Comparison of aspects of the physiology and morphology of diploid and triploid Atlantic salmon *Salmo salar*.

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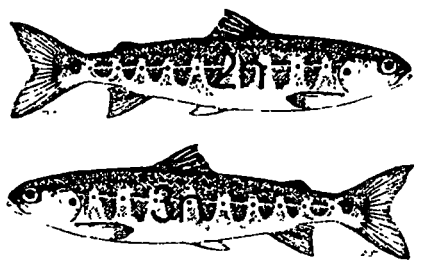

by

Joanne Sadler B.Sc. (Hons.)

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Doctor of Philosophy

School of Aquaculture
Tasmanian Fisheries and Aquaculture Institute
University of Tasmania at Launceston

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Chapter 6:

SKELETAL DEFORMITY IN DIPLOID AND TRIPLOID ATLANTIC SALMON

6. SKELETAL DEFORMITY IN DIPLOID AND TRIPLOID ATLANTIC SALMON

6.1 ABSTRACT

The skeletal morphology of commercially produced diploid and triploid Atlantic salmon was examined to elucidate the differential occurrence of skeletal deformities, including lower jaw deformity syndrome (LJD); short opercula and the absence of primary gill filaments (gill filament deformity syndrome (GFD)), in different genetic populations (mixed sex diploid, all-female diploid, all-female triploid and mixed sex triploid). Populations were produced and maintained under commercial conditions in freshwater until individuals had attained a wet weight of approximately 80g, at which time each population was divided and either retained in FW (FW smolt) or transferred to SW (SW smolt), where fish were held for a further 2 months. Whole fish were sampled throughout this period from hatching (470° days post-fertilisation) to assess the prevalence of deformity. Generally, the prevalence of skeletal deformities, was significantly higher in triploid populations. Jaw deformity, including lower jaw deformity syndrome (LJD), occurred in up to 2% of triploid fry, 7% of triploid FW smolt, 1% of diploid FW smolt and 14% of triploid SW smolt. Gross morphology of tissues affected by LJD is described. Short opercula were observed in up to 22% of triploids and 16.6% of diploids. Up to 60% of triploids and 4% of diploids suffered from GFD during FW development prior to SW transfer, then, up to 50% of triploid FW smolt and 60% of triploid SW smolt suffered from GFD. The severity of GFD varied between fish in terms of the number of branchial arches missing primary gill filaments and the number of gill filaments missing. An index of gill surface area (GSA) was significantly reduced in normal triploids and triploids afflicted with GFD, compared to diploid counterparts. It is likely that the reduction of GSA affects an individual's capacity for metabolic gas exchange under vigorous exercise or suboptimal environmental conditions.

6.2 INTRODUCTION

Skeletal deformities are common in cultured fish populations due to the absence of natural selective pressures that may result in the mortality of afflicted fish. The factors which contribute to the occurrence of skeletal deformity may be either genetic or environmental or both (reviewed by Hickey, 1972; McKay and Gjerde, 1986). Contributing factors within the culture environment may include the presence of pathogenic organisms, inappropriate physical parameters (light, temperature, salinity, dissolved oxygen, flow rate, pH) or the presence of heavy metals or other teratogenic substances (reviewed by Hickey, 1972; Chatain, 1994; Andrades *et al.*, 1996). It has been suggested that therapeutants such as malachite green and formaldehyde may have teratogenic effects (Alderman, 1991; Alderman and Clifton-Hadley, 1993; Edgell and Lawseth, 1993; McDonald, 1997). In addition, failure to achieve swimbladder inflation, often a result of suboptimal culture conditions, has been associated with an increased rate of deformity (Daoulas *et al.*, 1991; Chatain, 1994; Andrades *et al.*, 1996). Nutritional deficiencies, including phospholipids, vitamins A, B, C, or D and minerals such as manganese, phosphorous, magnesium and zinc (reviewed by McKay and Gjerde, 1986) may also contribute to abnormalities under culture conditions. An environmental disturbance at any stage of development, particularly during organogenesis, can cause the cessation or dissociation of growth processes of different tissues, which can result in abnormal development (Stockard, 1921).

The incidence of skeletal deformities in fish presents difficulty in the management of a reliable, high quality harvest and incurs certain financial loss to producers. Lower jaw deformity (LJD) (Bruno, 1990; Jungawalla, 1991; Hughes, 1992; Lee and King, 1994; King and Lee, 1993; Quigley, 1995; McGeachy *et al.*, 1996; Branson and Nieto, 1999; Goicoechea *et al.*, 1999) and other skeletal deformities, including short opercula (Sutterlin *et al.*, 1987; Baeverfjord *et al.*, 1997), scoliosis, lordosis (McKay and Gjerde, 1986) and gill deformities (Hughes, 1992; Baeverfjord *et al.*, 1997) have been previously recorded in cultured Atlantic salmon. In Tasmania, lower jaw

deformity occurs in up to 30% of commercially produced all-female triploid Atlantic salmon during the SW phase of the lifecycle, and subsequently represents considerable financial loss to sea farmers (Jungawalla, 1991; Hughes 1992).

Some studies suggest LJD is associated with the production of triploid Atlantic salmon (Jungawalla, 1991; Hughes, 1992; Lee and King, 1994; King and Lee, 1993, McGeachy *et al.*, 1996), whereas other studies do not specify the ploidy status of affected fish (Bruno, 1990; Quigley, 1995; Branson and Nieto, 1999; Goicoechea *et al.*, 1999), which may mean that diploid fish can also be affected by LJD. Therefore, it is unclear whether the incidence of LJD is associated directly with ploidy status and the potential differential physiology that may result from the differential cell morphology of triploid fish (reviewed by Benfey, 1999), or whether the incidence of LJD is associated with environmental factors. In addition, the majority of reports show that LJD occurs in all-female triploids (Hughes, 1993; King and Lee, 1993; Lee and King, 1994), and although one study showed LJD can occur in mixed sex triploid populations (McGeachy *et al.*, 1996), there was no indication as to whether the fish affected by LJD were male or female. Whether LJD is found only in female fish is unknown, but may indicate that the deformity is congenital and associated with the sex-determining locus. Furthermore, reports of the incidence of lower jaw deformity in Atlantic salmon stocks from Canada (McGeachy *et al.*, 1996), Scotland (Bruno, 1990), Ireland (Quigley, 1995), Norway (*pers comm.* Dr Tore Hastein, Central Veterinary Laboratory, Oslo, 1996) and Tasmania (Jungawalla, 1991; Hughes, 1993; King and Lee, 1993; Lee and King, 1994), indicate that the deformity is present following SW transfer, although the time of onset is unknown. More recently, scientists working in Chile have reported that LJD can occur in Atlantic salmon smolt just prior to SW transfer (Branson and Nieto, 1999; Goicoechea *et al.*, 1999). It remains unclear whether LJD is associated with culture conditions or a specific stage of development of the fish.

The aim of the current study was to describe the morphology, prevalence and temporal onset of various skeletal deformities, including LJD, in four different population types of Tasmanian Atlantic salmon (all-female diploid, all-female

triploid, mixed sex diploid and mixed sex triploid), which were maintained under standardised culture conditions, to determine whether the prevalence of LJD or other skeletal deformities were associated with either ploidy status or sex ratio. In addition, we examined whether the prevalence of the above deformities varied between smolt either held in FW or transfered to SW. Examining the incidence of deformity throughout ontogeny of the different population types is fundamental to determining the possible mechanisms by which these deformities occur, and thereby facilitates the improvement of management procedures and harvest quality. This study records for the first time a gill filament deformity (GFD) and compares the relative total gill surface area of fish afflicted with GFD to that of fish with normal gills.

6.3 METHODS AND MATERIALS

6.3.1 Fish production and husbandry

All-female diploid, all-female triploid, mixed sex diploid and mixed sex triploid populations were produced using commercial techniques and maintained in separate tanks under standardised husbandry conditions, during both the freshwater (FW) and seawater (SW) phases, as previously described in Chapter 2. Population sex ratios were determined to be 100% female for the all-female and 1 male : 1 female for the mixed sex populations (see results Chapter 2). Population ploidy status was found to be 96% and 100% triploid in all-female and mixed sex triploid populations, respectively (see results Chapter 2).

6.3.2 Prevalence of deformity during development

Fish were randomly sampled from each population at various stages throughout development (Table 1). Fish were killed by terminal anaesthesia in 25 ppm Benzocaine and total wet weight (TWWt.) and fork length (FL) were recorded. A number of different types of gross skeletal deformity were observed in the freshly

euthanased fish via examination of external morphology. The number of fish afflicted with each deformity type was recorded for each population, at each stage of development, and expressed as a percentage of the total number of fish sampled at each stage. Total percentage prevalence of deformity was defined as the total number of fish affected by one, or a number of different deformity types expressed as a percentage of the total number of fish observed within each population, at each stage of development. Fish were then fixed in 10% neutral buffered formalin, and the morphology of various deformities in fixed specimens was recorded either photographically, or diagrammatically using a camera lucida.

Table 1. Developmental stages and total number of fish sampled (n) for determination of deformity prevalence throughout the development of four Atlantic salmon populations; all-female triploids (FT), mixed sex triploids (MT), mixed sex diploids (MD) and all-female diploids (FD). Accumulated temperature units (ATU) in degree days represents development post-fertilisation.

Date	Development Stage	Weeks (post-fertilisation)	ATU (° days)	FT (n)	MT (n)	MD (n)	FD (n)
May 1996	Fertilisation		0				
July 1996	Hatching						
	ALEVINS	8	470	26	55	82	24
		9	528	123	29	58	86
		10	583	94	95	115	94
August 1996		11	642	125	15	99	113
		12	706	254	108	127	104
	Swim up & First Feeding						
	FRY	13	772	209	34	32	200
		14	843	20	30	110	20
September 1996		15	913	200	200	200	200
October 1996		19	1510	200	200	200	200
January 1997	PARR Populations Thinned	31	2350	200	200	200	200
April 1997		45	3118	200	200	200	200
June 1997	Populations Thinned						
September 1997			4026	30	30	30	30
October 1997	SMOLT		4202	100	100	100	100
	Sea transfer						
November 1997	SW SMOLT		4810	97	85	47	56
	FW SMOLT		4928	100	100	100	100

6.3.3 Staining and clearing for bone and cartilage morphology

Two normal female triploids and two female triploids afflicted with lower jaw deformity syndrome (LJD) from the SW smolt stage (Nov. 1997, 4810° days) were stained and cleared (Taylor and Van Dyke, 1985, Appendix D) to ascertain cartilage and bone morphology. The gross morphology of bones dissected from the lower jaw of both normal and deformed fish was recorded *in situ*, using a camera lucida.

6.3.4 Gill Histology

One normal branchial arch with a full complement of primary gill filaments was excised from each of 10 diploid and 10 triploid SW smolt (sampled Nov. 1997, 4810° days) which had been previously fixed in 10% neutral buffered formalin and stored in 70% ethanol. In addition, one branchial arch with missing primary gill filaments was excised from each of 10 triploid SW smolt (4810° days) afflicted with gill filament deformity syndrome (GFD). Branchial arches were dehydrated in an ascending ethanol series prior to embedding in paraffin wax and serial sectioning at 5μm in the longitudinal plane with respect to the ceratobranchial bone, upper branchial bone and the primary gill filaments. Sections were stained with Haematoxylin and Eosin prior to examination using a light microscope.

6.3.5 Gill Surface Area

A subsample of fixed diploid and fixed triploid SW smolt, selected at the same stage of development (Nov. 1997, 4810° days), was examined to determine the relative gill surface area of normal diploid (n = 26) and normal triploid (n = 18) fish and that of fish afflicted with gill filament deformity syndrome (GFD) (n = 48). The whole branchial apparatus (8 branchial arches) was carefully dissected from each fish. Individual branchial arches from each fish were then separated and laid flat under a glass plate, to expose the maximum 2-dimensional area represented by each branchial arch. Photographic contact prints were then made (see Appendix E) and an image analysis program (Analytical Imaging station 3) was used to determine the

total 2-dimensional gill surface area (GSA = mm^2) for each fish. The total wet weight (TWWt.) of each fish was recorded and was taken into consideration (used as a co-variate) during statistical analysis.

6.3.6 Sex and ploidy status of deformed fish

At the late FW parr (4202° days) and SW smolt (4810° days) stages, a section of gonad tissue was dissected from each of the fresh fish sampled (Oct. - Nov. 1997), and was fixed in Bouin's fixative for 24 hours, then stored in 70% ethanol. The sex of fish was determined histologically as described in Chapter 2, and the sex ratio of fish afflicted with each deformity type was recorded. Ploidy status of the deformed fish was determined by measurement of mean erythrocyte nucleus length (ENL) for each fish, as described in Chapter 2.

6.3.7 Statistical Analysis

A paired t-test (Microsoft Excel version 5 software) was used to compare the prevalence of deformity at each stage of development between; i) all-female diploids and all-female triploids, ii) mixed sex diploids and mixed sex triploids and iii) diploids and triploids (mixed sex and all-female populations pooled). In addition, for each population, comparisons of the incidence of deformity were made between FW smolt (4928° days) and SW smolt (4810° days).

Analysis of covariance, ANCOVA, was used to compare GSA between; i) normal triploid fish and triploid fish afflicted with GFD and ii) normal diploid and normal triploid fish, using total wet weight as a covariable in each case.

6.4 RESULTS

6.4.1 Lower Jaw Deformities

Lower jaw deformities detected at the gross level in all populations and throughout development, included laterally curved lower jaws, short lower jaws, long lower jaws and lower jaw deformity syndrome (LJD). Generic examples and the prevalence of each in the different ploidy and gender populations are provided below.

A laterally curved lower jaw was defined as a jaw in which the symphyseal joint was not aligned with the median longitudinal plane of bilateral symmetry (Fig. 1). In fish with short lower jaws, the relative length of the lower jaw was approximately 25 - 60% shorter than that of the upper jaw (Fig. 2). In fish with a long lower jaw, the lower jaw extended past the premaxilla of the upper jaw, giving the appearance of an 'overbite'.

In contrast to the previous jaw deformities, lower jaw deformity syndrome (LJD) was diagnosed by the downward curvature of the lower jaw (Fig. 3) (Hughes, 1991). In such cases, the anterior tip of the lower jaw did not meet with that of the upper jaw when the mouth was 'closed'. Less severe cases of LJD were referred to as mild lower jaw deformity (MLJD), and were characterised by downward curvature of the anterior end of the lower jaw (Fig. 4). Staining and subsequent dissection of individual bone and cartilage elements of fish affected by LJD revealed the shape of the lower jaw was defined by the shape of the dentary and angular bone in which mid sections were increased in dorso-ventral depth and were curved, resulting in the ventral position of the symphyseal joint relative to the articulation joint (Fig. 5). The gross morphology of the Meckel's cartilage was similar to that of normal individuals (Fig. 5). The position of the Meckel's cartilage, relative to the dentary and angular bones, in individuals with LJD was similar to that of normal individuals (Fig. 6).

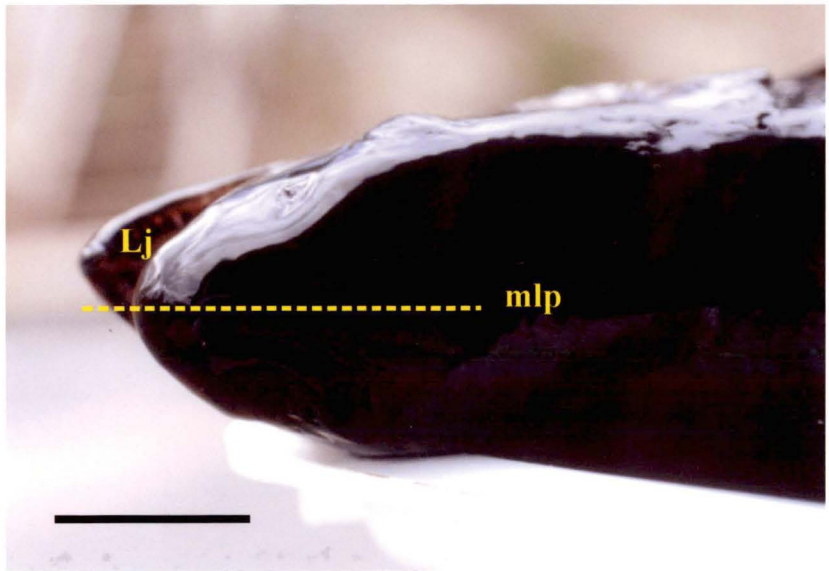


Figure 1. Dorsal view of a mature Atlantic salmon (TWWt. = 3.0 kg) with a laterally curved lower jaw (Lj) relative to the median longitudinal plane (mlp) of bilateral symmetry. Scale bar = 5.0 cm.

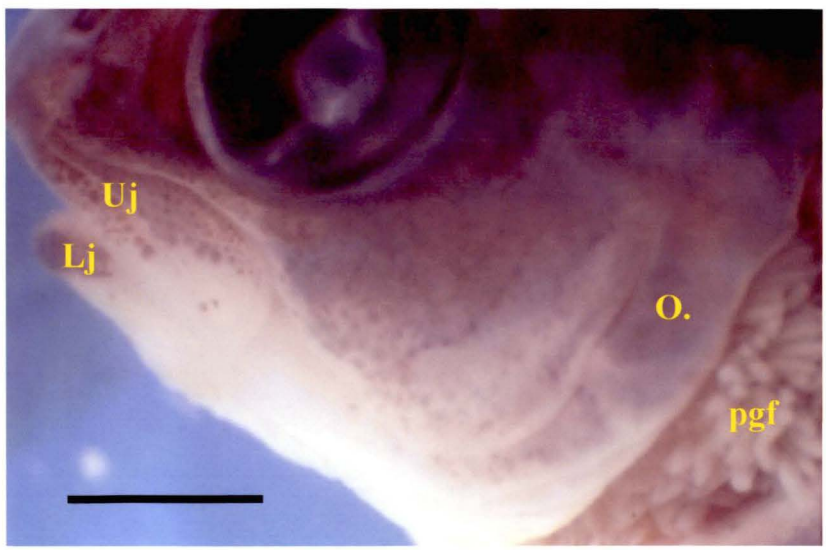


Figure 2. Lateral view of a Atlantic salmon parr (TWWt. = 30.0 g) with a short operculum (SO) and a short lower jaw relative to the upper jaw. Abbreviations: Uj = upper jaw, Lj = lower jaw, O. = operculum, pgf = primary gill filaments. Scale bar = 5.0 mm.

