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Keywords

Embryonic deformities, recirculating aquaculture system, Perca fluviatilis, histology, husbandry, rearing systems, embryonic development, Lorraine ponds.

Abstract

During the teleost ontogenesis, biotic and abiotic factors influence organs deformations. Despite of an important literature, the potential causes of deformities in fish are still poorly understood. Cultured fish in artificial conditions exhibit higher incidence of developmental impairments compared to wild ones. This study aims at describing exhaustively the deformity categories of newly hatched embryos of Eurasian perch and investigating at once all biotic and abiotic factors of two extremely different rearing systems [recirculating aquaculture system (RAS) vs. ponds] on their occurrence. The description and clustering of deformities according to the organs involved allowed not only to show that skeletal, cardiac and yolk anomalies are the most common ones, but also to highlight new functions undergoing developmental failure (locomotive, digestive and excretory systems). In total, 10 categories of abnormalities have been described. In addition, our data show a relationship between the cardiac and yolk deformities suggesting common causes. In a second part, we compared the incidence of deformities in the progeny of two distinct populations of fish, reared under extremely various conditions (RAS vs. pond). The total deformity rates did not differ between the groups but the nature of deformities was surprisingly different as the RAS reared fish (RRF) originating from fisheries presented a higher variety of deformity categories than the fish originating from Lorraine ponds (LPRF). Indeed, cardiac, yolk and skeletal axis deformities were more frequent in RRF embryos, whereas mouth abnormalities were preferentially represented in LPRF embryos. These findings may help understanding the potential causes of these developmental impairments in cultured animals and may lead to the improvement of husbandry conditions.

1. Introduction

During animals life cycle, several morphological, anatomical, physiological or behavioral deviations from a standard phenotype may occur (Divanach et al., 1996). Therefore, it derives from various intrinsic and/or extrinsic factors contributing to the decrease in the developmental success, especially for the early life stages during which the definitive phenotype is built (Balon, 1990). In that context, the occurrence of divergent phenotypes during the early development can increase the susceptibility of animals to predation and could lead to the decline and the extinction of a population (Westernhagen et al., 1988). The complex issue of understanding the potential causes of developmental impairments needs detailed investigations to reveal new processes potentially disturbed during the breeders life history and the offspring development (Divanach et al., 1996; Bonnet, Fostier & Bobe, 2007; Pulcini et al., 2015). This is important in farming animals where the production is particularly affected by these problems and in wild populations submitted to environmental changes (Divanach et al., 1996; Réalis-Doyelle et al., 2016).

Most of studies aimed at determining the effect of some specific abiotic or biotic factors on the developmental impairments, especially in species with external development as most of the finfish that are sensitive to the external factors. In that context, the effects of specific environmental factors (Westernhagen et al., 1988; Jezierska, Ługowska & Witeska, 2009; Villamizar et al., 2011; Bondarenko, Drozd & Policar, 2015; Réalis-Doyelle et al., 2016), nutritional factors (Fernández et al., 2008; Boglione et al., 2013) and husbandry practices (Aegerter & Jalabert, 2004) on the occurrence of deformities have already been reported for several species. However, these studies focused on the global deformity rates and several categories of deformities as the blastomeres cleavage, the skeletal axis, jaw, swim bladder or yolk deformities (Johnson & Katavic, 1984; Andrades, Becerra &

Fernandez-Llebrez, 1996; Divanach et al., 1996; Koumoundouros et al., 1997; Avery, Killen & Hollinger, 2009; Boglione et al., 2013). These deformities may compromise either the survival of the individuals or their growth rate.

As a whole, a high prevalence of deformed fish in captive conditions have been reported (Koumoundouros, 2010; Boglione et al., 2013). Considering the extreme, freshwater fish can be reared in ponds, close to the wild conditions, or in recirculating aquaculture system (RAS) in which all conditions are controlled to optimize fish growing and reproduction. As a consequence, cultured fish can confront various types of environments depending of the rearing system including the lack of predators, food, high density of fish and sometimes inappropriate captive conditions influencing several biological functions among which the reproduction (Andrades et al., 1996; Pulcini et al., 2015). In addition, fish maintained in one specific rearing condition are usually adapted to it. In other words, comparing fish reared in pond or RAS conditions involves to take into account not only all environmental modifications but also intrinsic factors of the breeders. Up to now, no study aimed at investigating the global effect of all these extrinsic and intrinsic parameters at once on the deformities occurrence in the progeny.

The Eurasian perch, Perca fluviatilis L., is a promising and valuable species for the diversification of freshwater aquaculture in Europe. It is reared in RAS and pond systems and is considered at a level 4 of the domestication process according to Teletchea & Fontaine (2014). Despite of the control of its reproduction cycle, the incidence of developmental impairments is still variable (Schaerlinger & Zarski, 2015). The Eurasian perch is an interesting species to study due to its high fecundity, a detailed description of its normal embryonic development (Alix et al., 2015), and the easiness to observe early developmental impairments thanks to the egg envelope transparency. Moreover, the perch spawn can easily be found from various origins (Zarski et al., 2016).

The present study aims at (1) investigating the morphological deformities in newly hatched embryos and (2) identifying the potential relationships between these abnormalities to (3) determine the global effect of all extrinsic and intrinsic factors of populations reared in two extreme rearing conditions (pond vs. RAS) on the occurrence of deformities in Eurasian perch.

2. Material and Methods

2.1. Broodstock and eggs management

Fish were handled in accordance with national and international guidelines for the protection of the animal welfare (Directive 2010/63/EU). The study was conducted during consecutive years (2013, 2014 and 2015). Fish originated either from Lorraine ponds (LPRF, three batches of spawn) or a fishfarmer broodstock reared in RAS conditions (RRF, two batches) (Table 1).

