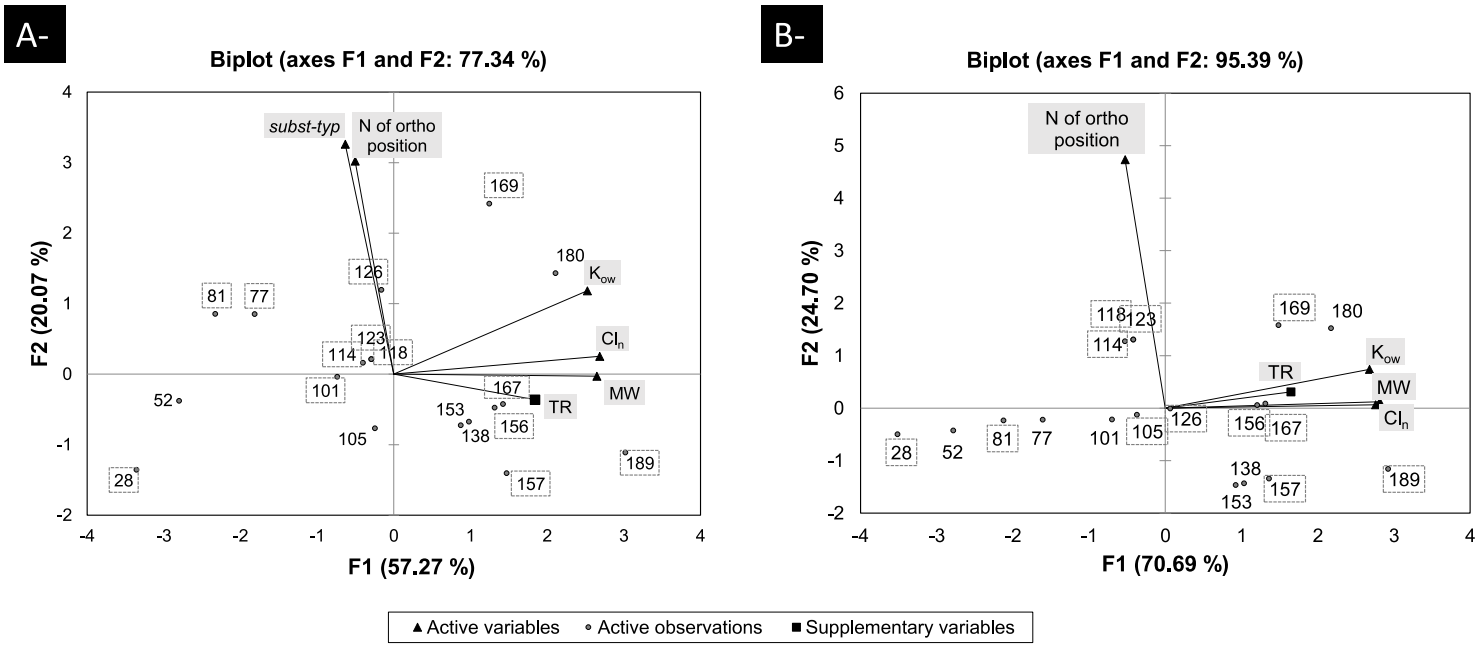

Figure 1. Titre du graphique

Indication: Numbers correspond to the PCB congeners. Framed congeners are dioxin-like PCBs. Bold numbers were congeners transferred at a high level ranking from 38 to 78% and from 30 to 80% respectively for milk and eggs.

References

Abdele, I., Abella, E., Lopez-albors, O., Valdes, P., Norte, M. J., & Garcia-Alcazar, A. (2004). Abnormalities in the juvenile stage of sea bass (Dicentrarchus labra x L.) reared at different temperatures: Types, prevalence and effect on growth. Aquaculture International, 12, 523– 538. https://doi.org/10.1007/s10499-004-0349-9

Afonso, J. M., Montero, D., Robaina, L., Astorga, N., Izquierdo, M. S., & Gines, R. 2000. Association of a lordosis-scoliosis-kyphosis deformity in gilthead seabream (Sparus aurata) with family structure. Fish Physiology and Biochemistry, 22,159–163. https://doi.org/10.1023/A:10078117

Alix, M., Chardard, D., Ledoré, Y., Fontaine, P., & Schaerlinger, B. (2015). An alternative developmental table to describe non-model fish species embryogenesis: Application to the description of the Eurasian Perch (Perca fluviatilis L. 1758) development. EvoDevo, 6, 39. https:// doi.org/10.1186/s13227-015-0033-3.