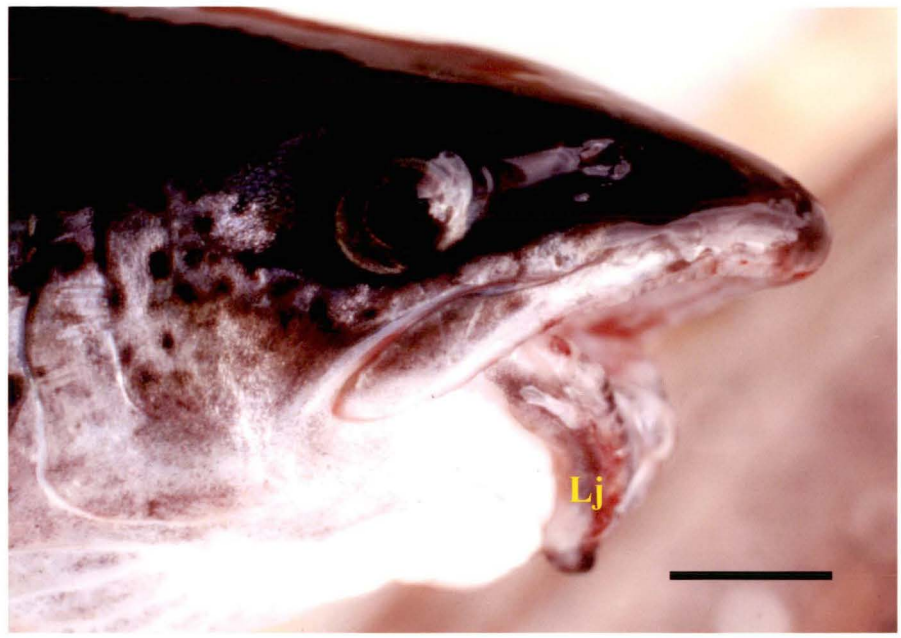


Figure 3. Lateral view of a mature Atlantic salmon (TWWt. ≈ 3.0 kg) with lower jaw deformity syndrome (LJD). Note downward curvature of anterior end of lower jaw. Lj = lower jaw. Scale bar = 5.0 cm

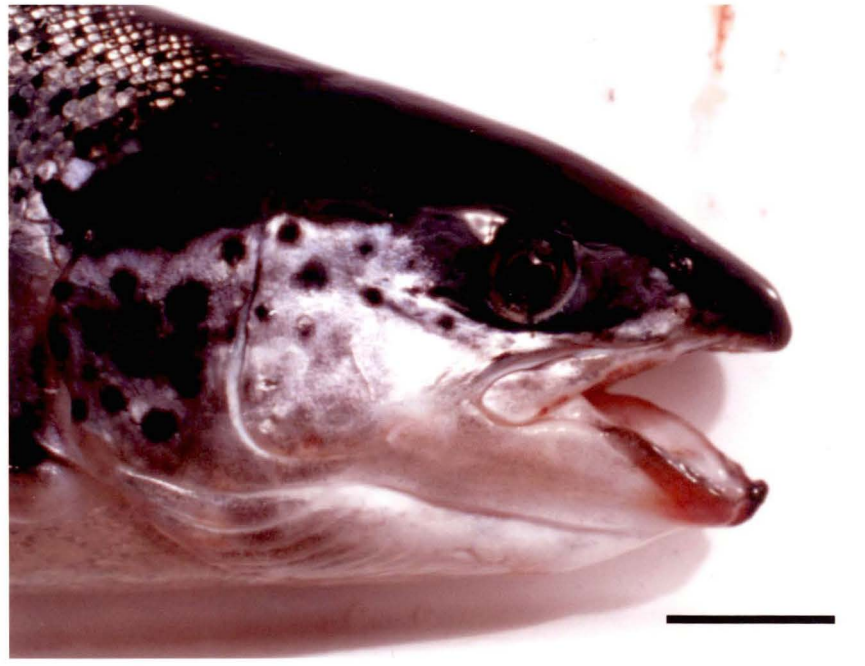
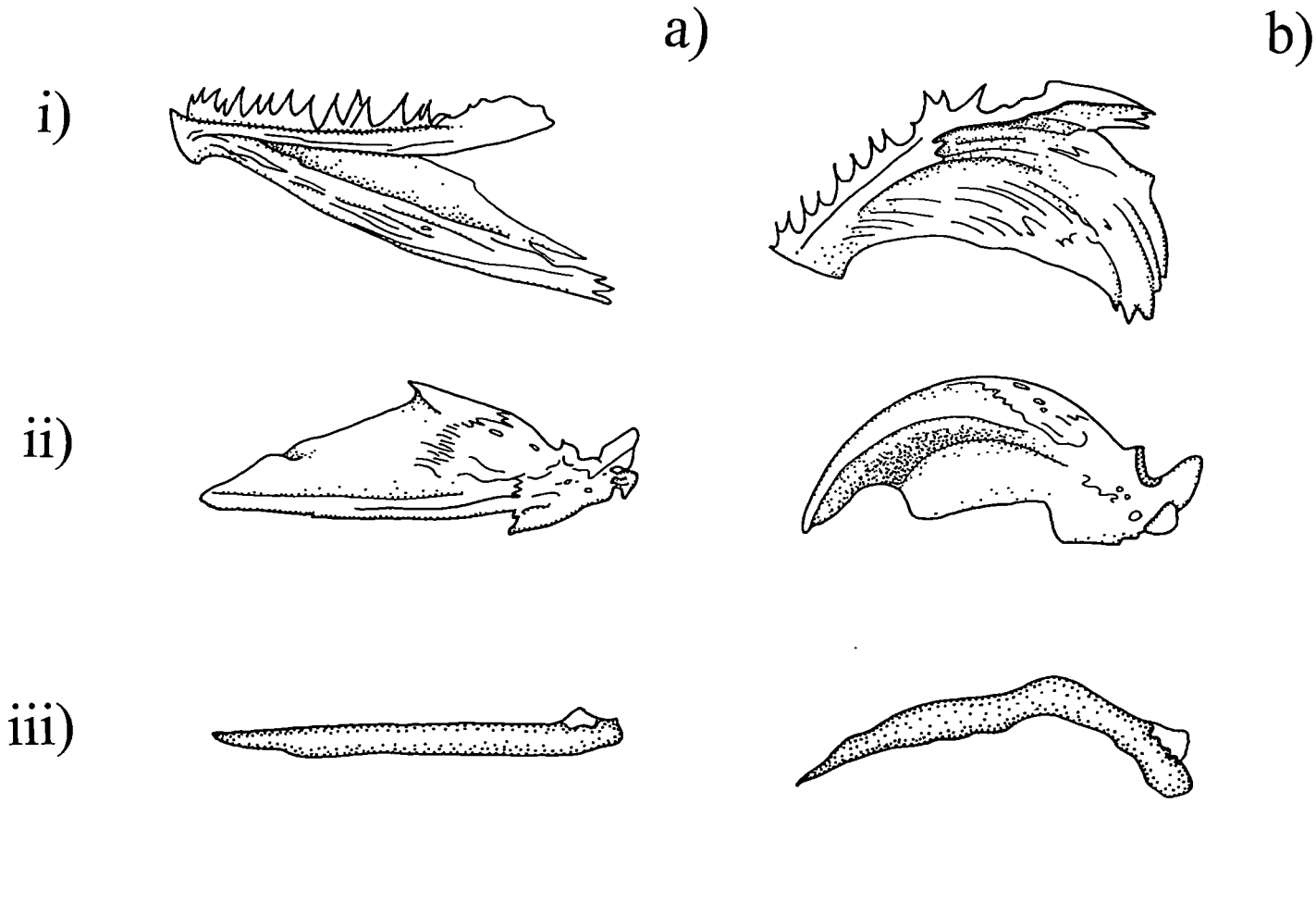


Figure 4. Lateral view of a mature Atlantic salmon (TWWt. ≈ 3.0 kg) with mild lower jaw deformity (MLJD). Scale bar = 5.0 cm.

Figure 5. Camera lucida diagrams of (i) the dentary bone, (ii) the angular bone and (iii) Meckel's cartilage, dissected from the lower jaw of a stained and cleared (Taylor and Van Dyke, 1985) normal Atlantic salmon SW smolt (a) and (b) an Altantic salmon SW smolt affected by lower jaw deformity syndrome (LJD). Scale bar = 10 mm.



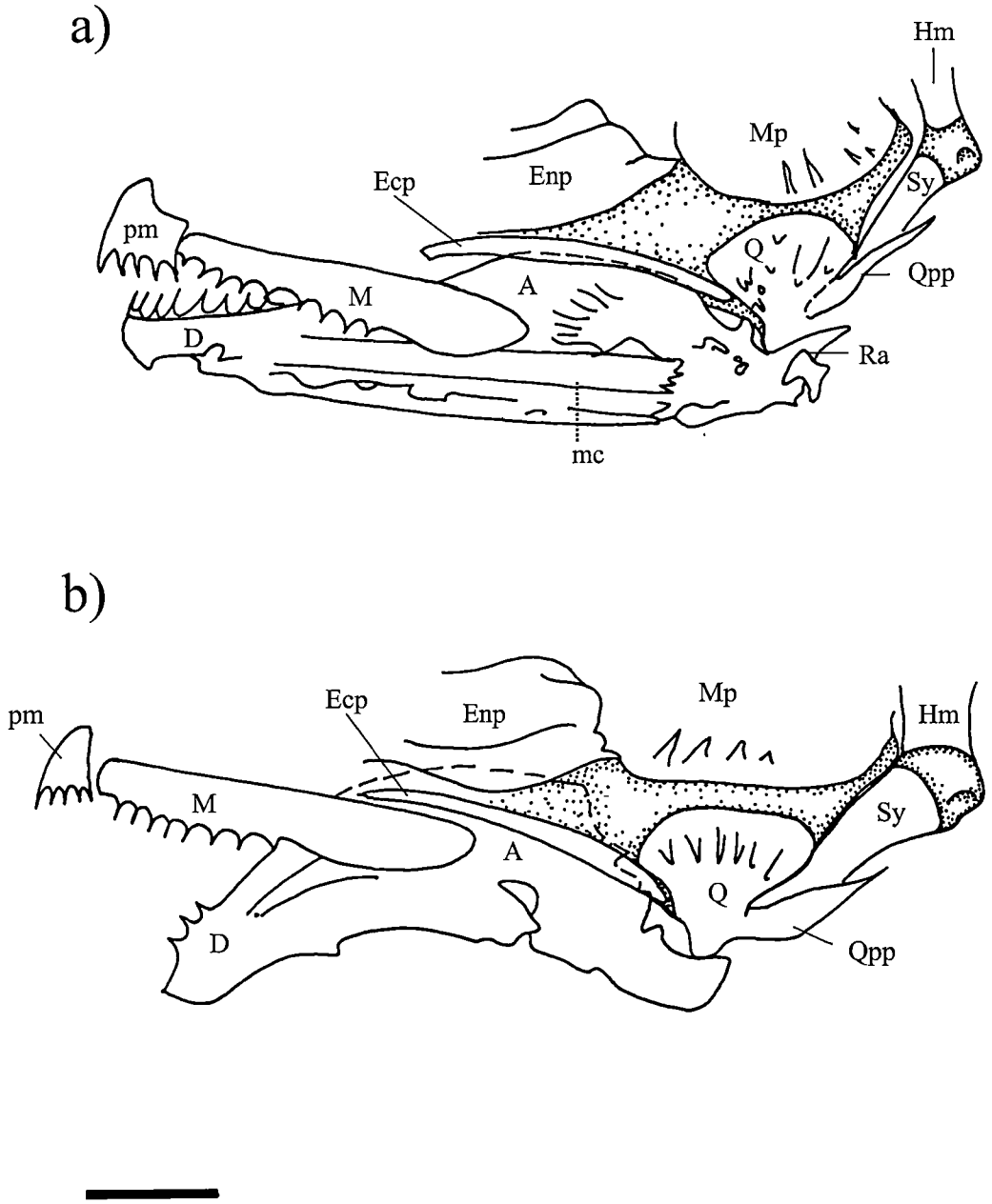


Figure 6. Camera lucida diagram of the gross morphology of bones and cartilages *in situ* in the lower jaw of stained and cleared (Taylor and Van Dyke, 1985) normal Atlantic salmon SW smolt (a) and (b) an Atlantic salmon SW smolt affected by lower jaw deformity syndrome (LJD). Abbreviations: A = angular bone, D = dentary, Ecp = ectopterygoid, Enp = endopterygoid, Hm = hyomandibular, M = maxilla, Mp = metapterygoid, pm = premaxilla, Q = quadrate, Qpp = posterior process of the quadrate bone, Ra = retroarticular bone, Sy = symplectic. Scale bar = 10.0 mm

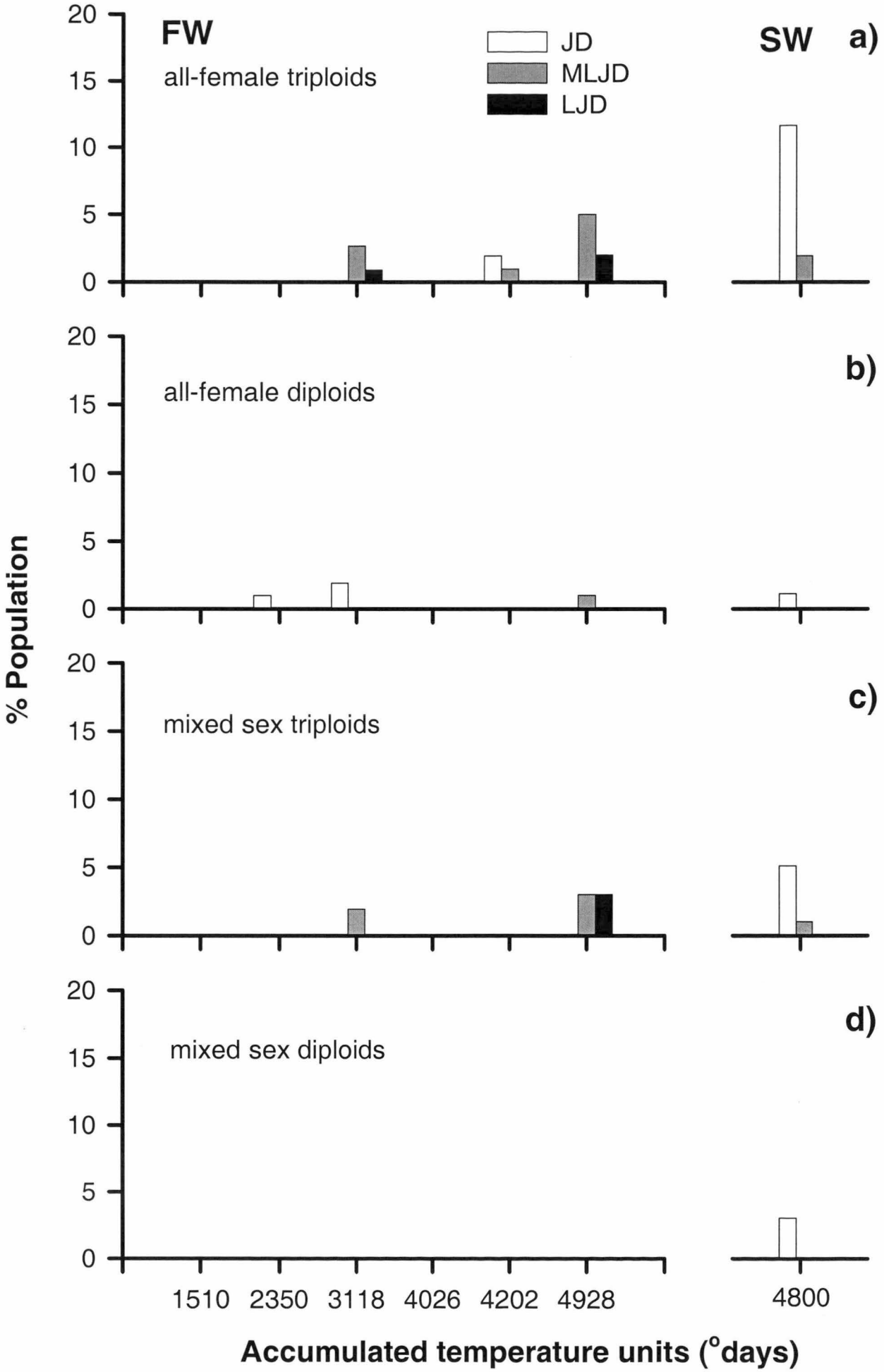
Prevalence of jaw deformities during development

Jaw deformities and deformities of the jaw suspensorium were detected as early as the embryonic stages (described in Chapter 5). These deformities included irregular development of the Meckel's cartilage, fusion of the quadrate and symplectic cartilages, dis-association of the symplectic from the hyomandibular, or a short symplectic cartilage (see Chapter 5). Such deformities were not detected at the gross level, but were detected using Taylor's staining and clearing technique (Taylor and Van Dyke, 1985, Appendix D).

Lower jaw deformities, including laterally curved lower jaws (JD), severe lower jaw deformity syndrome (LJD) and mild lower jaw deformity syndrome (MLJD, were observed in fish at relatively low levels in all age classes examined post-first feeding (Fig. 7). The prevalence of laterally curved lower jaw (JD), was up to 2% of all-female diploids at 2350° days and 3118° days, and up to 2% of all-female triploids detected at 4202° days (Fig. 7). In smolt reared under SW conditions (4810° days), JD was observed in 12% of all-female triploids, 1% of all-female diploids, 5% of mixed sex triploids and 3% of mixed sex diploids (Fig. 7), whereas, JD was not detected in smolt reared in FW, in any population.

The prevalence of mild lower jaw deformity syndrome (MLJD), was significantly higher in triploid populations compared to diploid populations ($P < 0.05$). MLJD was initially detected at 3118° days in 3% of all-female triploids, 2% of mixed sex triploids and 1% of all-female diploids (Fig. 7). This deformity was subsequently detected at 4202° days in 1% of all female triploids and at 4928° days in 5% of all-female triploids and 3% of mixed sex triploids. MLJD was not detected in mixed sex diploids at any stage during the FW phase. In smolt cultured under SW conditions (4810° days), 2% of all-female triploids and 1% of mixed sex triploids were afflicted with MLJD, but the deformity was not detected in diploid smolt (Fig. 7). The severe form of lower jaw deformity syndrome (LJD), was detected in triploid fish only. LJD was initially detected in 1% of all-female triploid fish at 3118° days and was subsequently observed in 2% of all-female triploids and 3% of mixed sex triploids at the FW smolt stage (4928° days). The severe form of LJD was not detected in SW smolt at 4810° days. The prevalence of deformities of the jaw in general (including JD, MLJD, LJD) in Atlantic salmon from hatching to 4800° days, increased during ontogeny.