2.2. Rearing protocol of LPRF

Lorraine pond reared fish were caught 1 month before the spawning season and transferred to outdoor facilities (tanks of 3000 L, Lorraine, France) to facilitate their manipulation. At that time, females were about to ovulate and males were slightly spermiating. During the spawning season, males and females were kept together in tanks, the photothermal conditions were natural to promote spontaneous oocytes maturation and water temperature records varied between 8 and 16°C. Once the first female spawned, each of them was checked daily to determine the ovulation time. The day of their ovulation, females were hand-striped into a dry container prior to the in vitro fertilization.

2.3. Rearing protocol of RRF

Recirculating aquaculture system reared fish provided by a fish farm (Lucas Perches, Lorraine, France), were reared in an intensive system and fed with commercial pellets (Sturgeon Grower, Le Gouessant) since the early juvenile stage and transferred to our experimental facilities until the beginning of the experiments. Males and females were kept together in 500 L (2013) or 2000 L (2015) tanks connected to a RAS, with an automatic system controlling the temperature and photoperiod (Fontaine, Wang & Hermelink, 2015). Following the induction of the gonadal cycle, fish were led to mature and spawn without any additional treatment but environmental stimulation (increment of the temperature up to 12°C and dark/light photoperiod up to 8:16). Once a female was recognized to be ready to spawn, the eggs were stripped manually as described above.

2.4. In vitro fertilization and incubation of the embryos

For each fish group, the same spawning protocol was applied. The fertilization was performed according to the 'wet method' (Zarski et al., 2012). A mix of sperm from three males was added to each spawn (0.10 ml sperm.g-1 dry eggs). During the embryogenesis, each spawn was incubated uninterruptedly until hatching at 13.0 f 0.5°C in a hatchery with automatic thermoregulation and UV sterilization. A constant dark/light photoperiod (8:16) was applied at a light intensity of 200 lx at the water surface. For each spawn, three samples (about 100 eggs per sample) were incubated separately to perform the experiment the same way on each sampling. The water quality of breeder's tanks and incubator are summarized in Table S1.

2.5. Characterization and occurrence of deformities

The embryonic period was considered from the fertilization to the onset of exogenous feeding according to Alix et al. (2015).

2.6. Observations of living embryos

Each spawn was monitored daily during the hatching period to collect embryos as soon as they hatch. Embryos captured on the day of their hatching were observed immediately using a stereoscopic microscope (Olympus, SZX7) at low magnification (92) to evaluate the presence of deformities. They were called newly hatched embryos (NHE). We checked several organs, such as the skeleton axis, mouth, eyes, yolk, fins-related structure (pectoral fins anlagen and finfold),

digestive and cardiac system, that are easily visible with this technique. To investigate more accurately which organs present an abnormality and describe the impairments, each abnormal individual was photographed the same day using a light upright optical microscope (Nikon Eclipse Ni-U) associated with a DS-Fi2 digital camera and the software NIS BR (Nikon France, France) at low magnification (920). Abnormalities occurrence was certified after a careful comparison with normal NHE morphology at the same age (Alix et al., 2015). Each NHE was assigned to one of 10 categories according to the organs involved [axis (Ad), cardiac (Cd), yolk sac (Yd), mouth (Md), eyes (Ed), digestive system (Dd), fins-related structure (Fd), urinary system (Ud), head (Hd) or multivariable deformed (MVd)]. Some embryos exhibited several deformations in various organs. They were taken into account in each category (except embryos exhibiting MVd).

2.7. Histological study

Deformed embryos were fixed the same day in Bouin's solution for 48 hours, prepared, cut and stained as described in Alix et al. (2015). Stained sections were examined using a light upright optical microscope (Nikon Eclipse Ni-U) at 9100 magnifications (Nikon France, Champigny-sur-Marne, France). To better characterize deformities involving the cardiac system, yolk, mouth and skeletal axis (abnormalities more often observed), five embryos per organ coming from 15 different spawn were longitudinally cut. In addition, five embryos were transversally cut for the cardiac and yolk deformities.

2.8. Data analyses

In total, 42 spawn were examined (30 from RRF and 12 from LPRF) among which 585 NHE exhibit macroscopic deformities (Table 1). For each spawn, the total number of NHE were taken into account to calculate the total deformity rate (Dr=number of deformed embryos/number of hatched embryos 9 100, in %). The mean deformity rate has been obtained for each of the fish group f SE (RRF and LPRF). In addition, specific deformity rates were calculated as the number of hatched embryos exhibiting a particular deformity in relation to the total number of abnormal embryos (in %). The assumption of normality (Shapiro test) and homogeneity of variances (Levene test) were tested for all the deformities variables. The specific deformities rates were represented with box plots in RRF or LPRF. When data did not respect the assumption of normality, anon-parametric Mann–Whitney test was used to determine a significant difference between the total deformities or specific deformities rates according to the global effect of abiotic and biotic factors linked to the rearing conditions. Statistical analyses were performed on the mean of deformity or specific deformities rates.

In order to determine potential relationships between categories of deformities, a principal component analysis (PCA) was performed using the specific deformities rates and represented on a correlation circle. Moreover, a Pearson's correlation table was generated. Data analyses were performed with "R" software (version 3.2.5; The R Foundation for Statistical Computing, Vienna, Austria).

3. Results

Investigating the deformities in newly hatched embryos (NHE) allows not only characterizing deformities commonly described in other species and in various environmental conditions, but also less described categories.

3.1. Macroscopic and histological descriptions of embryonic deformities

3.1.1. Axis deformities

Axis deformities were found to be the most frequent category of impairment (61.1 f 4.9%). Among them, one distinguishes lordosis (V-shaped dorsal-ventral curvature), kyphosis (A-shaped dorsal-ventral curvature) and scoliosis (lateral curvature) (Fig. 1b). The histological analysis did not reveal any cellular defect in the notochord or the tissues surrounding the deformed parts of the body (e.g. muscle).

3.1.2. Mouth deformities

Mouth deformities were the second most frequent deformity category recorded (33.6 f 4.8%). They were primarily observed on the lower jaw, with a shortening (Fig. 1c,d), elongation ora gaping mouth phenotype (Fig. 1e). No difference between normal and abnormal tissues was found at the histological level.