Alix, M., Zarski, D., Chardard, D., Fontaine, P., & Schaerlinger, B. (2017). Deformities in newly hatched embryos of Eurasian perch populations originating from two different rearing systems. Journal of Zoology, 302, 126–137. https://doi.org/10.1111/jzo.12447

Andrades, J. A., Becerra, J., & Fernandez-Llebre z, P. (1996). Skeletal deformities in fry, juvenile and adult stages of cultured gilthead sea bream (Sparus aurata L.). Aquaculture, 141, 1–11. https://doi. org/10.1016/0044-8486(95)01226-5.

Aunsmo, A., Guttvik, A., Midtlyng, P. J., Larssen, R. B., Evensen, O., & Skjerve, E. (2008). Association of spinal deformity and vaccine-induced abdominal lesions in Atlantic salmon, Salmo salar. L. Journal of Fish Diseases, 31, 515–524. https://doi.org/10.1111/j.1365-2761.00899.x

Blaustein, A., Kiesecker, J., Chivers, D., & Anthony, R. (1997). Ambient UV-B radiation causes deformities in amphibian embryos. Proceedings of the National Academy of Sciences of the USA, 94, 13735–13737. https://doi.org/10.1073/pnas.94.25.13735

Blaxter, J. H. S. (1992). The effect of temperature on fry fishes. Netherlands Journal of Zoology, 42, 336–357. https://doi. org/10.1163/156854291X00379

Boglione, C., Gavaia, P., Koumoundouros, G., Gisbert, E., Moren, M., Fontagné, S., & Witten, P. E. (2013). A review on skeletal anomalies in reared European larvae and juveniles. Part 1: Normal and anomalous skeletogenetic processes. Reviews in Aquaculture, 5, S99–S120. https://doi.org/10.1111/raq.12015

Boglione, C., Gisbert, E., Gavaia, P., Witten, P. E., Moren, M., Fontagné, S., & Koumoundouros, G. (2013). A review on skeletal anomalies in reared European larvae and juveniles. Part 2: Main typologies, occurrences and causative factors. Reviews in Aquaculture, 5, S121–S167. https://doi.org/10.1111/raq.12016

Boglione, C., Marino, G., Ferreri, F., Finoia, M. G., Scardi, M., & Cataudella, S. (1994). In P. Kestemont, F. Sevila, & P. Williot (Eds.), Measures for success (pp. 191–197). Antony, France: CEMAGREF.

Camus, P., & Koutsikopoulos, C. (1984). Incubation and embryonic development of gilthead bream, Sparus aurata (L.), at a range of temperatures. Aquaculture, 42, 117–128.

Chatain, B. (1994). Abnormal swimbladder development and lordosis in sea bass (Dicentrachus labra x) and sea bream (Sparus aurata). Aquaculture, 97, 169–180. https://doi.org/10.1016/0044-8486(94)90301-8

Cobcroft, J., & Battaglene, S. (2013). Skeletal malformations in Australian marine finfish, hatcheries. Aquaculture, 396, 51–58. https://doi.org/10.1016/aquaculture.2013.02.207

Cook, M. A., Guthrie, K. M., Rust, M. B., & Plesha, P. D. (2005). Effects of salinity and temperature during incubation on hatching and development of lingcod Ophiodon elongatus Girard, embryos. Aquaculture Research, 36, 1298–1303. https://doi. org/10.1111/j.1365-2109.2005.01346.x

Divanach, P., Papandroulakis, N., Anastasiadis, P., Koumoundouros, G., & Kentouri, M. (1997). Effect of Water currents on the development of skeletal deformities in sea bass (Dicentrarchus labrax L.) with functional swimbladder during post fry and nursery, phase. Aquaculture, 156, 145–155. https://doi.org/10.1016/S0044-8486(97)00072-0

Embody, G. (1934). Relation of temperature to the incubation periods of eggs of four species of trout. Transactions of the American Fisheries Society, 64, 281–292. https://doi.org/10.1577/1548.8659(1934)64(281:ROTTTI)2.0.CO;2