Figure 7. Prevalence (%) of lower jaw deformities in a) all-female triploid b) all-female diploid c) mixed sex triploid and d) mixed sex diploid populations throughout development. Accumulated temperature units (ATU = ° days) represent development post-fertilisation, FW = freshwater phase, SW = sea water phase, LJD = lower jaw deformity syndrome, MLJD = mild lower jaw deformity syndrome, JD = jaw deformity other than LJD. Sample sizes as per Table 1.


FW
SW
a)
b)
c)
d)
JD
MLJD
LJD
all-female triploids
all-female diploids
mixed sex triploids
mixed sex diploids
% Population
20
15
10
5
0
1510
2350
3118
4026
4202
4928
4800
Accumulated temperature units (°days)

6.3.2 Short Opercula

Short opercula deformity varied in morphology between individuals, but was generally characterised by the exposure of the distal tips of the gill filaments within the branchial chamber (Fig. 8). One or both opercula were affected in afflicted fish. In some fish, the opercula bone was reduced in anterio-posterior length and the dermal tissue surrounding the opercula bone extended posteriorly to the edge of the opercula bone (Fig. 9). In other cases, there was an inward folding of the posterior edge of the operculum so that the folded tissues projected into the branchial chamber (Fig. 9). The folded tissues included dermis and bone, with no indication of a fracture in the opercula bone along the fold line, but rather hypertrophy of the opercula at the inflexion point.

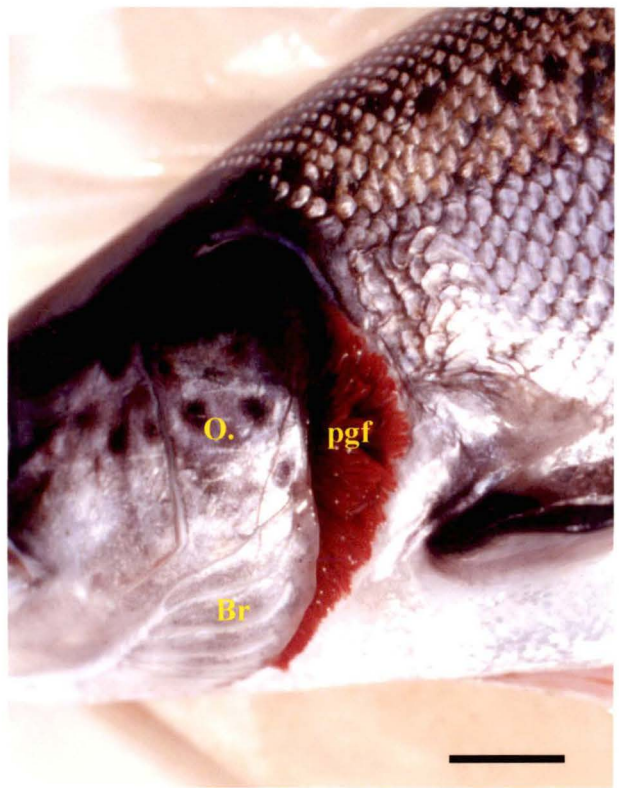


Figure 8. Lateral view of a mature Atlantic salmon (TWWt. ≈ 3.0 kg) with a short opercula deformity (SO). Abbreviations: O. = operculum, Br = branchiostegal rays, pgf = primary gill filaments. Scale bar = 3.0 cm.

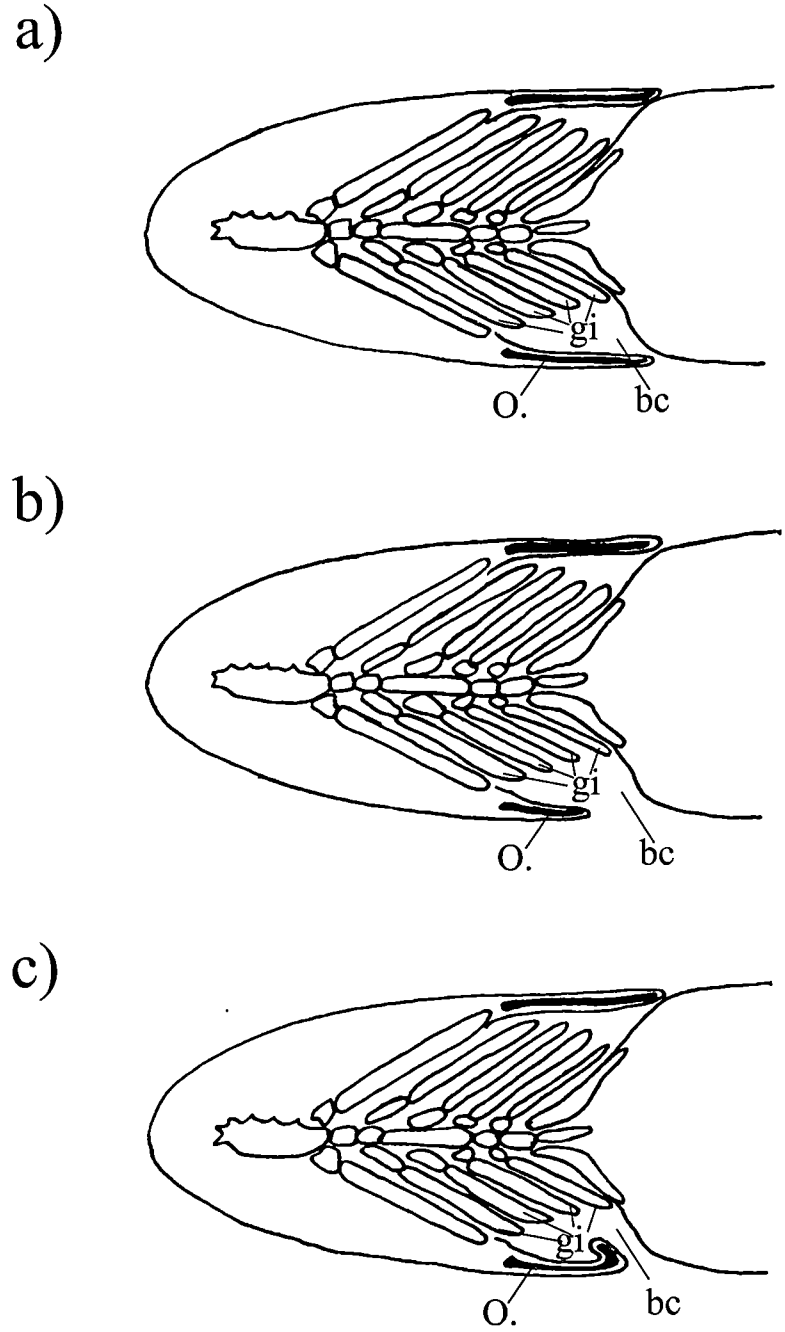
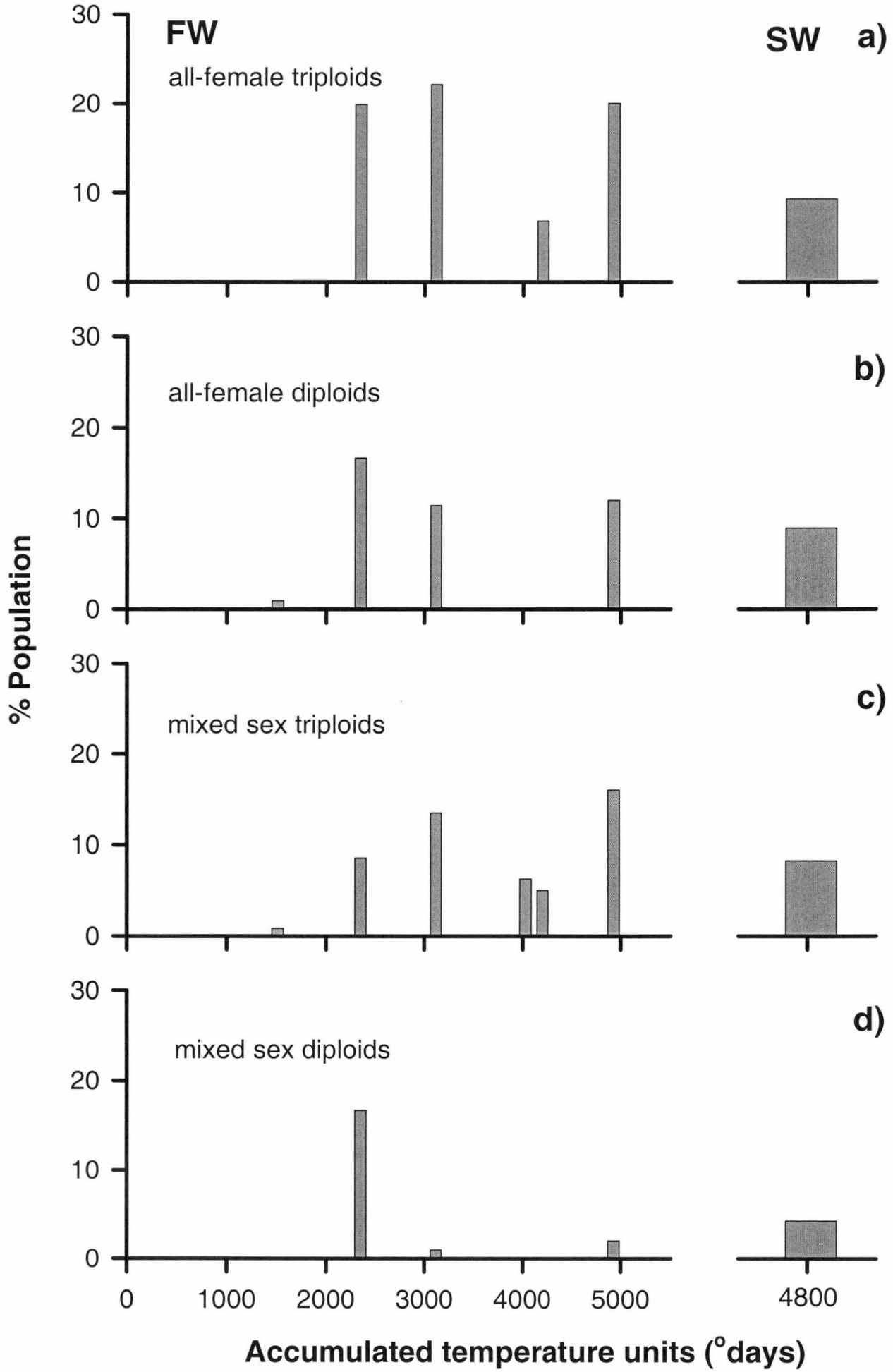


Figure 9. Diagrammatic representation of the dorsal view of an Atlantic salmon cranium with tissue pared away to expose the branchial chamber to show a) a normal opercula bone, b) a short opercula bone and c) an opercula bone with the posterior edge folded, projecting into the branchial chamber. Abbreviations: O. = opercula bone, bc = branchial chamber, gi = gills.

Prevalence of short opercula during development

Short opercula were initially observed after first feeding (≥ 1510° days), in both diploid and triploid fish; the prevalence of which fluctuated throughout development (Fig. 10). Short opercula were observed in 1 % of fish from the all-female diploid and the mixed sex triploid populations at 1510° days (Fig. 10). At 2350° days, up to 18% of both the all-female diploids and mixed sex diploids, 20% of the all-female triploids and 9% of the mixed sex triploids suffered from short opercula (Fig. 10). At 3118° days, short opercula were observed in 12% of the all-female diploids, 23% of the all-female triploids, 1% of the mixed sex diploids and 14% of the mixed sex triploids (Fig. 10). Short opercula were not detected in fish from either diploid population, nor in the all-female triploid population at 4026° days, but were present in up to 6.25% of mixed sex triploid fish at this stage. Similarly, at 4202° days, short opercula were observed in 6% of all-female triploids and 5% of mixed sex triploids only. At the smolt stage (4928° days), under FW conditions, 12% of all-female diploids, 20% of all-female triploids, 2% of mixed sex diploids and 16% of mixed sex triploids had short opercula (Fig. 10). In smolt cultured under SW conditions (4810° days), 9% of all-female diploids and all-female triploids, 4% of mixed sex diploids and 8% of mixed sex triploids had short opercula (Fig. 10). The prevalence of short opercula was significantly higher in triploid populations compared to diploid populations ($P < 0.05$), but did not differ with gender ($P > 0.05$).

Figure 10. Prevalence (%) of short opercula in a) all-female triploid b) all-female diploid c) mixed sex triploid and d) mixed sex diploid populations throughout development Accumulated temperature units (ATU = ° days) represent development post-fertilisation, FW = freshwater phase, SW = sea water phase. Sample sizes as per Table 1.


FW
SW
a)
all-female triploids
b)
all-female diploids
c)
mixed sex triploids
d)
mixed sex diploids
% Population
30
20
10
0
0
1000
2000
3000
4000
5000
4800
Accumulated temperature units (°days)

6.3.3 Gill Filament Deformity Syndrome (GFD)

Gill filament deformity syndrome (GFD) was diagnosed by the complete absence of a number of primary gill filaments on branchial arches (Fig. 11). Between one and five branchial arches from either side of the body were missing primary gill filaments in fish with GFD. The proportion of primary gill filaments missing from any one branchial arch varied between arches and between fish. Gross morphology indicated there were no residual proximal stumps of pre-existing filaments within the zone of missing gill filaments (Fig. 11b), which may otherwise indicate previous parasitic damage, including isopod damage (*pers comm*; Barry Munday, Reader, University of Tasmania, 1999). However, histological examination of gills affected by GFD revealed symptoms similar to those that occur in conjunction with a parasite load in 70% of the cases observed. These included the abnormal shape of primary gill filaments adjacent to the zone of missing filaments and hyperplasia of the secondary cartilage, the basal epithelium and the epithelium of the secondary lamellae (Fig. 12b).

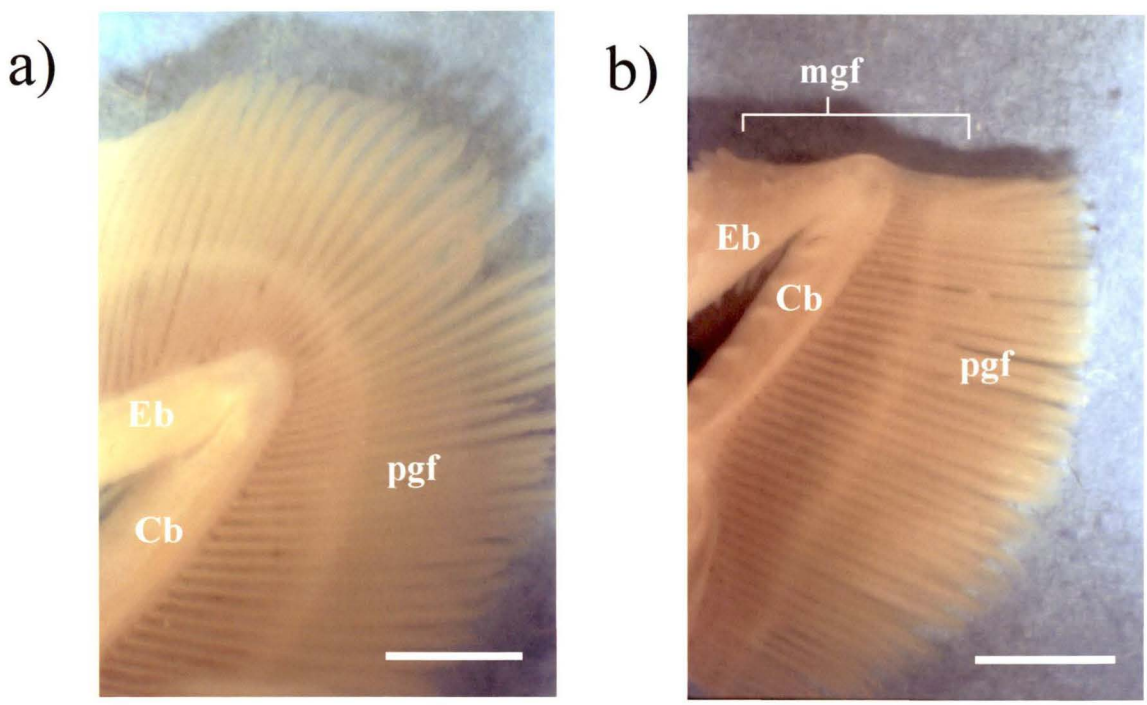


Figure 11. Gross morphology of a branchial arch from Atlantic salmon SW smolt with (a) a complete complement of primary gill filaments and (b) primary gill filaments absent, this being a characteristic of gill filament deformity syndrome (GFD). Abbreviations: Eb = epibranchial, Cb = ceratobranchial, pgf = primary gill filaments, mgf = zone of missing gill filaments. Scale bar = 3.0 mm