3.1.3. Yolk deformities

Yolk deformities represent 27.8 f 4.5% of the deformed embryos. NHE exhibit mainly yolk sac edema, pear-shape yolk or yolk detachment (Fig. 1b,c,e, star). This yolk detachment is accompanied by an abnormal morphology of the liver which is drawn out between the intestine and the yolk. In comparison with normal specimens (Fig. 2a,c,e), the histological study reveals that NHE with yolk edemas exhibit generalized edema in the body as described more accurately below (Fig. 2b,f).

3.1.4. Cardiac deformities

Two main types of deformities have been observed. First, cardiac cavity edemas (Fig. 1b,c,e), secondly, a modification of

the heart shape which is elongated and to a less extent heart atrophy (Fig. 1c,e). Cd were found in 27.3 f 4.7% of deformed embryos. As previously mentioned, cardiac and yolk edemas often occurred simultaneously (85% of the embryos exhibiting an edema). The histological study revealed also edemas in the head, upper jaw, around the eyes, under the esophagus and between organs of these NHE (Fig. 2b,d,f). In addition, it corresponds to the presence of loose connective tissue (Fig. 2d) and empty spaces observable around the pectoral fins anlagen (Fig. 2f).

3.1.5. Eyes deformities

Eyes deformities were found in 8.7 f 2.9% of the deformed embryos and could be subdivided into two main types. First, a total or partial fusion of the optic cup (cyclops) and second a pigmentation-deficient eyes (Fig. 1d,e). Moreover, the histological analyses showed that Ed could be observed on transversal or sagittal sections of embryos that did not present any macroscopic eyes deformities (Fig. 3). Nevertheless, it may coincide with other categories of deformities as generalized edemas. In these conditions, slight or total disorganization of the cellular layers (Fig. 3b, c,d,f) could be seen compared to the normal eyes structure (Fig. 3a). Actually, bipolar, ganglion and amacrine cells blended in the same cellular layer instead of three distinct ones (Fig. 3b,c). Moreover, the choroidal melanocytes layer was damaged at various levels and detached from the other ones (Fig. 3c). In more severe cases, a total disorganization of all cell layers especially for the choroidal melanocytes was observed (Fig. 3d,f). In some cases, a fusion of the optics cups with the merge of several layers was observed (Fig. 3e), even in embryos without any macroscopically visible eyes impairments. Finally, the NHE presenting a partial fusion of the eyes macroscopically, showed a merged optic cups with a complete disorganization of cellular structure (Fig. 3f).

3.1.6. Digestive system deformities

Digestive system deformities were found in 3.5 f 1.3% of the deformed embryos. Various types of abnormalities have been observed among which the digestive tract hypertrophy (Fig. 4b,c,d,g), clearly visible in the intestine with different scales of severity (Fig. 4b,c,d) and could be observed in the rectum (Fig. 4c,d,g). Digestive hypertrophy was often associated with the presence of other edemas (Fig. 4b,c,g,h). The second kind of deformities corresponds to an absence of folded intestinal villi (Fig. 4e) potentially leading to a decrease in the absorption surface. In spite of this, the mucosal folding pattern was visible in the rectum.

3.1.7. Fins-related structure deformities

Fins-related structure deformities correspond to 2.0 f 0.7% of the deformed embryos. They were either associated to the anal orifice absence and then mainly correspond to a continuous veil in the ventral part of the NHE (Fig. 4b,c,f,g). Another type of fins deformities is a lack of development of the pectoral fins anlagen (data not shown).

3.1.8. Urinary system deformities

Urinary system deformities correspond to 1.6 f 0.9% of the deformed embryos. These abnormalities correspond mainly to the absence of the urinary bladder (Fig. 4b,c,d,f,g). Rarely, hypertrophies of the posterior part of pronephric ducts and urinary bladder were noticed (Fig. 4h). As for the digestive system, the hypertrophy was often associated with the presence of edemas (Fig. 4h). Finally, an absence of urinary or anal orifice was observed after the rectum and the urinary bladder (Fig. 4f).

3.1.9. Head deformities

This designation mainly groups abnormalities involving defects of central nervous system or the sensitive organs placed on the head. For example, a displacement of the olfactory organs was observed (data not shown). They correspond to 1.1 f 0.7% of the deformed embryos.

3.1.10. Multivariable deformities

Finally, this category involves NHE lacking a large part of the normal body. For example, when the anterior or the posterior parts of the embryos are lacking, it will lead to individuals without the head or the tail (data not shown). These abnormalities are rare with 1.1 f 0.6% of the deformed embryos.

3.2. Potential relationships between the categories of deformities

In every spawn, most of the NHE exhibit multiple deformities. The next question was thus to determine whether some of them always occur in the meantime, suggesting some common causes and/or mechanisms impaired. To do so, correlations were performed between the specific deformities rates of each category (Fig. 5 and Table 2). Firstly, three defined axes explained 61.9% of the variance (29.8, 16.5 and 15.6% for the axis 1, 2 and 3, respectively) (Fig. 5). The first axis was strongly associated to the Yd and Cd ($r = 0.91$ and $r = 0.87$, respectively). The second axis was mainly described by the Md ($r = 0.62$), whereas the third axis was correlated with the MVd and Ud ($r = 0.83$ and $r = 0.65$). As these data suggest that predominant relationships could exist between deformities categories linked to the same axis, Pearson's correlation coefficient values were generated, indicating that only the Yd and Cd were highly and significantly correlated ($r = 0.84$, Table 2).