Fernandez, I., Garvaia, P. J., Laize, V., & Cancela, L. (2018). Fish as a model to assess chemical toxicity in bone. Aquatic Toxicology, 194, 208–226. https://doi.org/10.1016/aquatox.2017.11.015

Gill, C. D., & Fisk, D. M. (1966). Vertebral abnormalities in sockeye, pink and chum salmon. Transactions of the American Fisheries Society, 95, 177–182. https://doi.org/10.1577/1548-8659(1966)95[177:VAISPA]2.0.CO;2

Haya, K. (1989). Toxicity of pyrethroid insecticides to fish. Environmental Toxicology and Chemistry, 8, 381–391. https://doi.org/10.1002/ etc.5620080504

Herzig, A., & Winkler, H. (1986). The influence of temperature on the embryonic development of three cyprinid fishes, Abramis brama, Chalcalburnus chalcoides mento and Vimba vimba. Journal of Fish Biology, 28, 171–181. https://doi.org/10.1111/j.1095-8649.1986.tb05155.x

Holm, J., Palace, V., Siwik, P., Sterling, G., Evans, R., Baron, C., ... Wautier, K. (2005). Developmental effects of bioaccumulated selenium in eggs and frye of two salmonid species. Environmental Toxicology and Chemistry, 24, 2373–2381. https://doi.org/10.1897/04-402R1.1

Jezierska, B., Ługowska, K., & Witeska, M. (2009). The effects of heavy metals on embryonic development of fish (a review). Fish Physiology and Biochemistry, 35, 625–640. https://doi.org/10.1007/s10695-008-9284-4

Kihara, M., Ogata, S., Kawano, N., Kubota, I., & Yamaguchi, R. (2002). Lordosis induction in juvenile red sea bream, Pagrus major, by high swimming activity. Aquaculture, 212, 149–158. https://doi. org/10.1016/S0044-8486(01)00871-7

Kitajima, C., Tsukashima, Y., Fujita, S., Watanabe, T., & Yone, Y. (1981). Relationship between uninflated swim bladders and lordotic deformity in hatchery reared sea bream Pagrus major. Bulletin of the Japanese Society for the Science of Fish, 47, 1289–1294. https://doi. org/10.2331/suis an.47.1289

Koumoundouros, G. (2010). Morpho-anatomical abnormalities in Mediterranean marine aquaculture. Transworld Research. Network, 37, 661–695.

Koumoundouros, G., Maingot, E., Divanach, P., & Kentouri, M. (2002). Kyphosis in reared sea bass (Dicentrarchus labra x L.): Ontogeny and effects on mortality. Aquaculture, 209, 49–58. https://doi.org/10.1016/S0044-8486(01)00821-3

Koumoundouros, G., Oran, G., Divanach, P., Stefanakis, S., & Kentouri, M. (1997). The Opercular complex deformity in intensive gilthead sea bream (Sparus auratu L.) larviculture. Moment of apparition and, description. Aquaculture, 156, 165–177. https://doi.org/10.1016/S0044-8486(97)89294-0

Kranenbarg, S., Waarsing, J. H., Muller, M., Weinans, H., & Van Leeuwen, J. L. (2005). Lordotic vertebrae in sea bass (Dicentrarchus labra x L.) are adapted to increased loads. Journal of Biomechanics, 38, 1239– 1246. https://doi.org/10.1016/j.biomech.2004.06.011

Kurokawa, T., Shibahara, H., Gen, K., Nomura, K., & Tanaka, H. (2013). Determination of periods of sensitivity to low-salinity and low-temperature conditions during the early development of cultured Japanese eel Anguilla japonica fry with respect to the rate of morphological deformity at completion of yolk resorption. Fisheries Science, 79, 673–680. https://doi.org/10.1007/s12562-013-0636-3

Lahnsteiner, F. (2011). Limitations for prolonged chilled storage of ze-brafish (Danio rerio) embryos. Aquaculture Research, 42, 288–295. https://doi.org/10.1111/j.1365-2109.2010.02621.x

Lahnsteiner, F. (2012). Thermotolerance of brown trout, Salmo trutta, gametes and embryos to increased water temperatures. Journal of Applied Ichthyology, 28, 745–751. https://doi. org/10.1111/j.1439-0426.2012.01934.x