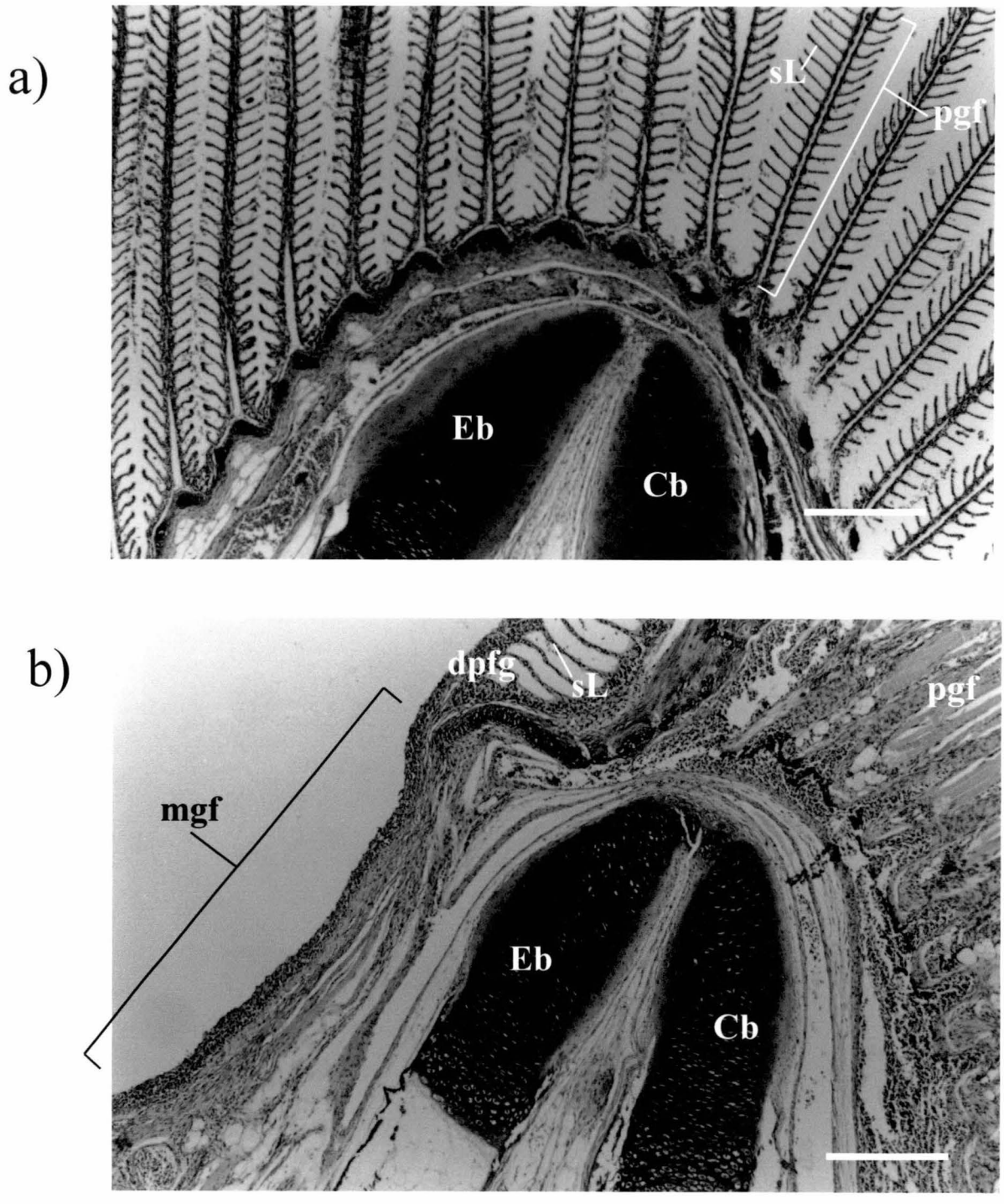


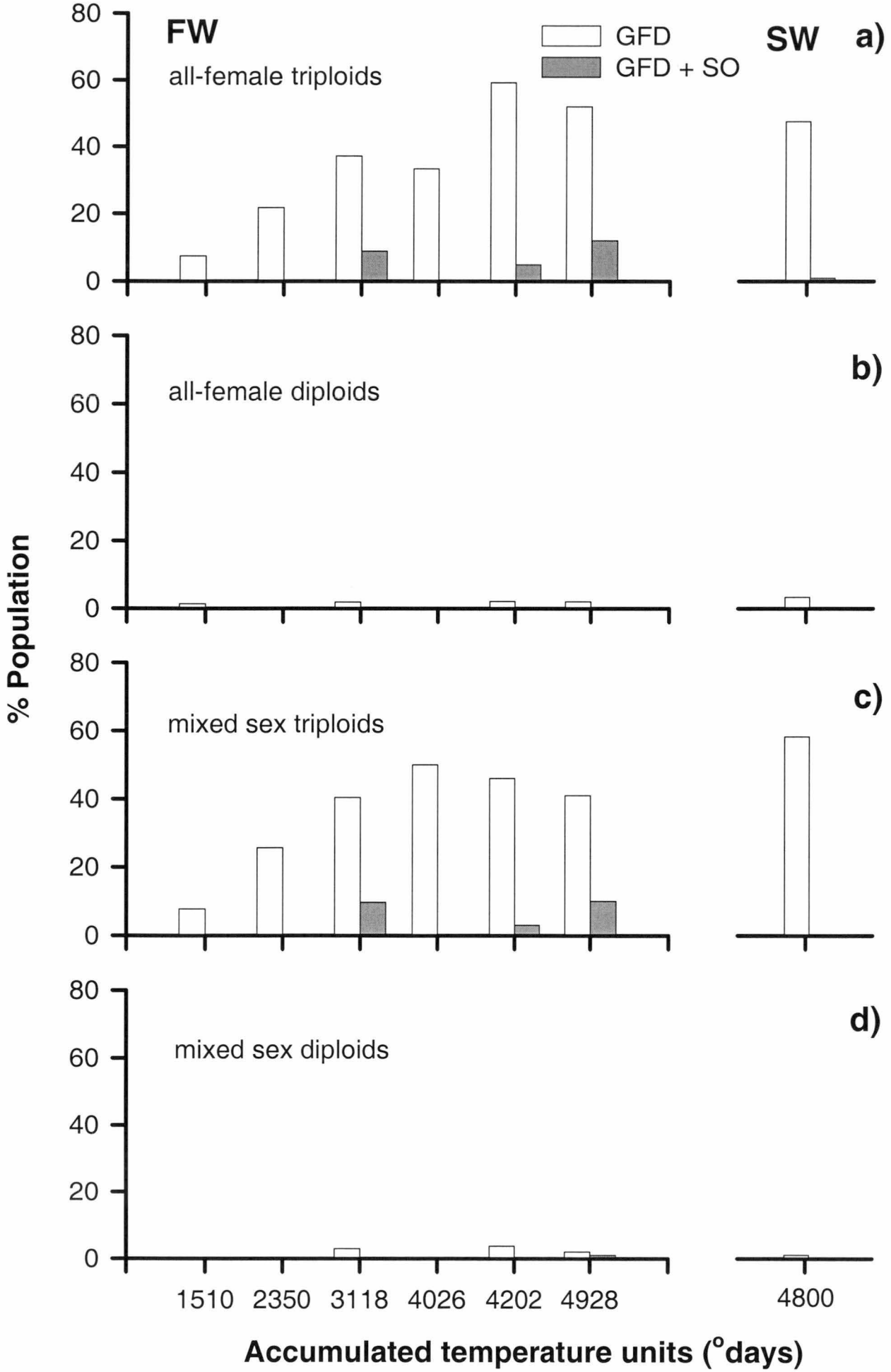
Figure 12. Photomicrograph of a longitudinal section of a branchial arch from an Atlantic salmon SW smolt with (a) a complete complement of primary gill filaments and (b) primary gill filaments absent, this being a characteristic of gill filament deformity syndrome (GFD). Sections were cut at 5 μm and stained with Haematoxylin and Eosin. Abbreviations: pgf = primary gill filament, Eb = epibranchial cartilage, Cb = ceratobranchial cartilage, sL = secondary lamella, mgf = zone of missing gill filaments, dpgf = deformed primary gill filament. Scale bar = 1.0 mm

Prevalence of GFD during development

The prevalence of gill filament deformity syndrome (GFD) in diploid fish ranged from 0 - 4% in mixed sex diploids and 0 - 2% in all-female diploids (Fig. 13), and was significantly lower than the prevalence of GFD in triploid fish throughout development ($P < 0.05$). There was no significant difference in the prevalence of GFD with population sex status. In triploid populations, GFD was initially observed at levels up to 8% at 1510° days (Fig. 13). The prevalence of GFD in triploid populations appeared to increase with development, reaching a maximum of 60% in all-female triploids at 4202° days, and 50% in mixed sex triploids at 4026° days. At the smolt stage, under FW conditions, levels of GFD were 52% in all-female triploids and 41% in mixed sex triploids, but remained at 2% in all-female diploids and mixed sex diploids at 4928° days. In smolt maintained under SW conditions (4810° days), GFD was observed in 1.8% of all-female diploids, 4.3% of mixed sex diploids, 57.8% of all-female triploids and 44.7% of mixed sex triploids.

Some fish were afflicted with both short opercula and GFD. The incidence of both short opercula and gill filament deformities in the same individual was observed predominantly in triploid fish (Fig. 13), with the exception of 1% of mixed sex diploids sampled at 4928° days. The number of fish displaying both deformity types varied, and did not increase throughout development with the increase in GFD. Between 4.8% - 12% of all-female triploids and 3% - 10% of mixed sex triploids, displayed both short opercula and GFD during the FW phase at 3118°, 4202° and 4928° days (Fig. 13). After 6 weeks in sea water (4810° days) only 1% of all-female triploids and 0% of mixed sex triploids had both short opercula and GFD (Fig. 13).

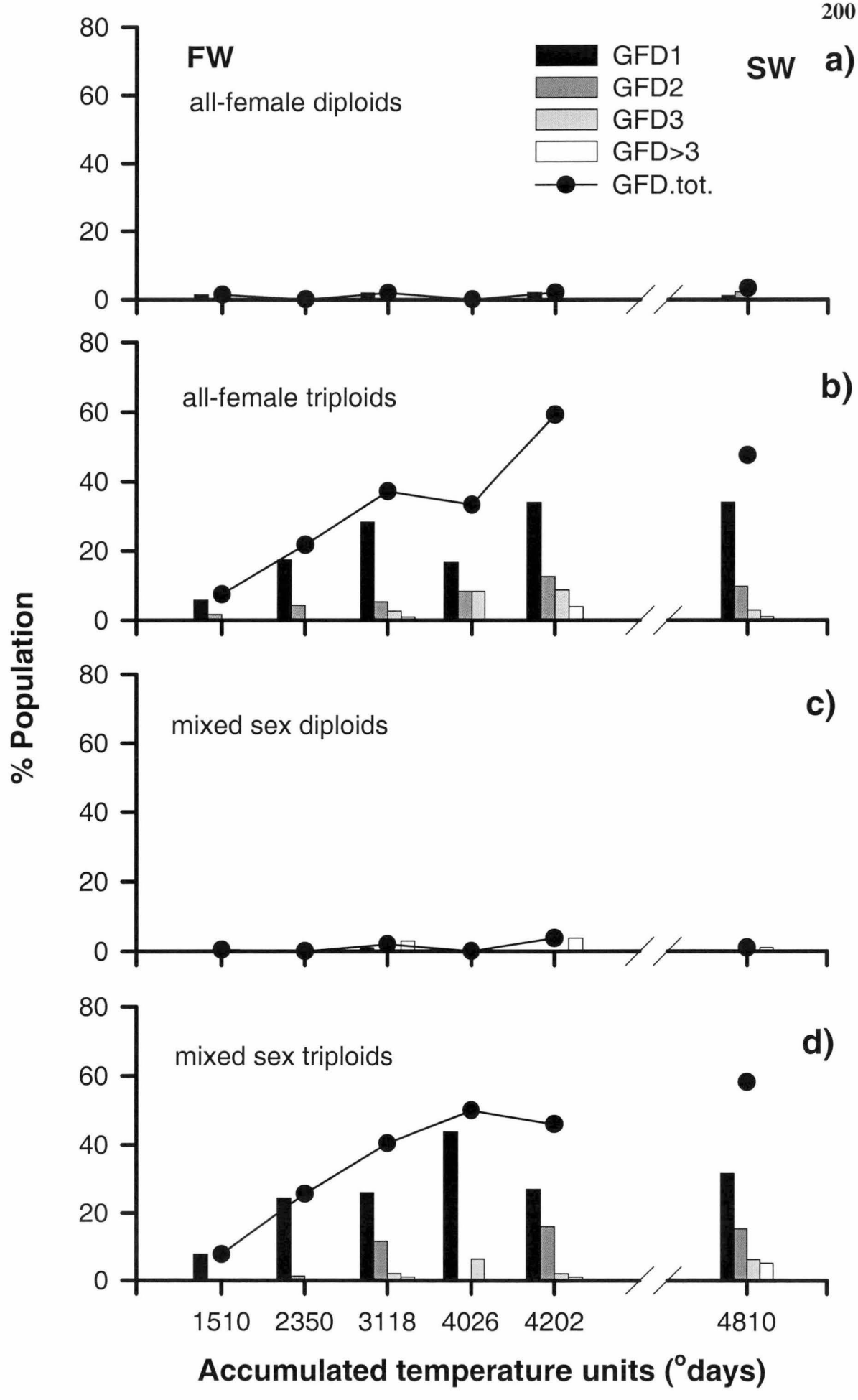
Figure 13. Prevalence (%) of gill filament deformity (GFD) in a) all-female triploid b) all-female diploid c) mixed sex triploid and d) mixed sex diploid populations throughout development. Accumulated temperature units (ATU = ° days) represent development post-fertilisation, FW = freshwater phase, SW = sea water phase, GFD = gill filament deformity syndrome, GFD + SO = simultaneous GFD and short opercula. Sample sizes as per Table 1.


FW
SW
GFD
GFD + SO
a)
all-female triploids
b)
all-female diploids
c)
mixed sex triploids
d)
mixed sex diploids
% Population
0
20
40
60
80
1510
2350
3118
4026
4202
4928
4800
Accumulated temperature units (°days)

Severity of GFD

The severity of GFD varied between afflicted fish in terms of the number of branchial arches missing gill filaments and the proportion of gill filaments missing from each arch. One to five branchial arches from either side of the body were missing primary gill filaments. Between 52% and 100% of fish with GFD had only one branchial arch with missing gill filaments, whereas between 0% and 40% of fish with GFD had two branchial arches affected by GFD, at any stage of development (Fig. 14). A lower percentage of fish from each population (< 10%) had more than 2 gill arches with missing gill filaments, at any one stage of development (Fig. 14).

Figure 14. Prevalence of fish with gill filament deformity syndrome (GFD), with one (GFD 1), two (GFD 2), three (GFD 3) or more than three (GFD > 3) branchial arches missing primary gill filaments in the a) all-female diploid, b) all-female triploid, c) mixed sex diploid and b) mixed sex triploid populations at different stages of development. Accumulated temperature units (ATU = ° days) represent development post-fertilisation. Filled circles denote total % prevalence of fish afflicted with GFD, irrespective of the level of severity. Sample numbers as per Table 1.


FW
all-female diploids
GFD1
GFD2
GFD3
GFD>3
GFD.tot.
SW
a)
b)
all-female triploids
c)
mixed sex diploids
d)
mixed sex triploids
% Population
0
20
40
60
80
1510
2350
3118
4026
4202
4810
Accumulated temperature units (°days)

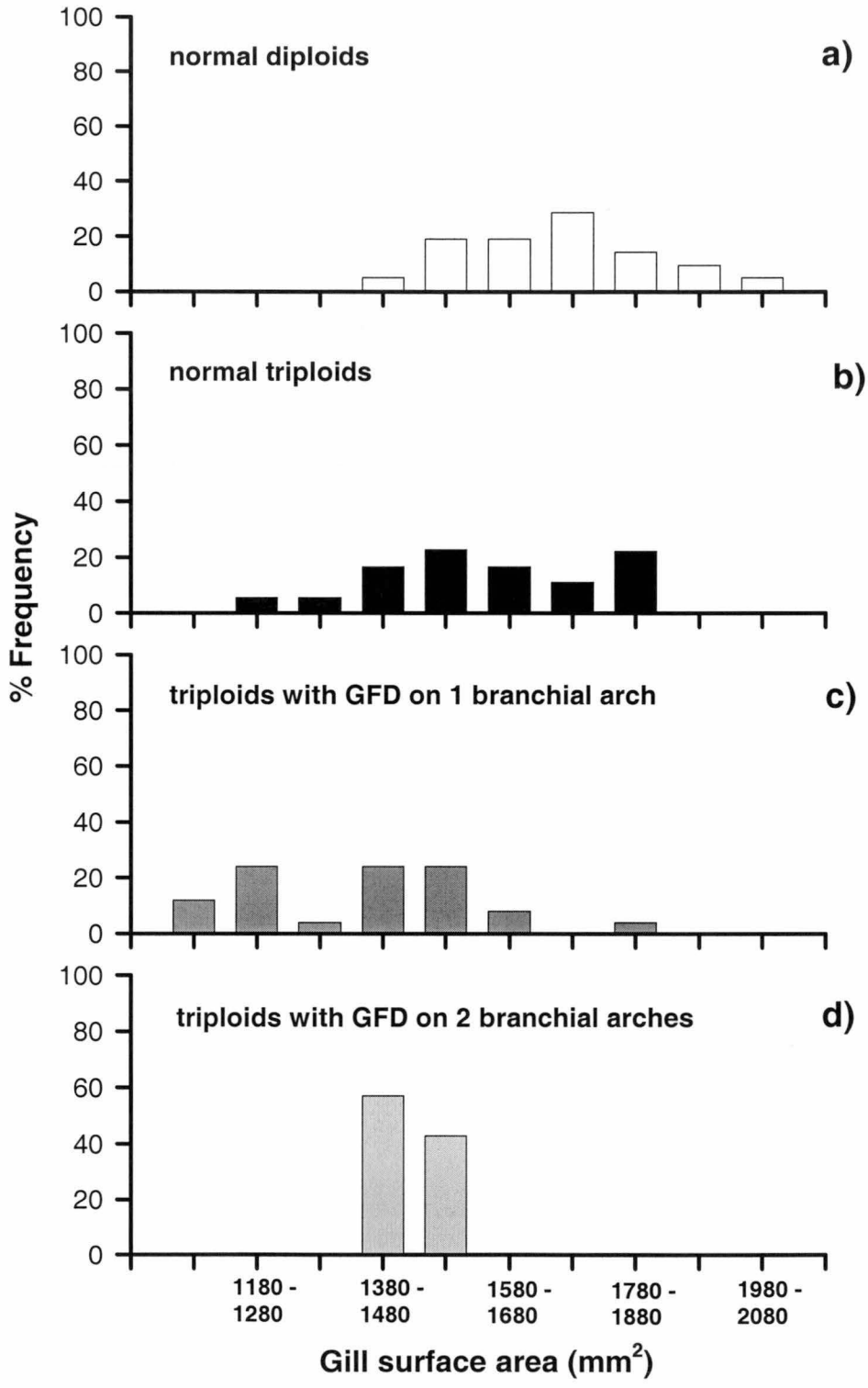
Gill surface area (GSA)

The total gill surface area (GSA) of diploid (n = 26) and triploid SW smolt (n = 18) with normal gills ranged between 1464.95 - 2040.81 mm^2 and 1248.73 - 1840.76 mm^2, respectively, whereas the GSA for triploid SW smolt with GFD (n = 48) ranged between 1132.37 - 1845.38 mm^2 (Fig. 15).

In total 20.8% of fish with GFD had a GSA that was lower than the range for normal triploid fish. An increase in the number of branchial arches affected by GFD was not indicative of a concomitant decrease in gill surface area compared to normal fish. Afflicted individuals with a GSA below the range for normal triploid fish had either one or three branchial arches affected by GFD. Thirty two percent of triploid SW smolt with one branchial arch affected by GFD (n = 25) and 28.5% of triploid SW smolt with more than three branchial arches affected by GFD (n = 7) had a GSA that was lower than the range for normal triploid SW smolt (Fig. 15).