3.3. Incidence of deformities categories according to the global effect of biotic and abiotic factors linked to the rearing system

This work shows that the Øand LPRF present various developmental impairments. Within a spawn, several categories of abnormalities were observed and numerous embryos presented several deformities. The total deformity rate (Dr) ranged from 0.8 to 20.8%, depending on the spawn. No significant difference was observed in the Dr according to the broodstock groups and mean values amounted to 6.2 f 0.99% and 5.8 f 1.66% for the RRF and LPRF, respectively. In addition, the deformity categories with an occurrence lower than 3.5% (Dd, Fd, Ud, Hd and MVd), varied between 0 and 30% according to the spawn. The deformities with occurrences higher than 8.5% (Ad, Cd, Yd, Md) varied between 0 and 100%. A detailed statistical analysis revealed that the Md and Ad correspond to the main deformities found in LPRF fish, while the RRF present a higher variety of organs deformed (Fig. 6a,b). Moreover, Ad, Cd and Yd were significantly higher in RRF than in LPRF ($P < 0.05$) (Fig. 6c,d,e). Interestingly, the Md was more often frequent in LPRF than RRF (Fig. 6f). No significant difference was found between the LPRF and RRF for other categories of deformities.

4. Discussion

Enhancing the knowledge of embryonic deformities helps to improve the rearing conditions. Current works mainly focused on the effect of some specific intrinsic/extrinsic factors on some specific deformities. These studies did not take into account the potential interactions between all factors that could lead to deformities occurrence. As a starting point, we thus compared two populations of fish adapted to extremely variable environments. We thus took into account all abiotic and biotic factors varying in the two conditions as a whole. As a consequence, several hypotheses could explain differences between the two populations as the feeding regime, the genetic, especially family structure or consanguinity levels (Afonso et al., 2000; Izquierdo, Fernandez-Palacios & Tacon, 2001; Boglione et al., 2013) as well as other factors. Our exhaustive approach allowed highlighting various developmental impairments depending on the rearing conditions.

So far, it was generally accepted that artificial rearing conditions may have a significant impact on the occurrence of developmental impairments in several species and that the deformities incidence appears to be significantly lower in wild populations (Koumoundouros, 2010; Boglione et al., 2013). Nevertheless, here, we did not find any significant differences in the Dr between the Øand LPRF even though a large number of parameters were different between these populations. This may stem from the fact that deformities can appear throughout the fish's life and the difference of deformity occurrence related to rearing conditions could emphasize later. Even if the mean Dr appear low, huge variations occur between the spawn even within a group of fish depending on the organs or systems involved. This extreme variation has huge consequences on the offspring survival or health.

Among the several types of deformity studied in the literature, those involving the skeleton (body axis and mouth), the yolk and the swim bladder are more often described and now well defined for several species (Boglione et al., 2013). Here, we show that in perch also, the main deformities categories observed correspond to the skeletal axis and the mouth deformities as well as the cardiac and yolk deformities. Nevertheless, these last categories were more significantly frequent in the RRF than in LPRF.

Concerning the yolk and cardiac anomalies, we found that they were usually associated together and, to a larger extent, to global edemas. It was already observed in the Atlantic halibut (Hippoglossus hippoglossus) larvae where it was probably related to unfavorable temperatures without any clear link to the broodstock origin (Ottesen & Bolla, 1998). Relationships between broodstock rearing conditions and yolk abnormalities was reported in Atlantic salmon (Salmo salar), because of an excessive stress during the pre-spawning period (Eriksen et al., 2006). It can thus be suggested that higher incidence of yolk deformities observed in this study in the case of RRF could be related to the stress level experienced by the breeders.

Conversely to the deformity categories previously mentioned (e.g. Ad, Cd, Yd), the incidence of Md was significantly higher in NHE originating from LPRF than from RRF. Little information is available about the potential determinants of this type of deformity, except the nutrition and the genetics (Boglione et al., 2013). Here, the significantly high mouth deformity frequency in LPRF compared to RRF NHE may be explained by pollutants potentially present in the pond system (Gaillard, 2014). Indeed, the accumulation of environmental contaminant in fish oocytes may affect the developmental success and provoke mouth deformities (Jezierska et al., 2009).

Ed were observed in some species but have poorly been studied. It has been shown that they can result from several factors (e.g. ultraviolet light exposure, post-ovulatory aging, nutrition and rearing conditions) and can impact fish health, behavior, survival and growth (Bonnet et al., 2007; McElwain et al., 2013). Our histological studies reveal various level of cellular layers disorganization. Interestingly, some macroscopically normal eyes exhibit abnormalities in the cellular structure. They may lead to individuals with huge visual impairments, potentially compromising their survival as the Eurasian perch is a visual predator (Vlavonou, 1996). In order to further investigate these deformities, it would be interesting to couple more detailed cellular structure of the eyes study with behavioral experimentations. It would allow understanding the consequences of these structural anomalies on the visual and biological responses of the larvae and potentially explain some lethalities observed in hatcheries by the time of first exogenous feeding.

Other categories of deformities (digestive, fins, urinary, head and multivariable deformities) have never or very poorly been described in the literature. It may be due to their little frequency and difficulties in observation compared to other anomalies. However, they may explain, at least partly, some survival troubles at some specific stages. These anomalies may alter some important biological functions such as the digestion, excretion and fish locomotion. The results observed in this study indicate that the fin-related-structure deformities, widely observed in the perch hatcheries, may stem from the impairments determined already at the embryonic stages. According to the normal perch developmental table (Alix et al., 2015), the anus closure or urinary bladder development always occur at the same time (7 dpf for the urinary bladder and 10 dpf for the anus opening). In the present study, embryos were collected at their hatching date (6–15 dpf) and compared to normal embryos of the same age. As a consequence, in our case, these variations rather reflect developmental defects than normal variations in the chronology as it could happen in other fish species. Further works are needed to better characterize all these developmental abnormalities.