Lahnsteiner, F., & Mansour, N. (2012). The effect of temperature on sperm motility and enzymatic activity in brown trout Salmo trutta, burbot Lota lota and grayling Thymallus thymallus. Journal of Fish Biology, 81, 197–209. https://doi.org/10.1111/j.1095-8649.2012.03323.x

Lee, C. S., & Menu, B. (1981). Effects of salinity on egg development and hatching in grey mullet Mugil cephalus L. Journal of Fish Biology, 19, 179–188. https://doi.org/10.1111/j.1095-8649.1981.tb05822.x

Linares-Casenave, J., Werner, I., Van Eenennaam, J. P., & Doroshov, S. I. (2013). Temperature stress induces notochord abnormalities and heat shock proteins expression in fry green sturgeon (Acipenser medirostris Ayres 1854). Journal of Applied Ichthyology, 29, 958–967. https://doi.org/10.1111/jai.12220

Loffler, J., Ott, A., Ahnelt, H., & Keckeis, H. (2008). Early development of the skull of Sander lucioperca (L.) (Teleostei: Percidae) relating to growth and mortality. Journal of Fish Biology, 72, 233–258. https:// doi.org/10.1111/j.1095-8649.2007.01699.x

Meeuwig, I., Bayer, J., & Seelye, J. (2013). Effects of temperature on survival and development of early life stage Pacific and Western brook Lampreys. Transactions of the American Fisheries Society, 134, 119–127. https://doi.org/10.1577/FT03-206.1

Nowosad, J., & Kucharczyk, D. (2019). Fish report of the occurrence and different types of Siamese twins in common whitefish Coregonusmaraena larvae originating from the Baltic sea. Diseases of Aquatic Organisms. http://www.int-res.com/prepress/d03314.html

Nowosad, J., Sikora, M., & Kucharczyk, D. (2018). Survival rate and the occurrence of larval malformations, including Siamese twins, following fertilization of postovulatory aged oocytes in ide Leuciscusidus. Diseases of Aquatic Organisms, 127, 237–242. https:// doi.org/10.3354/dao03208

Ojanguren, A. F., & Braña, F. (2003). Thermal dependence of embryonic growth and development in brown trout. Journal of Fish Biology, 62, 580–590. https://doi.org/10.1046/j.1095-8649.2003.00049.x

Paperna, I. (1978). Swimbladder and skeletal deformations in hatchery bred Sparus aurata. Journal of Fish Biology, 12, 109–114. https://doi. org/10.1111/j.1095-8649.1978.tb04157.x

Peterson, R. H., & Martin-Robichaud, D. J. (1989). First feeding of Atlantic salmon (Salmo salar L.) fry as influenced by temperature regime. Aquaculture, 78, 35–53. https://doi. org/10.1016/0044-8486(89)90004-5

Pittman, K., Skiftesvik, A., & Harboe, T. (1989). Effect of temperature on growth rates and organogenesis in the fry of Halibut (Hypoglossus hypoglossus L.). Conseil Permanent International Pour L'exploration De La Mer, 191, 421–430.

Pörtner, H. O., & Peck, M. A. (2010). Climate change effects on fishes and fisheries: Towards a cause-and-effect understanding. Journal of Fish Biology, 77, 1745–1779. https://doi. org/10.1111/j.1095-8649.2010.02783.x

Poynton, S. (1987). Vertebral column abnormalities in Brown trout Salmo trutta. Journal of Fish Disease, 10, 53–57. https://doi. org/10.1111/j.1365-2761.1987.tb00718.x

Rana, K. J. (1990). Influence of incubation temperature on Oreochromis niloticus (L.) eggs and fry I. Gross embryology, temperature toler-ance and rates of embryonic, development. Aquaculture, 87, 65–181. https://doi.org/10.1016/0044-8486(90)90273-P

Realis-Doyelle, E., Pasquet, A., De Charleroy, D., Fontaine, P., & Teletchea, F. (2016). effects of temperature on the early life stages of a cold stenothermal fish species, brown trout Salmo trutta: Possible implications for climate-warming predictions. PLoS ONE, 11(5), e0155487. https://doi.org/10.1371/journal.pone.0155487

Romansic, J., Waggener, A., Bancroft, B., & Blaustein, A. (2009). Influence of ultraviolet-B radiation on growth, prevalence of deformities, and susceptibility to predation in Cascades frog (Rana cas- cadae) fry. Hydrobiologia, 624, 219–233. https://doi.org/10.1007/s10750-009-9703-2