Generally, fish with a low GSA also had a low total wet weight (Fig. 16). The relationship between gill surface area and total wet weight was similar in triploid fish with normal gills and triploid fish with deformed gills, such that the respective regressions had the same slope (Fig. 16). This fulfilled one of the assumptions required for ANCOVA analysis, and subsequently, it was possible to compare GSA values of each group using means that were adjusted to account for the covariance of GSA with weight. There was no significant difference in mean GSA values between triploid SW smolt with normal gills and triploid SW smolt with deformed gills, when comparing fish of a similar weight ($P > 0.05$, Fig. 17).

In the same fashion, ANCOVA analysis was used to compare the mean GSA value of normal diploid and normal triploid fish, again accounting for the covariance with total wet weight. The mean GSA of triploid SW smolt was significantly lower than that of diploid SW smolt when comparing fish of a similar weight ($P < 0.05$, Fig. 18). In addition, there was no difference in condition factor between normal diploid and normal triploid SW smolt ($P > 0.05$).


normal diploids
a)
normal triploids
b)
triploids with GFD on 1 branchial arch
c)
triploids with GFD on 2 branchial arches
d)
% Frequency
100
80
60
40
20
0
1180 - 1280
1380 - 1480
1580 - 1680
1780 - 1880
1980 - 2080
Gill surface area (mm2)

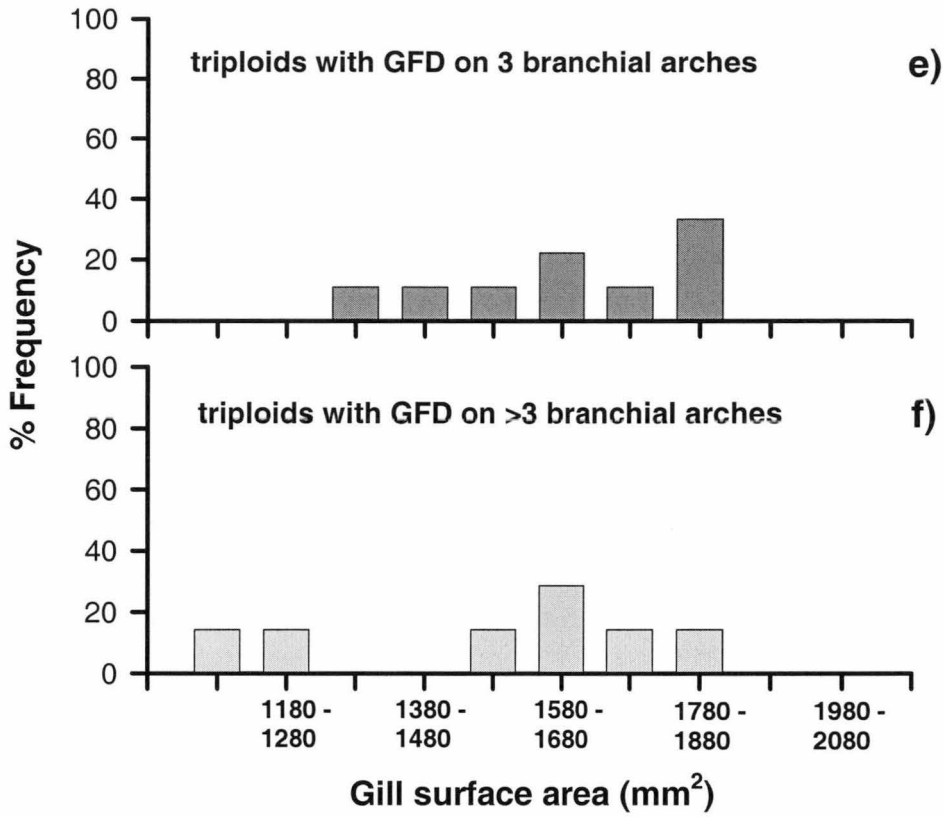
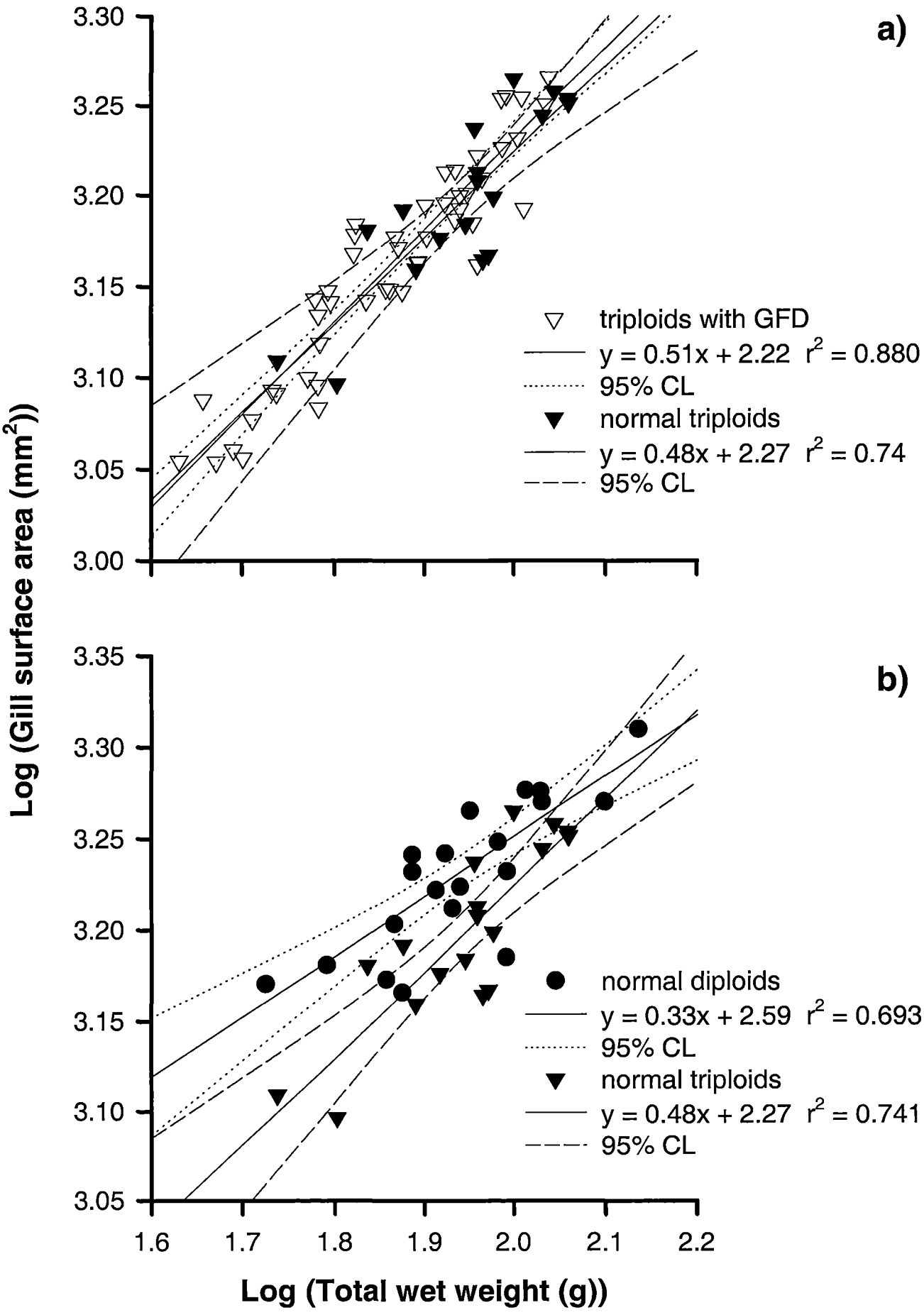


Figure 15. Frequency distribution (%) of total gill surface area (GSA) values (size classes are 100 mm^2) for a) normal diploid, b) normal triploid, and triploid SW smolt (4810° days) with either c) 1, d) 2, e) 3 or f) more than 3 branchial arches affected by GFD.

Figure 16. Change in total gill surface area (GSA) with total wet weight (TWWt.) of a) normal triploids and triploids with GFD, and b) normal triploid and normal diploid Atlantic salmon SW smolt (4810° days). Black filled circles denote normal diploid fish, black filled triangles denote normal triploid fish, Open triangles denote triploid fish with filament deformity (GFD). Dashed lines indicate 95% confidence limits (CL).


a)
b)
Log (Gill surface area (mm²))
Log (Total wet weight (g))
3.30
3.25
3.20
3.15
3.10
3.05
3.00
3.35
1.6
1.7
1.8
1.9
2.0
2.1
2.2
triploids with GFD
y = 0.51x + 2.22 r² = 0.880
95% CL
normal triploids
y = 0.48x + 2.27 r² = 0.74
95% CL
normal diploids
y = 0.33x + 2.59 r² = 0.693
95% CL
normal triploids
y = 0.48x + 2.27 r² = 0.741
95% CL

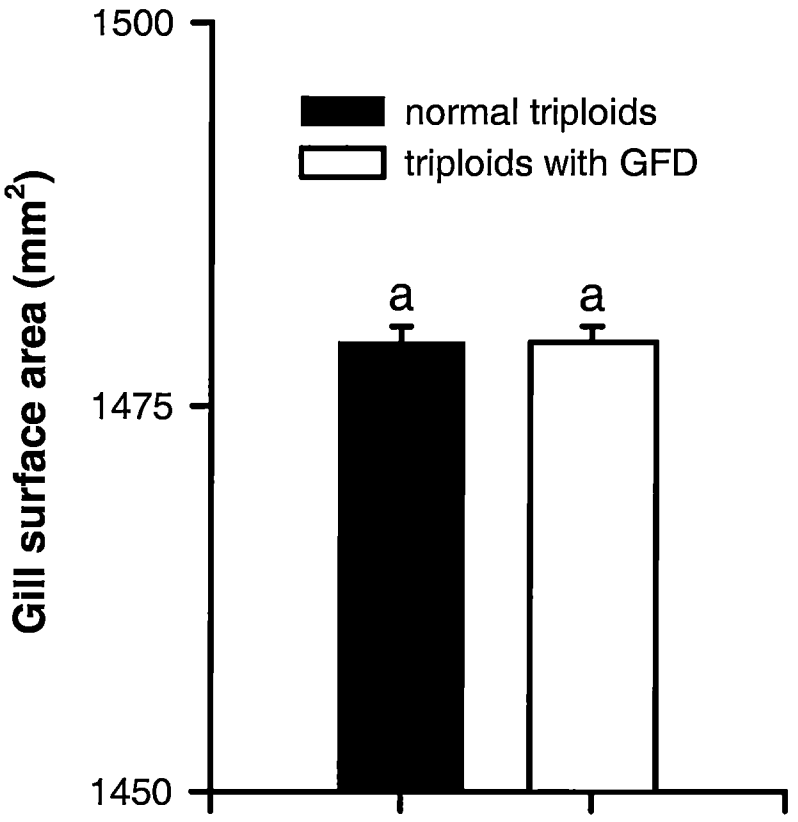


Figure 17. Relative mean total gill surface area (GSA ± SE) for triploid Atlantic salmon SW smolt (4810° days) with either normal gills (normal triploids) or with gill filament deformity (GFD). Means are adjusted to account for covariance with total wet weight of the fish.

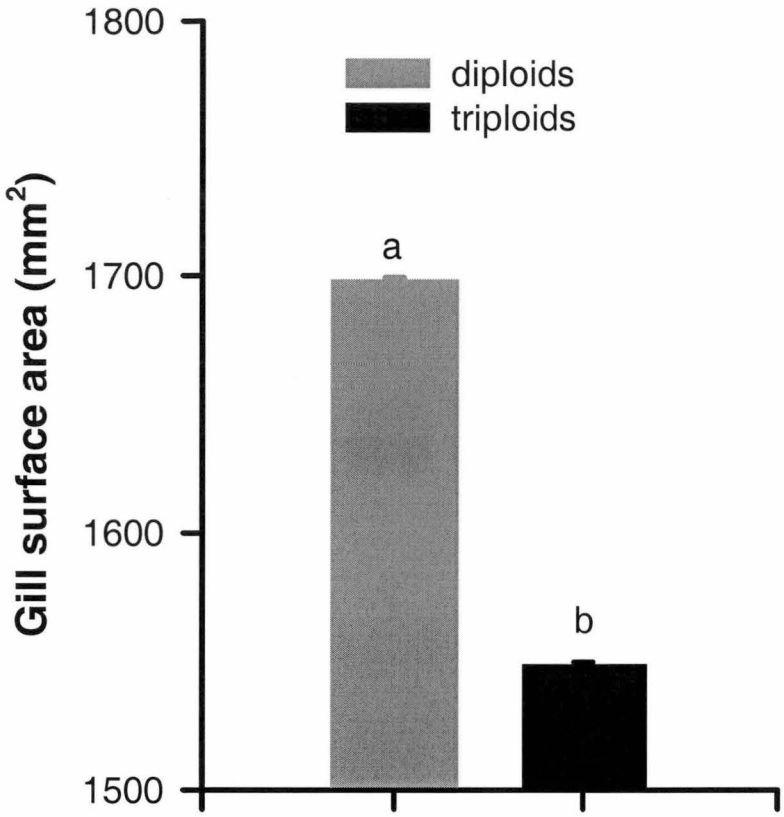


Figure 18. Relative mean total gill surface area (GSA ± SE) for diploid and triploid Atlantic salmon SW smolt (4810° days) with normal gills. Means are adjusted to account for covariance with total wet weight of the fish.

6.3.4 Other types of deformity

Other types of deformity observed at the gross level in fish from each population affected the vertebral column and /or the size, shape and number of muscle myomeres present in each fish. These deformities included kyphosis, scoliosis, lordosis, spiralled trunks, reduced number of muscle myomeres and Siamese (double headed) fish. They are hereafter collectively referred to as "non-cranial" deformities for descriptive purposes. Kyphosis is distinguished by the dorsal curvature or upward growth of the tail at the posterior edge of the yolk sac during the alevin stage of the lifecycle (Bruno and Poppe, 1996) (Fig. 19). Scoliosis is characterised by the lateral curvature of the body relative to the median longitudinal plane of bilateral symmetry, resulting in 'waves' along the body length (Bruno and Poppe, 1996) (Fig. 20). Fish with spiralled trunks had lateral curvature of the body relative to the median longitudinal plane of bilateral symmetry, resulting in the "corkscrew-like" appearance of the body. Siamese fish had two heads which shared common organs (Fig. 21).

Figure 19. Lateral view of an Atlantic salmon alevin with kyphosis. Note the dorsal curvature of the tail. Scale bar = 5.0 mm

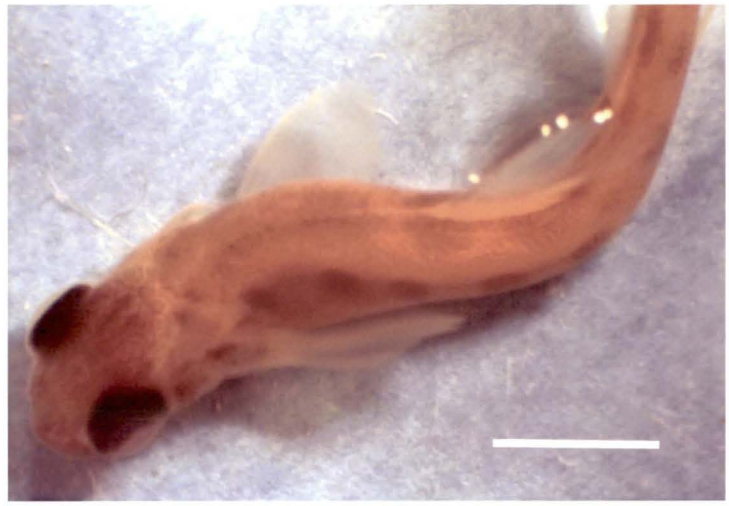


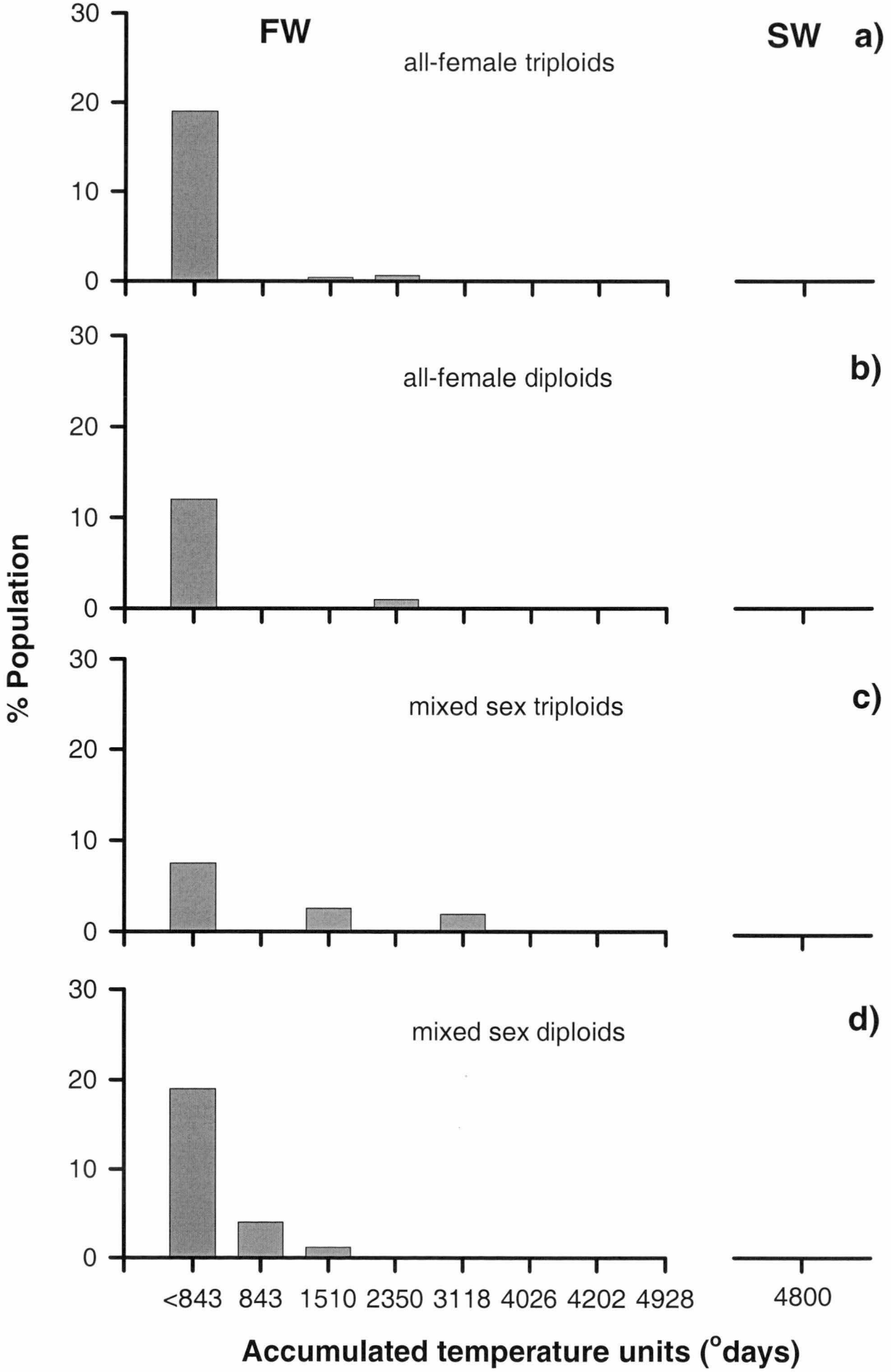
Figure 20. Dorsal view of an Atlantic salmon fry with scoliosis. Note the lateral curvature of the body relative to the median longitudinal plane of bilateral symmetry. Scale bar = 7.0 mm

Figure 21. Lateral view of a siamese Atlantic salmon alevin. Scale bar = 5.0 mm

Prevalence of non-cranial deformities throughout development

Non-cranial deformities occurred in up to 19% of all-female triploids and mixed sex diploids, 7.3% of mixed sex triploids and 1.7% of all-female diploids between 470° - 706° days post-fertilisation (Fig. 22, < 843° days). At the first sample date following swim-up (843° days) and during first feeding, the incidence of all non-cranial deformity types fell to zero. Thereafter and prior to 4026° days, non-cranial deformities were detected at very low rates (1% - 3%) in all populations (Fig. 22).