As a conclusion, this study is the first one characterizing various deformities categories according to the rearing conditions of breeders. For the freshwater fishes, the pond and RAS correspond to the extreme opposite rearing conditions in term of human control of the fish environment. In addition, fish populations adapted to these conditions present phenotypic and genetic divergences that could also influence the deformities occurrence. In the literature, most of studies tented to understand the effect of individual factors on the deformities occurrence. However, the combination of several parameters may have additive or antagonist effects. Taking into account in the meantime all factors allows making a state of place of the differences occurring between the two rearing systems. Even if some of the abnormalities may be related to some specific factors, further studies are needed to improve our knowledge and the fish rearing conditions in fishfarms

Table 1. Characteristics of fish groups with the names, their rearing conditions and number of samples used in this study

up	Origin	Environmental control of the reproductive cycle	Fish farm	Period of spawning	Number of spawn studied	Total embryos	Deformed embryos
RRF	RAS fish farm	Artificial	Lucas Perches	April 2013	7	9133	382
				June 2015	23		
LPRF	Lorraine ponds	Natural	GAEC Piscicole du Saulnois	April 2013	1	3515	203
			GAEC Piscicole du Saulnois	March 2014	5		
			GFA du Kuhweg	March 2014	6		

LPRF, Lorraine pond reared fish; RRF, recirculating aquaculture system reared fish.

Table 2. Pearson's correlations table on the deformities rates in all newly hatched embryos

	Ad	Ed	Yd	Cd	Md	Ud	Dd	Fd	Hd	MVd
Ad		0.23	0.40	0.3	−0.58	−0.08	0.09	0.17	0.22	0.09
Ed			0.53	0.45	−0.25	−0.06	−0.05	−0.04	−0.03	−0.10
Yd				**0.84**	−0.17	0.13	0.34	0.37	0.30	−0.10
Cd					−0.03	0.25	0.36	0.43	0.26	−0.04
Md						0.07	0.04	−0.04	0.06	−0.07
Ud							0.04	0.14	−0.06	0.54
Dd								0.47	0.03	−0.06
Fd									0.26	−0.10
Hd										−0.07

Ad, axis deformities, Cd, cardiac deformities, Dd, digestive deformities, Ed, eyes deformities, Fd, fins deformities, Hd, head deformities, Md, mouth deformities, MVd, multivariable deformities, Ud, urinary deformities, Yd, yolk deformities. The bold number corresponds to the highly correlated variables ($r > 0.80$).

Figure 1. Description of the most frequent deformities in NHE.
(a) Normal NHE. (b) NHE with an axis deformity, kyphosis in the anterior part and lordosis in the posterior part of the body, (black arrowhead) and an edema in the cardiac cavity and around the posterior intestine (stars) leading to the deformation of the yolk (pear-shape yolk). (c) NHE with a mouth deformity with a shortening of the lower jaw (dotted arrow) and a yolk and cardiac edema (stars). (d) Detail of a NHE's head with eyes (large arrow) and jaw deformities (dotted arrow). (e) Anterior part of an abnormal NHE with eyes deformity (large arrow), gaping jaw deformity (dotted arrow, the anterior cartilage bent down and the mouth could not be closed) and multiple edemas (stars). Cc, cardiac cavity; E, eyes; En, encephalon; Ff, finfold; Int, intestine; J, jaw; Ld, lipid droplet; Myo, myotome; Nt, notochord; Y, yolk. Scale bars represent 1000 lm for a–c and 500 lm for d, e.