Samarin, A. M., Zarski, D., Palinska-Zarska, K., Krejarzeff, S., Blecha, M., Kucharczyk, D., & Policar, T. (2017). In vitro storage of unfertilized eggs of the Eurasian perch and its effect on egg viability rates and the occurrence of larval malformations. Animal, 11, 78–83. https:// doi.org/10.1017/S1751731116001361

Schultz, A., & Bonar, S. (2009). Growth and survival of fry and juvenile gila chub at different temperatures. North American Journal of Aquaculture, 71, 1–5. https://doi.org/10.1577/A06-084.1

Sfakianakis, D. G., Georgakopoulou, E., Papadakis, I. E., Divanach, P., Kentouri, M., & Koumoundouros, G. (2006). Environmental determinants of haemallordosis in European sea bass, Dicentrarchus labra x (Linnaeus, 1758). Aquaculture, 254, 54–64. https://doi.org/10.1016/j.aquaculture. 2005.10.028

Sokal, R. R. & Rohlf, F. J. (1995). In R. R. Sokal & F. J. Rohlf (Eds.),Biometry: The principle and practice of statistics in biological research. Analysis of three-way and multiway tables (pp. 743–760). New York, NY: Freeman and

Company.
Steinarsson, A., & Björnsson, B. (1999). The effects of temperature and size on growth and mortality of cod fry. Journal of Fish Biology, 55, 100–109. https://doi.org/10.1111/j.1095-8649.1999.tb01048.x
Teletchea, F., Fostier, A., Kamler, E., Gardeur, J. N., Le Bail, J. Y., Jalabert, B., & Fontaine, P. (2009). Comparative analysis of reproductive traits in 65 freshwater fish species: Application to the domestication of new fish species. Reviews in Fish Biology and Fisheries, 19, 403–430. https://doi.org/10.1007/s11160-008-9102-1
Teletchea, F., Gardeur, J. N., Kamler, E., & Fontaine, P. (2009). The relationship of oocyte diameter and incubation temperature to incuba-tion time in temperate freshwater fish species. Journal of Fish Biology, 74, 652–668. https://doi.org/10.1111/j.1095-8649.2008.02160.x
Treasurer, J. W. (1983). Estimates of egg and viable embryo in a lacus-trine perch, Perca fluviatilis. Environmental Biology of Fishes, 8, 3–16. https://doi.org/10.1007/BF00004941
Wang, L., & Tsai, C. (2000). Effects of temperature on the deformity and sex differentiation of tilapia, Oreochromis mossambicus. Journal of Experimental Zoology, 286, 534–537. https://doi.org/10.1002/(SICI)1097-010X(20000401)286:5<534:AID-JEZ11>3.0CO;2-2
Wiegand, M. D., Hataley, J. M., Kitchen, C. L., & Buchanan, L. G. (1989). Induction of developmental abnormalities in fry goldfish, Carassius auratus L., under cool incubation conditions. Journal of Fish Biology, 35, 85–95. https://doi.org/10.1111/j.1095-8649.1989.tb03395.x
Witten, P. E., Gil-Martens, L., Hall, B. K., Huysseune, A., & Obach, A. (2005). Compressed vertebrae in Atlantic salmon (Salmo salar): Evidence for metaplastic chondrogenesis as a skeletogenic response late in ontogeny. Diseases of Aquatic Organisms, 64, 237–246. https:// doi.org/10.3354/dao064237
Witten, P., Gil-Martens, L., Huysseune, A., Takle, H., & Hjelde, K. 2009. Towards a classification and an understanding of developmental relationships of vertebral body malformations in Atlantic salmon (Salmo salar L.). Aquaculture, 295, 6–14. https://doi.org/10.1016/j.aquaculture. 2009.06.037 .
Yamagami, K. (1988). Mechanisms of hatching in fish. In W. S. Hoar & D. J. Randall (Eds.), Fish physiology (pp. 447–499). New York, NY: Academic Press.
Yamamoto, T. S., Kobayashi, W., & Kuramoto, T. (1996). Twin malformation induced chum salmon eggs by elevated water temperature, with a suggestion as to its mechanism. Canadian Journal of Zoology, 74, 485–491. https://doi.org/10.1139/z96-057