Figure 22. Prevalence (%) of "non-cranial" deformities including kyphosis, scoliosis, lordosis, reduced myomere numbers, spiralled trunks and Siamese fish in a) all-female triploid b) all-female diploid c) mixed sex triploid and d) mixed sex diploid populations throughout development. Accumulated temperature units (ATU = ° days) represent development post-fertilisation, FW = freshwater phase, SW = sea water phase. Sample sizes as per Table 1.


FW
SW
a)
all-female triploids
b)
all-female diploids
c)
mixed sex triploids
d)
mixed sex diploids
30
20
10
0
% Population
<843
843
1510
2350
3118
4026
4202
4928
4800
Accumulated temperature units (°days)

6.4.5 Total prevalence of skeletal deformity during development

During the period between hatching and swim-up (470° - 706° days post-fertilisation) the total prevalence of skeletal deformity (LJD, JD, GFD, short opercula and non-cranial deformities pooled) was up to 20% in each population, irrespective of ploidy status ($P > 0.05$, Fig. 23). Following swim-up and during first feeding (≤ 913° days), total deformity rates decreased to zero in all populations (Fig. 23).

Following first feeding (> 1000° days), the incidence of skeletal deformity increased and was significantly higher in triploid populations compared to diploid populations ($P < 0.05$, Fig. 23). Up to 65% of triploid individuals and 20% of diploid individuals suffered from gross skeletal deformities towards the end of smoltification (4202° days, Fig. 23). These levels of deformity were subsequently maintained in populations of smolt regardless of whether the fish were maintained under FW conditions (4928° days), or were transferred to SW (4810° days, Fig. 23).

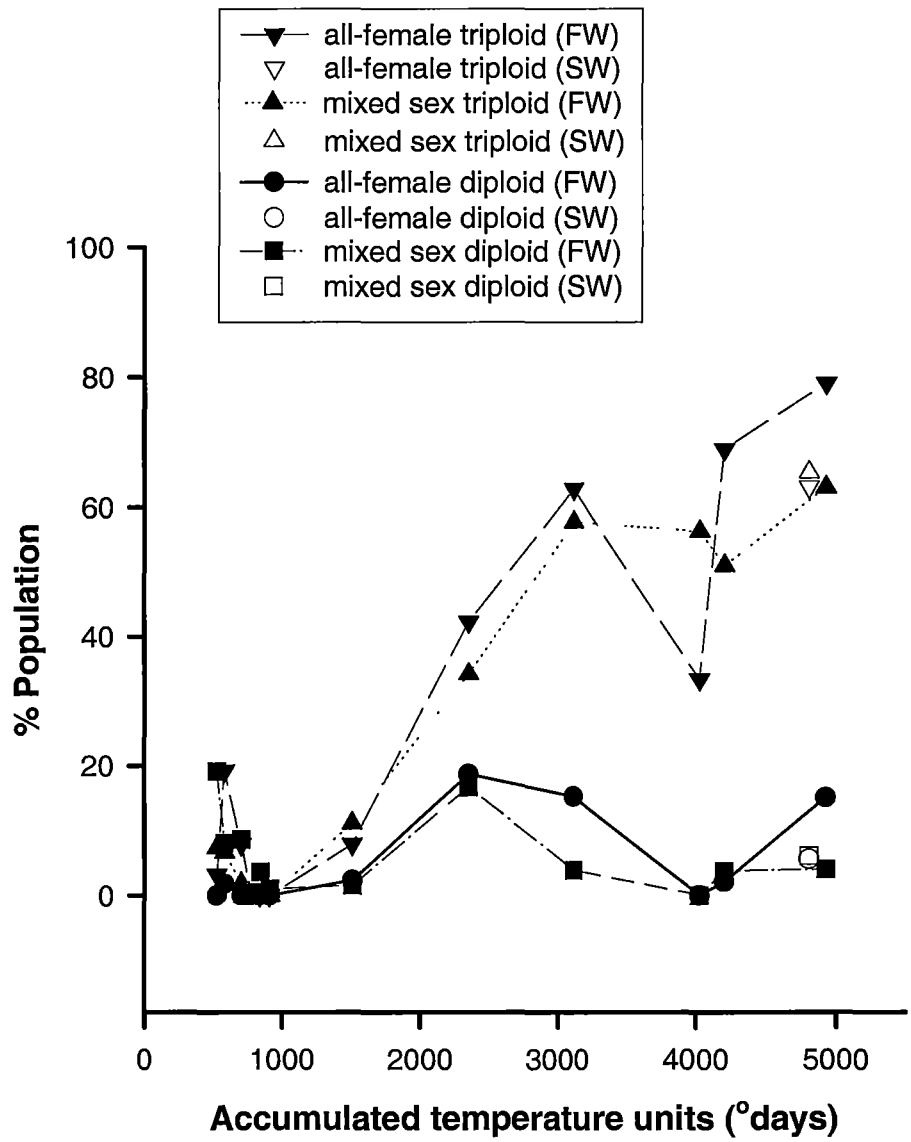


Figure 23. Change in the total percentage prevalence of gross skeletal deformities in four Atlantic salmon populations (all-female diploids, all-female triploids, mixed sex diploids and mixed sex triploids) during development. Accumulated temperature units (ATU = ° days) represent development post-fertilisation. Black symbols represent values during FW culture conditions. White symbols represent values during SW culture conditions (SW smolt). Sample numbers as per Table 1.

Relative contribution of different deformity types to the total deformity prevalence

Short opercula was the predominant type of deformity observed in diploid populations, but not in triploid populations (Fig. 24). At 2350° days short opercula contributed to 90% and 100% of all deformities observed in the all-female diploid and mixed sex diploid populations, respectively (Fig. 24). At the same stage of development short opercula represented 48% and 26% of all deformities in the all-female triploid and mixed sex triploid populations, respectively (Fig. 24). Short opercula contributed to between 75 - 79% of all deformities observed in diploid FW smolt and 27 - 31% of deformities in triploid FW smolt (4928° days, Fig. 24). Of the deformities observed in SW smolt, 83% in all-female diploids, 50% in mixed sex diploids, 14% in all-female triploids and 12.6% in mixed sex triploids were short opercula (Fig. 24). The predominant prevalence of short opercula in diploid populations was less obvious mid-FW phase (Fig. 24), concomitant with population thinning. The contribution of short opercula to deformity prevalence in triploid populations remained relatively low at this time.

The predominant deformity type observed in triploid populations was GFD. The contribution of GFD to the high total deformity rates observed in triploid populations ranged between 51% - 100% of total deformity rates in all-female triploids and 68% - 92% of that in mixed sex triploids, throughout development (Fig. 24). In contrast, the relative proportion of deformed diploid fish with GFD was low at stages of development characterised by more than 5% total deformity prevalence. GFD contributed to between 0 - 13% of all deformities in all-female diploids, and between 0 - 38% of deformities in mixed sex diploids. Stages of development characterised by very low total deformity prevalence in diploid populations (2 - 4%), as seen at 4202° days, had an apparently high proportion of fish afflicted with GFD; up to 100% in all-female diploids and 75% of that in mixed sex diploids (Fig. 24).

The relative contribution of all forms of jaw deformity, including laterally curved lower jaws and mild and severe LJD, to total deformity prevalence remained below 20% in all populations throughout development in FW (Fig. 24). At the SW smolt stage (4810° days), jaw deformity contributed to 25.4% of deformity in all-female triploids, 20% in mixed sex triploids, 10% in all-female diploids and 37.5% in mixed sex diploids.

All deformities observed prior to first feeding consisted entirely of non-cranial deformities, including kyphosis, scoliosis, lordosis, reduced myomere numbers and Siamese fish (Fig. 25). The contribution of non-cranial deformities to total deformity prevalence was negligible in all populations after first feeding (Fig. 25).

Figure 24. Percentage contribution of short opercula (SO = diagonal stripe fill), gill filament deformity (GFD = grey fill) and jaw deformities (JD = black fill) to the total prevalence of skeletal deformity in the a) all-female triploid (FT), b) all-female diploid (FD), c) mixed sex triploid (MT) and d) mixed sex diploid (MD) populations of Atlantic salmon, at different stages throughout development. Accumulated temperature units (ATU = ° days) represent development post-fertilisation, FW = freshwater phase, SW = sea water phase.

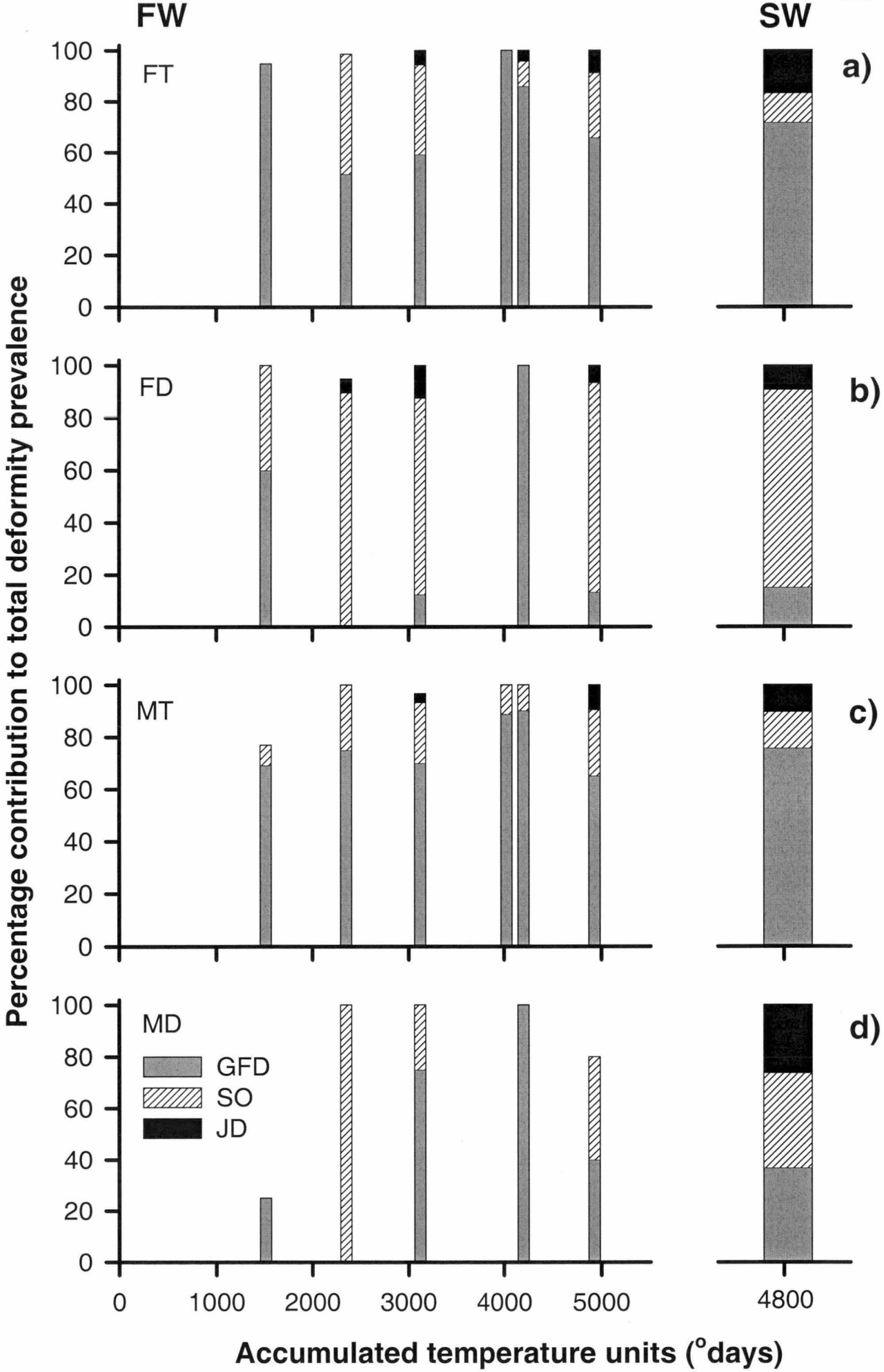
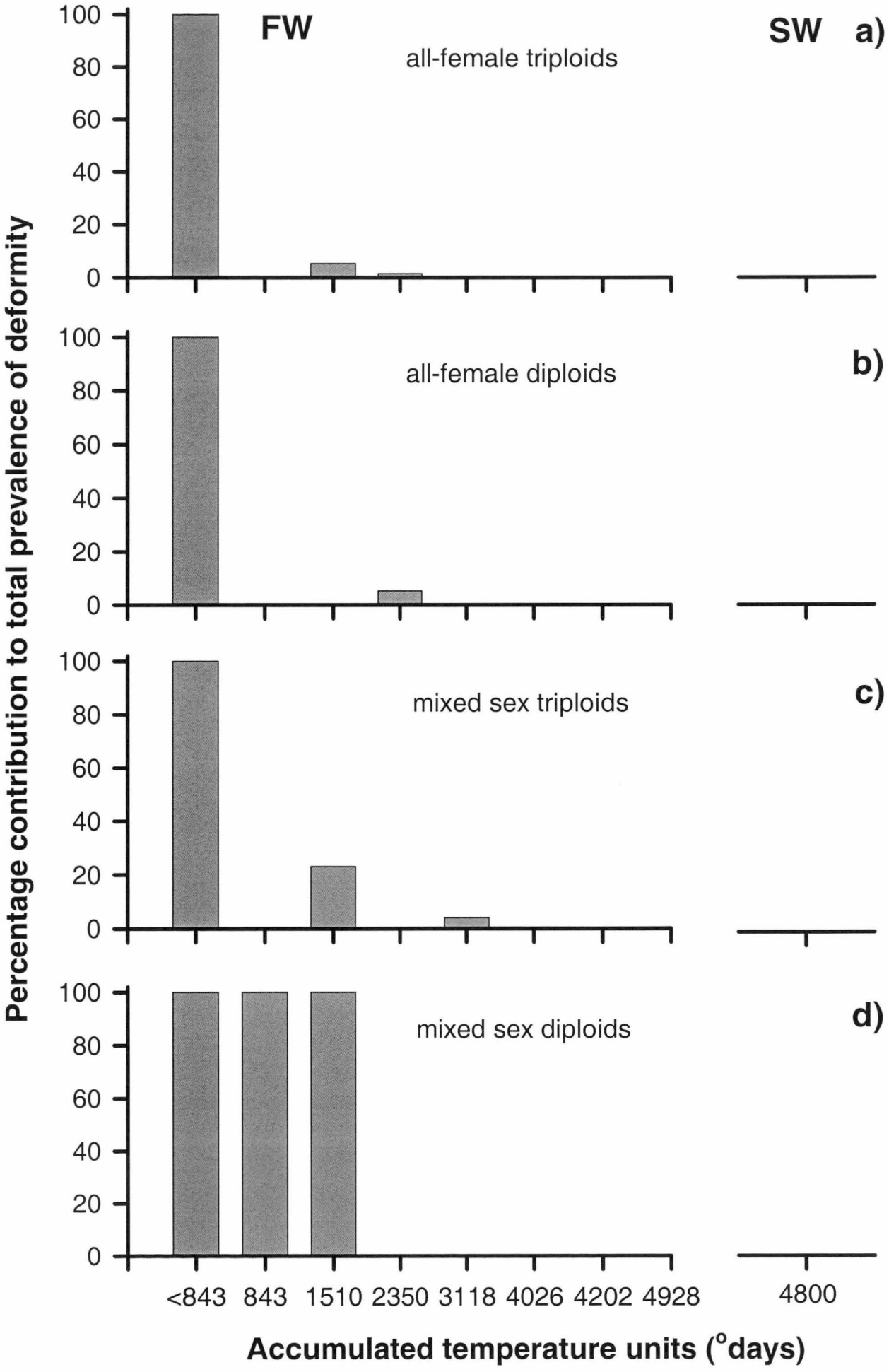

FW
SW
FT
FD
MT
MD
a)
b)
c)
d)
GFD
SO
JD
Percentage contribution to total deformity prevalence
Accumulated temperature units (°days)
0
20
40
60
80
100
0
1000
2000
3000
4000
5000
4800