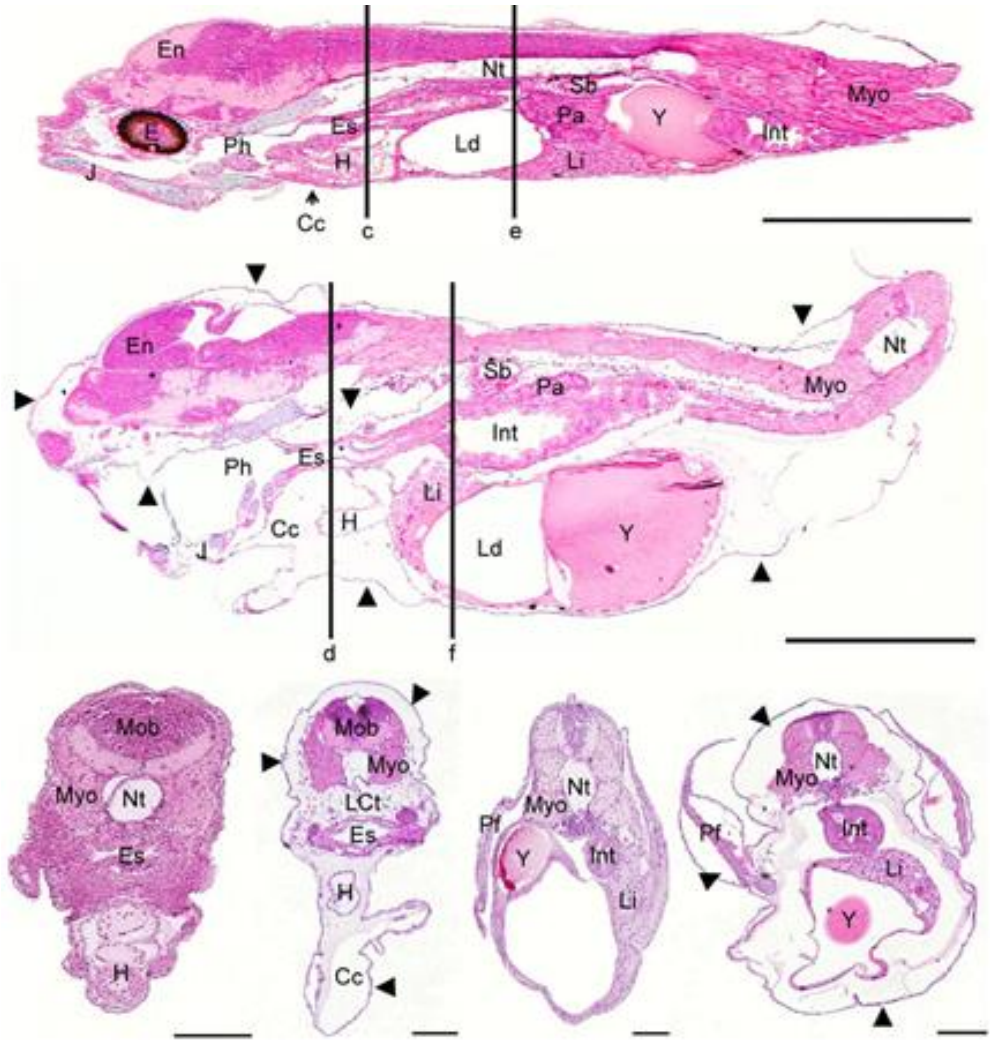

Figure 2. Histological description of NHE presenting cardiac and yolk edema.
(a) Sagittal section of the head and the trunk of a normal NHE. Vertical black lines represent the location of transverse sections of other embryos shown in c and e. (b) Sagittal section of the head and the trunk of a NHE presenting a generalized edema. The epithelium is detached from the connective tissue or the underlying myotome in the head and the upper jaw. This edema is also visible in the ventral part of the esophagus, in the cardiac cavity, around the yolk sac and in the ventral part of the tail (black arrowheads). Vertical black lines represent the location of transverse sections of similar other embryos shown in d and f. (c, d) Transverse section presenting the region of the cardiac cavity of (c) a normal NHE and (d) a NHE presenting a generalized edema (black arrowheads) with an epithelial detachment as well as loose connective tissues between the notochord, the esophagus and the mass of the myotomes. The cardiac cavity is elongated because of this edema. (e, f) Transverse section at the level of the trunk of (e) a normal NHE and (f) a NHE presenting a generalized edema (black arrowheads) with an epithelial detachment around all the tissues. Cc, cardiac cavity; E, eyes; En, encephalon; Es, esophagus; H, heart; Int, intestine; J, jaw; Lct, loose connective tissue; Ld, lipid droplet; Li, liver; Mob, medulla oblongata; Myo, myotome; Nt, notochord; Pa, pancreas; Pf, pectoral fin; Ph, pharynx; Sb, swim bladder; Y, yolk. Scale bars represent 500 lm for a-b and 100 lm for c–f.