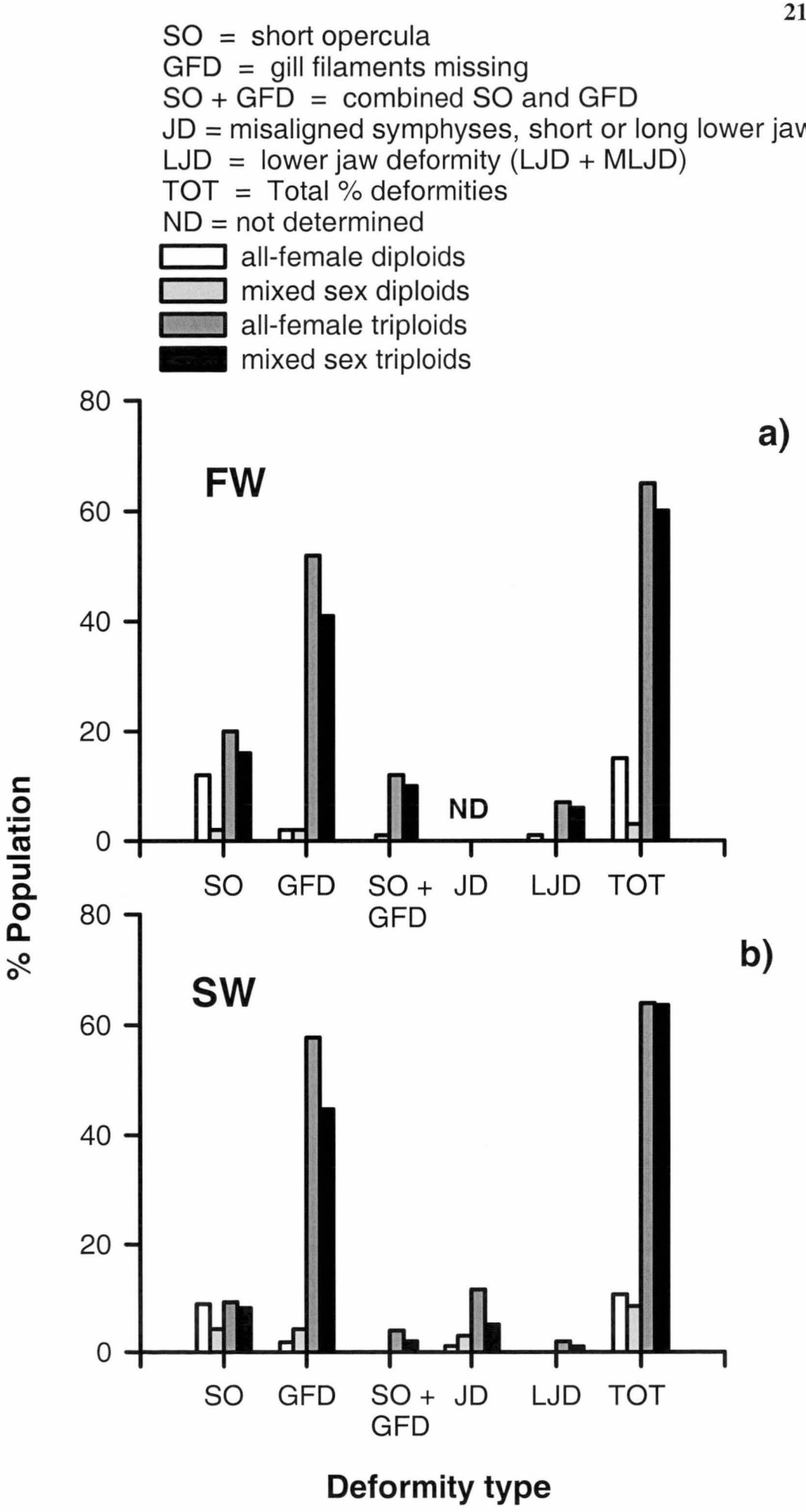
Figure 25. Percentage contribution of non-cranial deformities, including kyphosis, scoliosis, Siamese fish and reduced myomere numbers, to the total prevalence of skeletal deformity in a) all-female triploid, b) all-female diploid, c) mixed sex triploid and d) mixed sex diploid populations of Atlantic salmon at different stages throughout development. Accumulated temperature units (ATU = ° days) represent development post-fertilisation. FW = freshwater phase, SW = sea water phase.


FW
SW
a)
all-female triploids
b)
all-female diploids
c)
mixed sex triploids
d)
mixed sex diploids
100
80
60
40
20
0
Percentage contribution to total prevalence of deformity
<843
843
1510
2350
3118
4026
4202
4928
4800
Accumulated temperature units (°days)

6.4.6 Deformity of freshwater and seawater smolt

The diversity of deformities did not differ between FW and SW smolt (Fig. 26). The prevalence of short opercula and jaw deformity was slightly higher in smolt grown in FW compared to smolt grown in SW ($P = 0.069$) from the same populations. The specific growth rate of FW smolt from each population (SGR = 0.143 - 0.176 %/day) was significantly higher than that for SW smolt in each population (SGR = 0.041 - 0.063 %/day, $P < 0.05$), as described in Chapter 2.

Figure 26. Prevalence of different deformity types in Atlantic salmon smolt (4810 - 4928° days) from four populations (all-female diploid, all-female triploid, mixed sex diploid and mixed sex triploid) maintained in either a) freshwater (FW) or b) seawater (SW). GFD = gill filament deformity, SO = short opercula, SO + GFD = combined SO and GFD, LJD = lower jaw deformity syndrome (including mild cases (MLJD))


SO = short opercula
GFD = gill filaments missing
SO + GFD = combined SO and GFD
JD = misaligned symphyses, short or long lower jaw
LJD = lower jaw deformity (LJD + MLJD)
TOT = Total % deformities
ND = not determined
all-female diploids
mixed sex diploids
all-female triploids
mixed sex triploids
a)
FW
ND
b)
SW
% Population
80
60
40
20
0
SO
GFD
SO + GFD
JD
LJD
TOT
Deformity type

6.4.7 Sex ratio of different deformity types

The occurrence of short opercula, GFD and jaw deformity, was not associated with any one sex. Mixed sex triploid FW parr (4202° days) that were afflicted with short opercula were 100% female; however, sample numbers were low (Fig. 27). Triploid SW smolt (4810° days) afflicted with short opercula and from the same mixed sex population were 100% female, whereas diploid SW smolt with short opercula were 1:2 male to female (Fig. 28). Parr afflicted with GFD had a sex ratio of 2:1 (male : female) in the mixed sex diploid population and 1:1 in the mixed sex triploid population (Fig. 27). Smolt afflicted with GFD from the mixed sex diploid population were all female, whereas mixed sex triploid smolt with GFD were 1:1 male to female (Fig. 28). In the mixed sex diploid and mixed sex triploid populations, fish afflicted with jaw deformity at the SW smolt stage had a sex ratio of 2:1 and 1:3 (male: female), respectively (Fig. 28).

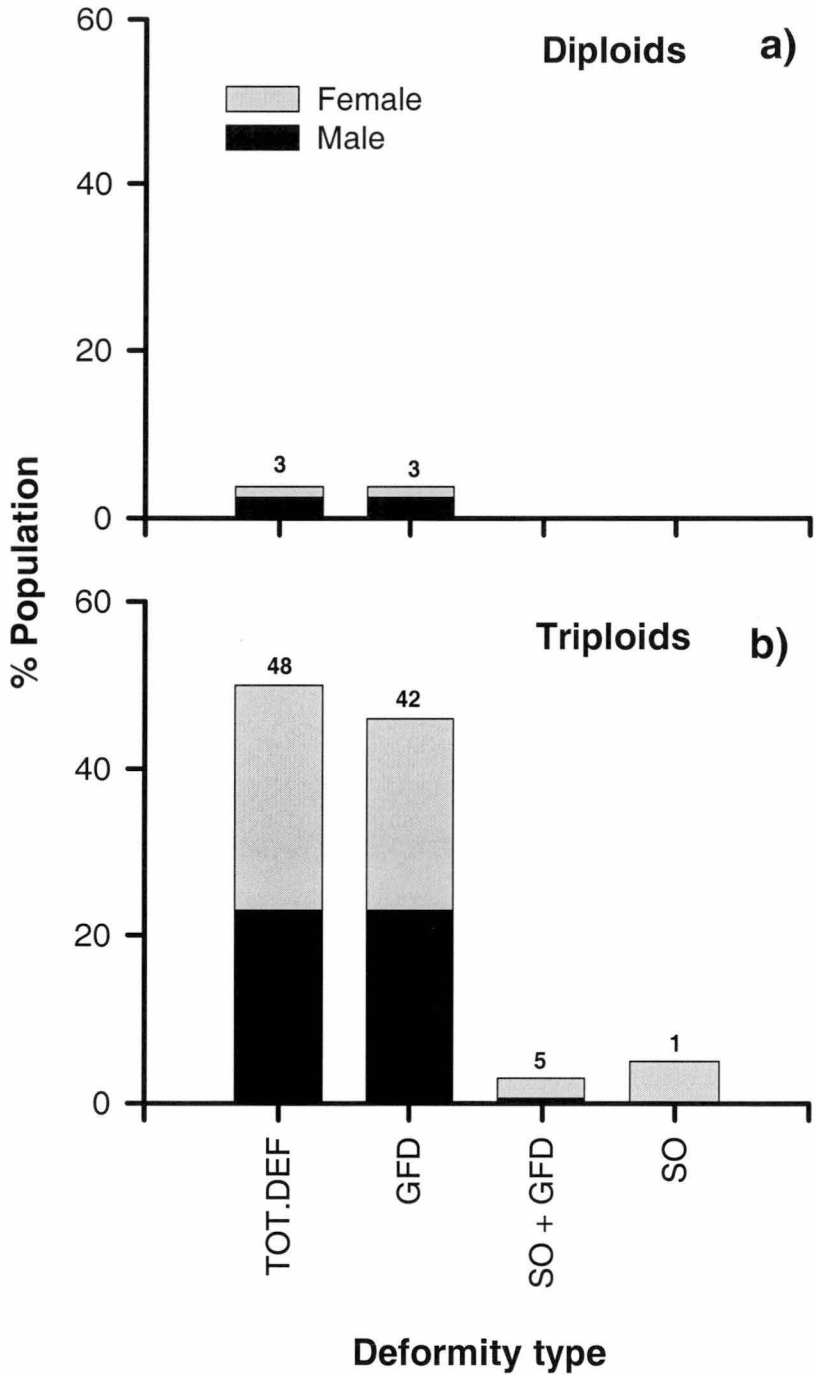


Figure 27. Proportion of male and female FW parr (4202° days) afflicted with different types of deformity in the a) mixed sex diploid and b) mixed sex triploid populations. TOT.DEF = total deformity prevalence, GFD = gill filament deformity, SO = short opercula, SO + GFD = combined SO and GFD. Superscripts denote sample numbers (n).

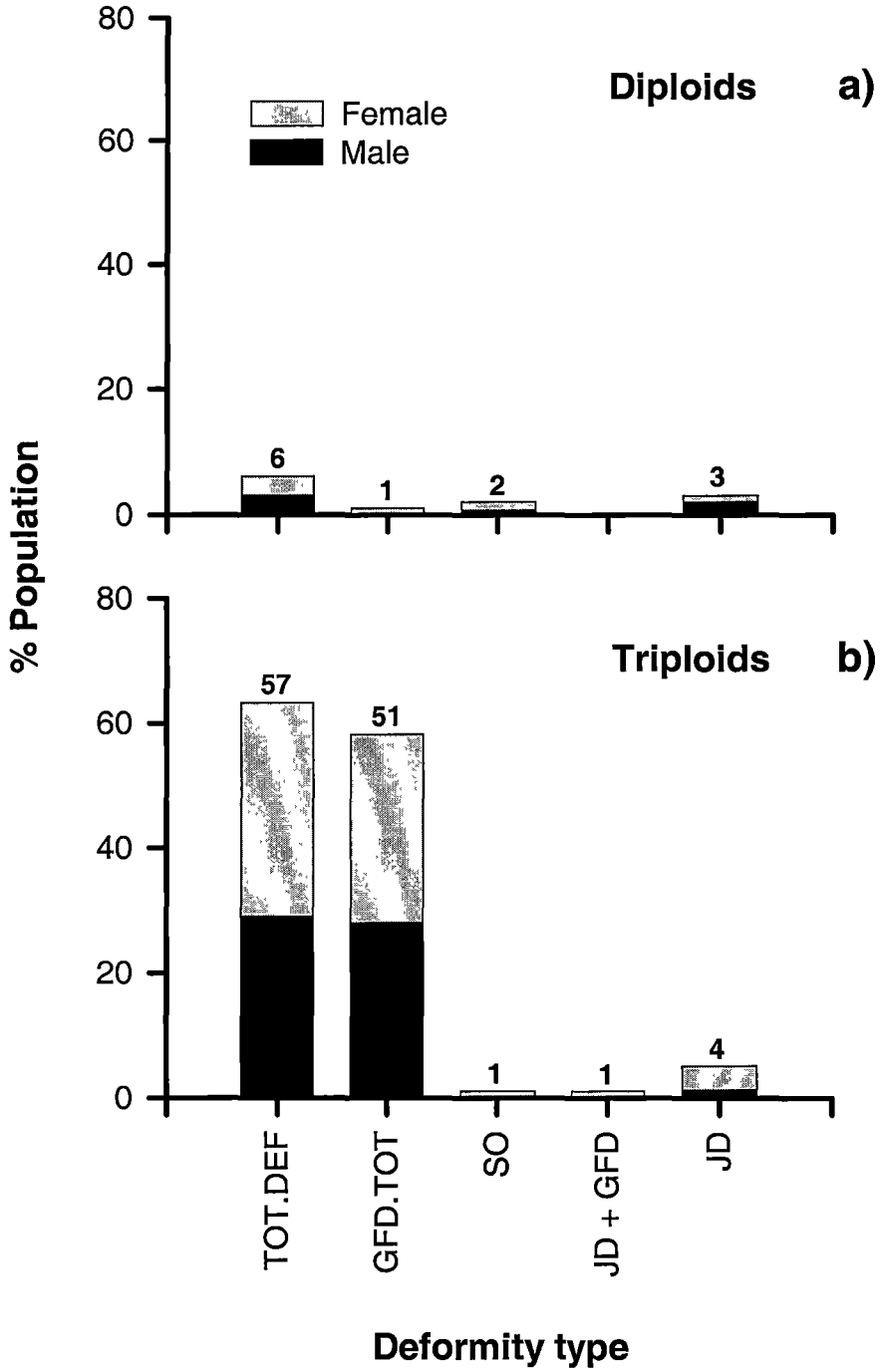


Figure 28. Proportion of male and female SW smolt (4810° days) afflicted with different types of deformity in the a) mixed sex diploid and b) mixed sex triploid populations. TOT.DEF = total deformity prevalence, GFD = gill filament deformity, SO = short opercula, JD = jaw deformity, JD + GFD = combined JD and GFD. Superscripts denote sample numbers (n).

6.4.8 Specific growth rate and deformity

No correlation was found between specific growth rate determined at each stage of development and the percentage prevalence of lower jaw deformity (Fig. A, Appendix F) and short opercula (Fig. B, Appendix F) at each stage. There was a negative correlation between specific growth rate, as determined for each stage of development, and the prevalence of gill filament deformity in triploid populations at each stage (Fig. 29).

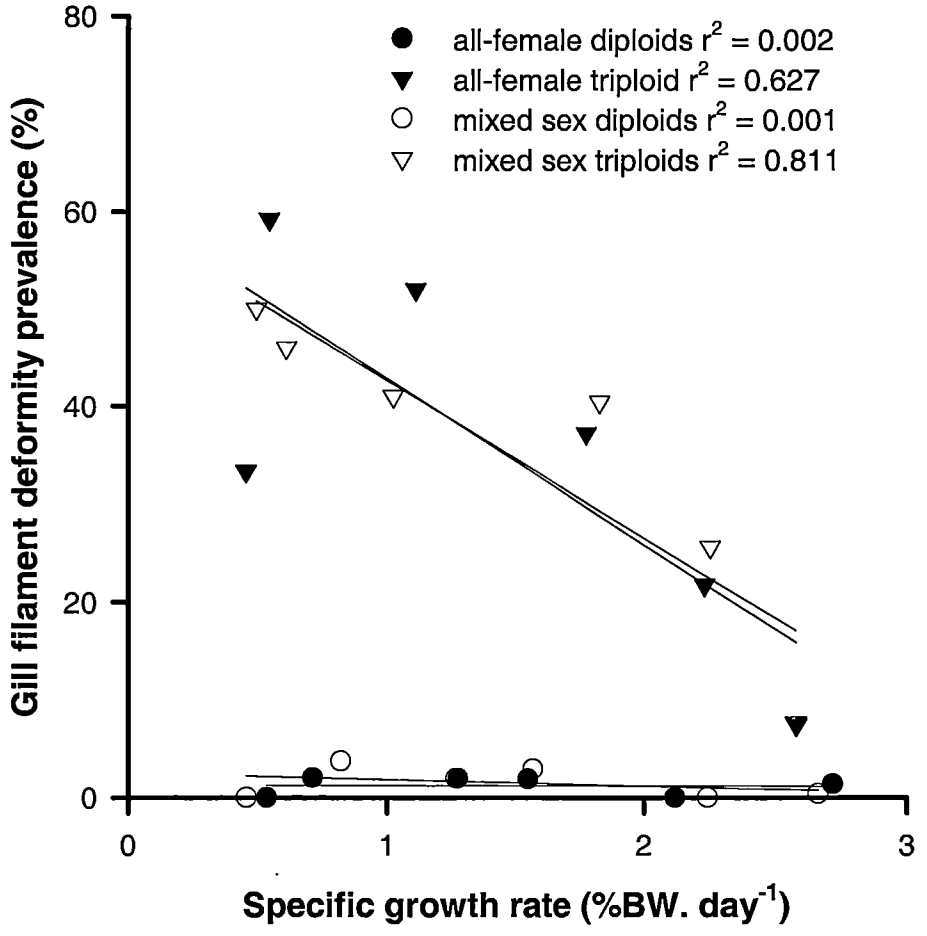


Figure 29. Variation in prevalence of gill filament deformity at each stage of development with specific growth rate of each population at each stage.

Furthermore, the mean total wet weight (Fig. C, Appendix F), mean fork length (Fig. D, Appendix F) and mean K factor (Fig. E, Appendix F) of SW smolt affected by GFD, short opercula or jaw deformities did not vary significantly to that of normal individuals at the same stage of development.

6.5 DISCUSSION

6.5.1 LJD

This study reports LJD (albeit at very low levels) in smolt both retained in FW and transferred to SW. Previous studies, including those of Tasmanian Atlantic salmon, indicate that LJD occurs at rates of up to 30% during the SW phase of the lifecycle (Bruno, 1990; McGeachy *et al.*, 1996; Lee and King, 1994; King and Lee, 1993), but the prevalence in earlier life stages was unknown locally. Freshwater smolt displayed a higher SGR than SW smolt and the presence of LJD in higher degree in FW smolt in this study suggests that LJD is more likely associated with an increase in SGR rather than with water salinity or the effects of seawater transfer *per se*. Lee and King (1994) also suggested that growth rate may affect the incidence of LJD in triploid Tasmanian Atlantic salmon, although the results of their study were inconclusive. A recent study by Goicochea *et al.* (1999) showed LJD can occur at high levels (90%) during the pre-smolt FW phase of diploid Chilean Atlantic salmon. The disparity in the time of onset of LJD in the latter study and the current study may be due to differences in stock genetics and/or growth rates.