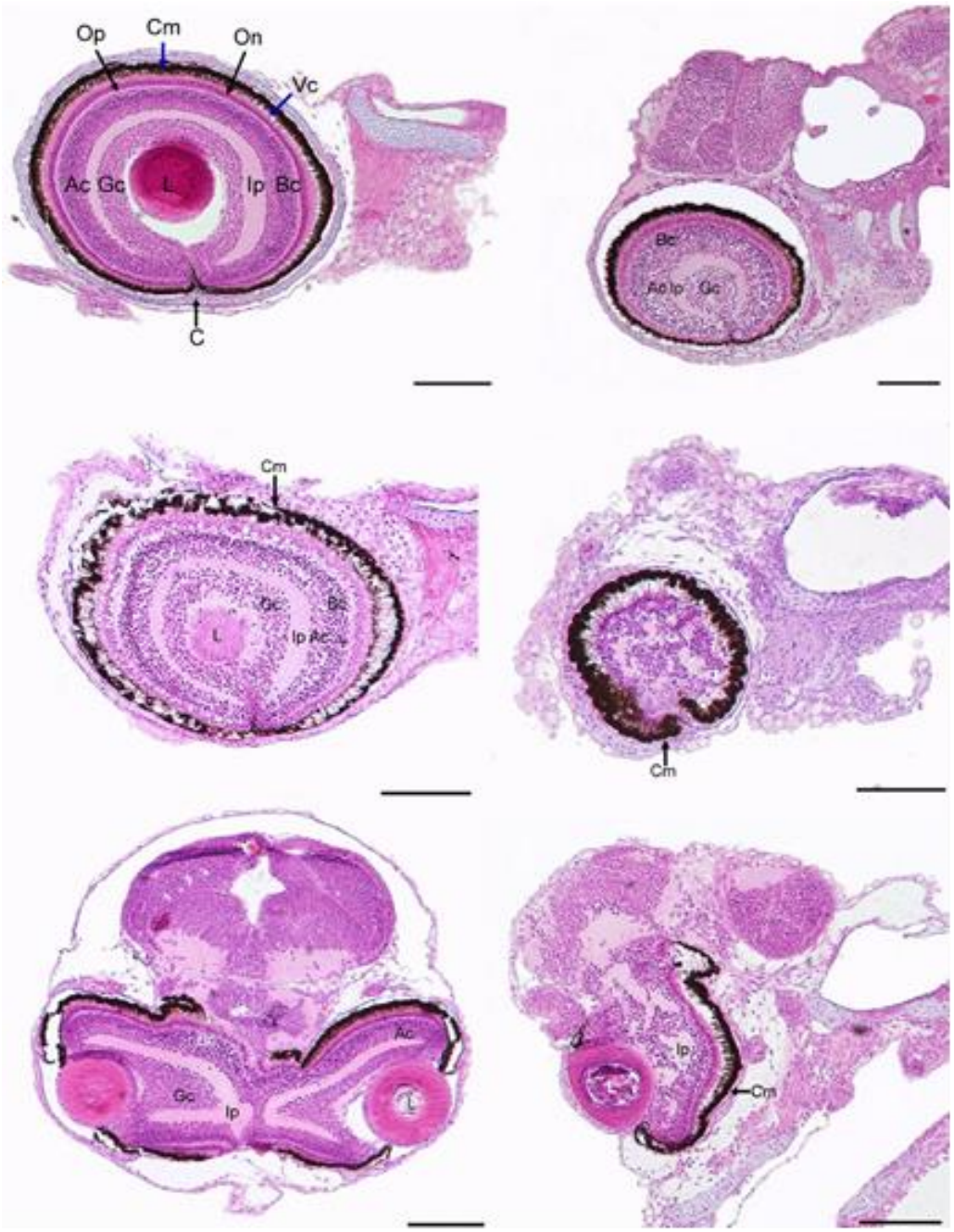

Figure 3. Histological description of several kinds of eyes deformities in NHE.
(a) Sagittal section of a normal eye in NHE. (b, c) Sagittal section of abnormal eyes with a blending between several cellular layers as interneuron bipolar (Bc), ganglion (Gc) and amacrine cells (Ac). (b) Slight disorganization with a blending between the amacrine, ganglion and bipolar cells layers. Bipolar cells are visible in the amacrine and ganglion cells layers. (c) Severe disorganization in the amacrine, ganglion, bipolar cells and choroidal melanocytes layers. Bipolar cells are visible in the amacrine and ganglion cells layers. Moreover, the choroidal melanocytes layer is detached from the other ones. (d) Sagittal section of an abnormal eye with a total disorganization of all cellular layers. (e) Transversal section of NHE's head with merged eyes combined with edema inducing epithelial detachment all around the head. (f) Sagittal section of merged eye with total disorganization of cellular layers except Op, On and Vc layers and potential encephalon deformity. Some cellular layers are not visible in this case. Ac, amacrine cells; Bc, bipolar cells; C, cartilage; Cm, choroidal melanocytes; Gc, ganglion cells; Ip, inner plexiform; L, lens; On, outer nuclear layer; Op, outer plexiform; Vc, visual cells. Scale bars represent 100 lm.

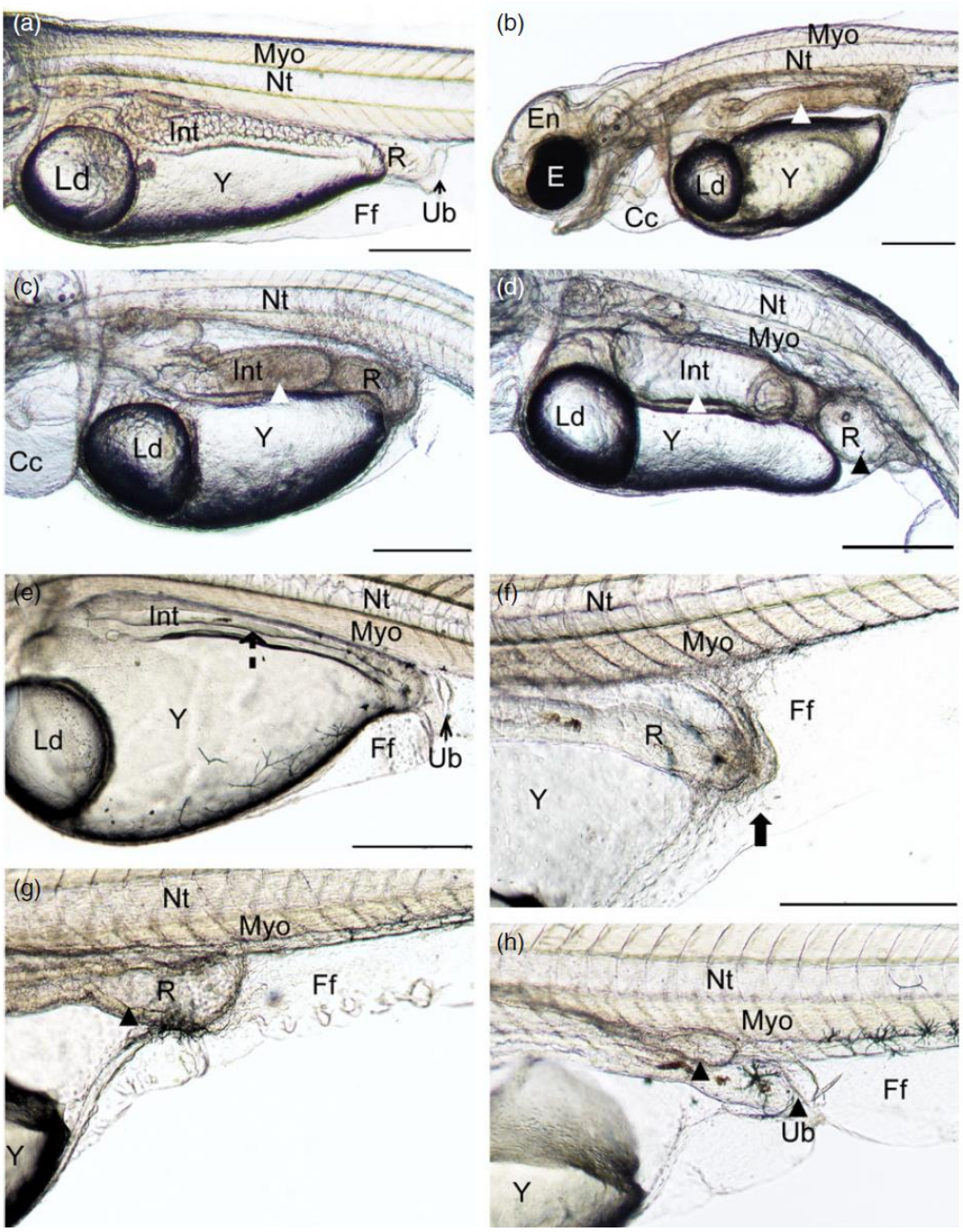

Figure 4. Description of the digestive, urinary and fins-related-structures deformities in NHE.
(a) Normal digestive and urinary system in a NHE. (b–d) Different levels of hypertrophy of the digestive system. (b) NHE with a slight hypertrophy of the intestine (white arrowhead) combined to cardiac edema. (c) Hypertrophy of the intestine (white arrowhead) and the rectum combined to cardiac edema. (d) Severe hypertrophy of the intestine (white arrowhead) and the rectum (black arrowhead) with an axis deformity. (e) Absence of primary folds in the intestinal mucosa (dotted arrow). (f) Absence of the urinary and anal orifice (large arrow). (g) Hypertrophy of the rectum (black arrowhead) and absence of a visible urinary bladder combined with a yolk edema. (h) Hypertrophy of urinary ducts and bladder (black arrowhead) combined with a yolk edema. Cc, cardiac cavity; E, eyes; En, encephalon; Ff, finfold; Int, intestine; Ld, lipid droplet; Myo, myotome; Nt, notochord; R, rectum; Ub, urinary bladder; Y, yolk. Scale bars represent 500 lm.

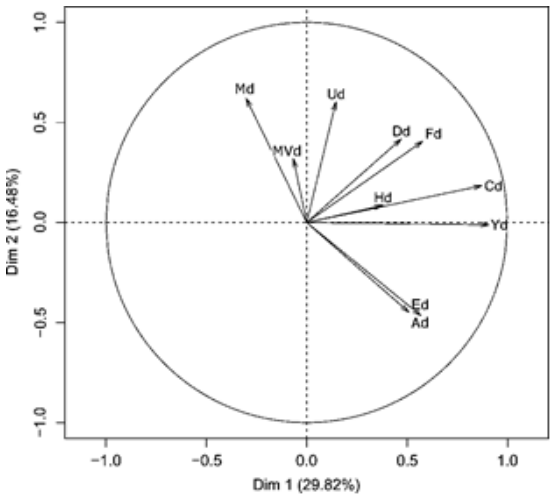

Figure 5. Study of the potential correlations between the categories of deformities. Correlation circle of the principal component analysis (PCA) presenting the potential relationships between the types of deformities observed in all NHE.

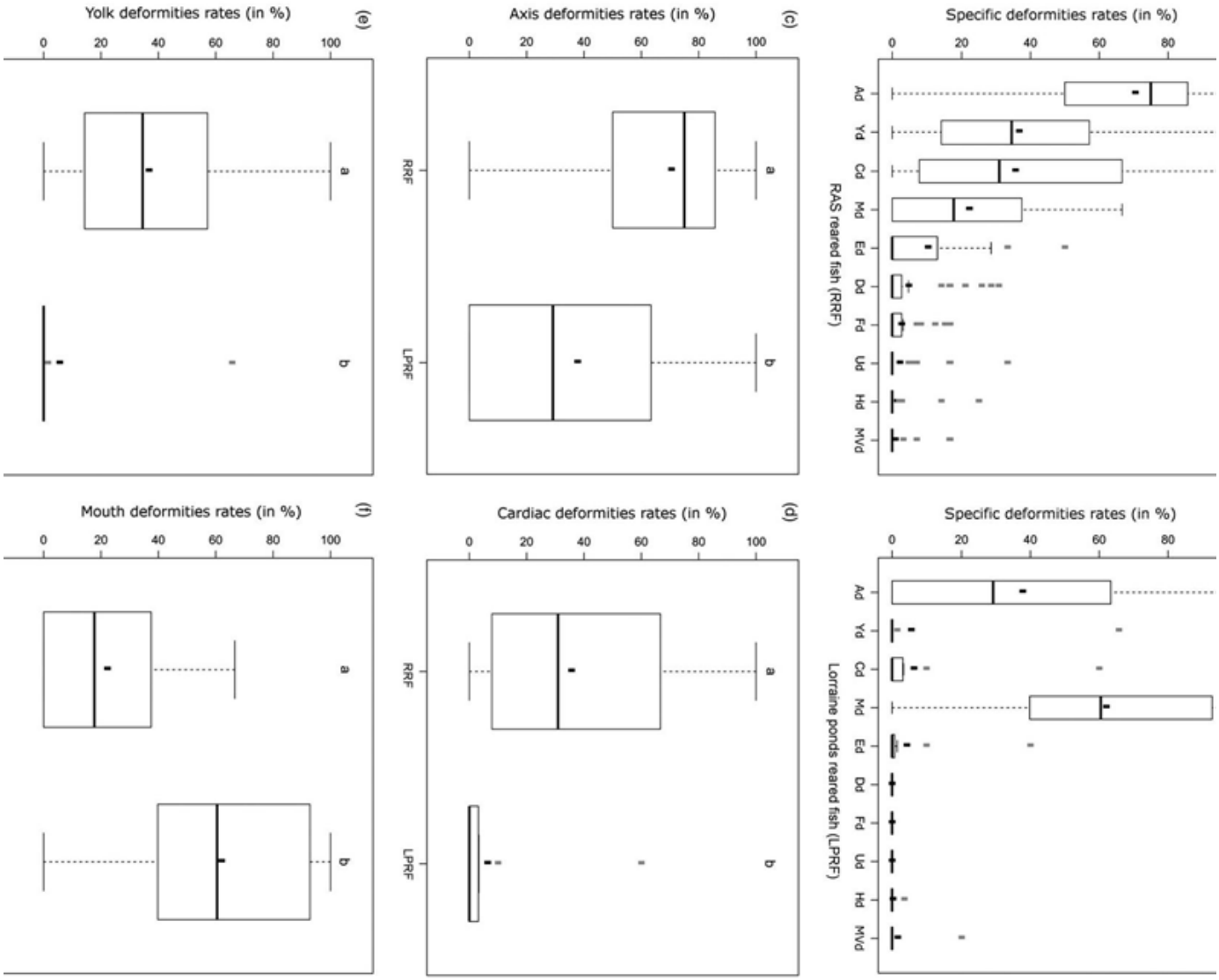

Figure 6. Incidence of the deformities according to the organs and the rearing conditions of breeders (RAS vs. pond).

(a, b) Box plots representing the percentage of deformities occurring in each organ in relation to the total number of deformed NHE from (a) RAS reared fish (n = 382) and (b) Lorraine pond reared fish (n = 203). (c–f) Box plots representing the percentage of four types of deformities according to the rearing conditions of the breeders. (c) Axis deformities rates (in %). (d) Yolk deformities rates (in %). (e) Cardiac deformities rates (in %). (f) Mouths deformities rates (in %). Some NHE present multiple deformities and are thus included in the counting for several organs. The bold black line represents the median deformities rates, the small black rectangles are the mean specific deformities rates and the small gray rectangles represent atypical spawn with specific deformities rates (outliers or observations considered as abnormal in the population). Different letters indicate significant differences between rearing conditions of reproductive cycle at $P < 0.05$ using anon-parametric Mann–Whitney test. LPRF, Lorraine pond reared fish; RAS, recirculating aquaculture system; RRF, RAS reared fish

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