It is possible that LJD is the result of either the expression of an autosomal recessive gene or the altered expression of a homeiotic gene, particularly following triploid induction. The possible heritable nature of LJD within the Tasmanian Atlantic salmon stock is supported by the recurrence of LJD in triploids produced each year, regardless of growout site (Jungawalla, 1991; Hughes, 1992; King and Lee, 1993; Lee and King, 1994); however, the constancy of Tasmanian culture conditions cannot be disregarded as a contributing factor. The possible heritability of LJD has

been refuted by Bruno (1990), who showed that LJD can be site specific. In the Shetland Islands, high levels of LJD occurred in one group of Atlantic salmon derived from an otherwise normal population, such that a varying prevalence of LJD between sibling fish was associated with the grow-out site (Bruno, 1990). The influence of environmental conditions on the incidence of LJD warrants consideration.

It has previously been suggested that vitamin C deficiency may cause LJD (Hughes, 1992; King and Lee, 1993), since vitamin C is essential for the formation of hydroxyline, a consituent of collagen. As a result vitamin C deficiency can result in the formation of weak and defective collagen and bones (reviewed by Hughes, 1992). King and Lee (1993) examined the effects of a reduced vitamin C intake on the incidence of LJD in triploid Atlantic salmon, but the results of this preliminary study were inconclusive. It remains unclear as to whether vitamin C deficiency contributes to the high incidence of skeletal deformities in triploid fish. In cases where skeletal deformity is associated with nutritional deficiency, the supplementation of broodstock feed with vitamins or phospholipids has reduced the incidence of deformity in subsequent progeny (Soliman *et al.*, 1986a).

A previous study of the histopathology of LJD in Chilean Atlantic salmon described symptoms including high levels of hepatic transaminases (Goicoechea *et al.*, 1999), which may indicate hepatocyte dysfunction due to exposure to toxic substances (Hugget *et al.*, 1992); however, Davies' (1995) assessment of water quality of the Derwent and Florentine River catchments ruled out the likelihood of teratogenic substances in the SALTAS hatchery water supply. Malachite green, a therapeutic bath treatment used by the industry for the treatment of *Saprolegnia* sp. fungus infestation, cannot however be discounted as a causative agent.

The other types of jaw deformity observed in this study; misaligned symphyses, short or long lower jaws, had a higher prevalence in triploids than diploids post-SW transfer. Misaligned symphyses have been previously reported in diploid and triploid Atlantic salmon populations at rates of up to 12.3% (Hughes, 1992; King and Lee,

1993; Lee and King,1994; Branson and Nieto, 1999). Similarly, short lower jaw (Lee and King, 1994) and protrusion of the lower jaw beyond the upper jaw (Sutterlin *et al.*, 1987) in Atlantic salmon have previously been recorded. Protrusive jaw deformity (PJ) was previously associated with the triploid condition rather than the triploid induction treatment, as diploid individuals that emerged from the triploid treatment did not display the PJ deformity (Sutterlin *et al.*, 1987). The aetiology of these deformities in Atlantic salmon remains unclear.

Jaw deformities observed in other species have been attributed to various causes. It has been suggested that open jaw syndrome in Chinook salmon *Oncorhynchus tshawytscha*, characterised by a gaping mouth in conjunction with a short lower jaw, is possibly caused by genetic abberation, teratogenic substances and/or environmental factors such as water quality and temperature (Crouch *et al.*, 1973). A report on "lockjaw" syndrome in diploid sable fish *Anoplopoma fimbria* larvae, a syndrome characterised by a gaping mouth that cannot be closed, indicated that the problem was alleviated by the greater control (decrease) of rearing temperatures (McFarlane, 1989). Similarly, a "lockjaw" deformity in Atlantic halibut *Hippoglossus hippoglossus* has been shown to increase in incidence with increased water temperatures (Bolla and Holmefjord, 1988; Pitmann *et al.*, 1989; Ottesen and Bolla, 1998) and light intensity (Bolla and Holmefjord, 1988), but is not affected by salinity (Ottesen and Bolla, 1998) or water flow rates (Opstad and Bergh, 1993). It has been suggested that bacterial infection (Opstad and Bergh, 1993) and/or other pathogenic organisms (Morrison and MacDonald, 1995) may be responsible for jaw deformity in halibut, but egg disinfection with glutaric dialdehyde does not affect the incidence of the deformity in larvae (Harboe *et al.*, 1994). Higher water temperatures appear to be responsible for jaw deformity in gilthead seabream, *Sparus aurata*, but the deformity was not associated with higher growth rates (Polo *et al.*, 1991).

In light of the manner in which each skeletal element develops (Chapter 5), we may suggest possible mechanisms which contribute to the incidence of jaw deformity. The bones of the lower jaw, including the dentary, angular and retroarticular, are primarily formed by membranous secretion (Chapter 5), and as such, their shape may

change according to the structural integrity and strength of the dermal tissues in which they are formed and that of the bone matrix secreted by these tissues. Potential differential strength between the soft connective tissue and the musculature that supports and facilitates movement of the lower jaw may create distorting tensions which, in combination with weak bone matrix, could progressively result in the downward curvature of the lower jaw, as seen in fish with LJD.

Fish afflicted with jaw deformity in this study had a total wet weight similar to that of normal fish; however, sample numbers were low. Salmonids afflicted with jaw deformity are able to feed and in some cases there appears to be no affect on growth (McGeachy *et al.*, 1996), whereas in other cases the average SGR is reduced in fish with JD (Crouch *et al.*, 1973; Bruno, 1990; Quigley, 1995; Al-Harbi, 1999). In contrast, "lockjaw" deformity in marine finfish larvae, often results in starvation and subsequently high mortality rates (MacFarlane, 1989; Polo *et al.*, 1991; Morrison and MacDonald, 1995).

6.5.2 Short Opercula

In the current study, the decline in prevalence of short opercula deformity at 4026 - 4202° days coincided with the non-selective thinning of each population. The reason for the decline at this stage, and again at the FW smolt and SW smolt stage (> 4810° days) is unknown. The incidence of short opercula was not associated with population type, although prevalence in triploid populations was slightly higher than that in diploid populations. Similarly, Sutterlin *et al.* (1987) also found that the incidence of short opercula was not associated with ploidy status in Atlantic salmon. Short opercula deformity is common to other cultured species, including sea bass *D. labrax* (Barahona-Fernandes, 1982), sea bream *Sparus aurata* (Galeotti *et al.*, 1997), striped trumpeter *Latris lineata* (Cobroft *et al.*, in preparation), tilapia *Oreochromis niloticus* (Soliman *et al.*, 1986b; Mair, 1992) and channel catfish *Ictalurus punctatus* (Gannam and Lovell, 1991).

As previously discussed, the assymetrical manifestation and the occurrence of short opercula after the larval stage in the current study, and in previous studies (Barahona-Fernandes, 1982), indicates environmental factors during ontogeny may contribute to the deformity. It has been suggested that short opercula may be the result of genetic mutation (Mair, 1992), pantothenic acid (vitamin B_3 or B_5) deficiency (Sutterlin *et al.*, 1987), vitamin C deficiency (Soliman *et al.*, 1986b), methyltestosterone treatment (Gannam and Lovell, 1991) or high rearing temperatures (Baeverfjord *et al.*, 1997). As discussed for gill deformity, vitamin deficiency may affect the integrity of the dermal tissues in which the dermal bones of the operculum are formed.

The bones of the operculum are formed by membranous secretion (Chapter 5), and their shape may change according to the structural integrity and shape of the dermal tissues in which the bone matrix is formed. Consequently, mechanical or physiological disturbance to these tissues may result in the opercula deformities observed in the current study. The inward curvature of the opercula, as seen in diploid and triploid Atlantic salmon in this study, has previously been observed in sea bream *Sparus aurata* (Galeotti *et al.*, 1997). Our findings agree with those of Galeotti *et al.*(1997), in that it appears that the shape of the abnormal opercula bone results from subsequent ossification of an abnormal opercula lamellae. Ultrastructural observations of the abnormal opercula lamellae in sea bream revealed an alteration of collagen fibrillae and fibroblasts with swollen mitochondria and disorganised cristae (Galeotti *et al.*, 1997).

It is important to note that opercula deformities may lower a fish's oxygen stress tolerance, since the subsequent reduced area of the operculum may reduce the effectiveness of the opercula and/or buccal pumps, each of which is of equal importance for generating water flow over the gills for respiratory purposes (Hughes and Shelton, 1958; Saunders, 1961; Davis and Randall, 1972). In addition, short opercula may predispose fish to parasitic attack of the gills, such as that of isopods or amoebic gill disease (AGD, Clark and Nowak, 1999), in SW. Subsequently, it is

possible that short opercula, in combination with reduced gill surface area contributes to the differential mortality of triploid fish under suboptimal conditions.

6.5.3 GFD

The pathology of gill tissue in fish affected by GFD was similar to that of fish suffering from parasitic damage; for example isopod damage (*pers comm.* Barry Munday, Reader, University of Tasmania, 1999; Nowak and Bryan, 1998), which can occur in fish under SW conditions, but not FW conditions. Given that GFD occurred in juvenile triploid Atlantic salmon early in the FW phase, and there are no known FW parasites which exist in Tasmania, it is highly unlikely that GFD occurs as a result of parasitic attack. Furthermore, the absence of primary gill filament remnants and the epithelial structure at the proximal site of missing gill filaments suggests that primary filaments never developed at these sites. Therefore we hypothesise that GFD syndrome is a developmental deformity of the gills rather than a secondary condition arising from prior gill parasite infestation.

The manifestation of GFD was often asymmetrical. Asymmetrical deformities are thought to be induced by environmental factors at the embryological or immediate post-embryological stage because the same genes determine characteristics of paired organs (Barahona-Fernandes, 1982). There is a possibility that water temperature during embryogenesis may contribute to GFD in triploid fish. Gill deformities (and other deformities) have previously been recorded in Atlantic salmon fry reared at 10° C (Baeverfjord *et al.*, 1996; 1997). Although the routine incubation temperature for eggs and fry at the SALTAS hatchery is within the optimal range (8° C), a temperature of 10° C is applied to fertilised eggs during triploidy induction. It is possible that the water temperature of the triploid treatment is a causative factor of deformity in triploid fish. In another study, diploid Atlantic salmon subject to triploid induction treatment did not display deformities; however, the temperature of the latter triploid induction treatment is unknown (Sutterlin *et al.*, 1987).

Alternatively, gill deformities affecting the branchial arches have previously been attributed to genetics (Hughes, 1993) and nutritional deficiency (Branson and Nieto, 1999) in Atlantic salmon and, more specifically to vitamin C deficiency in carp *Cyprinus carpio* (Dabrowski *et al.*, 1988). Other than genetic predisposition, nutrition is likely to impact post-embryology and as such may explain the increasing prevalence of GFD later in development, especially if it impacts on the gill arch growth zones. The ceratobranchial and epibranchial bones of the gill arch were formed by endochondral ossification (Chapter 5); that is calcium deposits are formed earliest in cartilagenous areas containing apparently normal chondrocytes and osteocytes are formed by transformation of chondrocytes in the zone of calcium deposition (Norris *et al.*, 1963). The branchial bones have cartilagenous joints (Chapter 5) and their growth is reminiscent of mammalian appendages (Norris *et al.*, 1963), in which the epiphyseal region of new bone development is at either end of the longitudinal axis of each bone, adjacent to the cartilagenous joints. New bone is formed at each epiphyseal region as the branchial bones increase in length during ontogeny (Norris *et al.*, 1963, Chapter 5), and presumably primary gill filaments, which extend along the lateral edge of these bones, are recruited and develop in conjunction with the new bone as the bones grow. Little is known about the potential recruitment of additional primary gill filaments during fish development. The incidence of GFD at later stages of development may be caused by the failure of new primary gill filaments to develop in the zone of new bone growth at the cartilagenous epiphyseal region, as the ceratobranchial and epibranchial bones increase in length. If the cause is not nutritional deficiency, some other environmental disturbance during post-embryological development may disrupt the development of new primary gill fillaments culminating in the cumulative prevalence of GFD.

The incidence of GFD did not significantly decrease the total gill surface area of afflicted triploid fish compared to that of triploid fish with apparently normal gills. This could indicate that fish with missing primary gill filaments are able to compensate by increasing the length of existing gill filaments; however, this was not assessed in the current study. Alternatively, fish with significantly reduced gill

surface area may have succumbed to natural selection for a critical gill surface area and died.

Notwithstanding this, all triploids had significantly reduced gill surface area compared to diploids, regardless of whether they had apparently normal gills or gills affected by GFD. In light of the absence of differences of blood parameters and stress physiology of diploid and triploid populations, this reduction in relative gill surface area may explain high mortality levels of triploid fish under suboptimal culture conditions, such as high water temperatures, low dissolved oxygen levels and crowding. The repercussions of reduced relative gill surface area on respiration and/or ionic regulation in triploid fish may be detrimental under traumatic conditions of exhaustive exercise, seawater transfer, or infection with AGD (Clark and Nowak, 1999) under SW conditions. It has been shown that fish regulate blood circulation in the gills, and although they do not utilise their full gill surface area under optimal pre-stress conditions, fish may require the full surface area of their gills for efficient blood gas exchange under conditions of hypoxia or physical exertion (Hughes, 1966; Booth, 1978, 1979). This phenomenon may in part explain why triploid fish which have a reduced gill surface area compared to their diploid counterparts, were subject to high mortality rates during transport and following seawater transfer in the present study, and similarly under suboptimal conditions in previous reports (Quillet *et al.*, 1987; Quillet and Gaignon, 1990; Aliah *et al.*, 1991; Johnstone *et al.*, 1991; Ojolick *et.al.*, 1995, reviewed by Benfey, 1999). Furthermore, it is possible that reduced gill surface area may affect Ca^{+} uptake via the chloride cells of the gill lamellae (Payan *et al.*, 1984). The effect of reduced gill surface area on respiration and ionic regulation in triploid fish under suboptimal conditions requires further investigation. The cause of reduced gill surface area in triploids remains unknown.

The negative correlation between SGR at each stage of development and GFD prevalence most likely reflects the decrease in SGR with development (Fig. F, Appendix F), and the concurrent increase in the prevalence of GFD with development, described in section 6.3.3, rather than a direct covariance in SGR and GFD prevalence.

6.5.4 Other deformities

Kyphosis, lordosis and scoliosis were among the other types of deformity detected in each population within this study. Spinal deformity is one of the most common types of deformity to be reported for cultured fish of various species. The heritable nature of spinal deformity in Atlantic salmon has been shown (McKay and Gjerde, 1986) and may occur in conjunction with environmental factors such as nutritional deficiencies (McKay and Gjerde, 1986; Soliman *et al.,* 1986a), the presence of toxins (McKay and Gjerde, 1986), or changes in water salinity or temperature (Vagsholm and Djupvik, 1998; Baeverfjord *et al.*, 1997).

In species other than Atlantic salmon, environmental factors such as the presence of heavy metal pollution (Bengtsson *et al.*, 1988), kepone pesticides (Couch *et al.*, 1977), trifluralin herbicide (Couch *et al.*, 1979), pathogenic organisms (Bucke and Andrews, 1985), hypervitamintosis or excess of vitamin A (Dedi *et al.*, 1995), vitamin C deficiency (Soliman *et al.,* 1986a), excessive water flow (Backiel *et al.*, 1984), sub-optimal salinity (Doroshev and Aronovich, 1974), temperature, radiation/ light, mechanical disturbance (reviewed by Hickey, 1972) or low dissolved oxygen (DO) levels (Alderdice *et al.*, 1958), have been attributed as the cause of spinal deformity. The onset of Lordosis in gilthead sea bream *Sparus aurata* (Chatain, 1994; Andrades *et al.*, 1996) and sea bass *Dicentrarchus labrax* (Daoulas *et al.*, 1991; Chatain, 1994) has been associated with the failure of swimbladder inflation, itself usually associated with suboptimal culture conditions. Further to this, it has been suggested that the skeletal form of fish afflicted with lordosis was influenced by the mechanical pressure exerted by muscle (Chatain, 1994).

Deformities observed prior to first feeding were not detected following swim up and first feeding, presumably because deformed larval fish died. A previous study which examined body deformation in hatchery reared sea bass (*Dicentrarchus labrax* L.) showed that abnormalities which appear in larval fish are lethal (from 0 to 40 days), while those which become apparent only in post-larval fish (after 40 days) do not interfere with the survival of the fish (Barahona-Fernandes, 1982).

6.5.5 Sex ratio of different deformity types

The skeletal deformities observed in this study occurred in both male and female fish and therefore cannot be the result of a sex-linked gene or physiological condition, but rather may be due to an autosomal condition (non-sex linked genotype). Other studies on Atlantic salmon have shown that deformities such as LJD (Sutterlin *et al.*, 1987; Quigley, 1995) and short opercula (Sutterlin *et al.*, 1987) can occur in both male and female fish. Similarly, opercula, spine, cranium, muscle and eye deformities detected in common carp *Cyprinus carpio* (Al-Harbi, 1999) and tilapia *Oreochromis niloticus* L.(Mair, 1992), have been shown to occur equally in male and female fish. In contrast, a higher incidence of spinal deformity in male fourhorn sculpins, *Myoxocephalus quadricornis* L., compared to female fish has been attributed to the longer near-shore occupation of male than female fish and therefore longer exposure to heavy metal pollution (Bengtsson *et al.*, 1988). Alternatively, it has been suggested that differential growth rates in male and female Atlantic salmon may contribute to the higher incidence of a congenital spinal defect in male compared to female fish (McKay and Gjerde, 1986).

6.5.6 Ploidy and skeletal deformity

The incidence of skeletal deformities in diploid and triploid Tasmanian Atlantic salmon stock may be caused by any of three possible mechanisms. Firstly, it is possible that Tasmanian Atlantic salmon are genetically prone to the aforementioned deformities since the Phillip River stock from which the Tasmanian Atlantic salmon stock originates, has a history of high deformity rates (*pers comm.* Brian Glebe, Atlantic Salmon Federation, Canada, 1999). That triploid fish displayed higher prevalence of skeletal deformity compared to diploid populations may be a result of increased heterozygosity of genes that control skeletal morphology in triploid fish (Allendorf and Leary, 1984). Secondly, deformities may result from the altered expression of genes under suboptimal environmental conditions, with modulation generated by morphogenic agents such as retinoic acid and growth factors (reviewed by Koumoundouros *et al.*, 1997; Hall and Miyake, 1995). If suboptimal

environmental conditions are the trigger for skeletal deformity then the higher susceptibility of triploid fish to deformity under standardised culture conditions indicates that triploids may have different environmental requirements to diploids. Finally, altered metabolism may contribute to the higher incidence of skeletal deformity in triploid fish. The capacity to metabolise nutrients, including the ability to absorb and utilise dietary phospholipids, vitamins A, B, C, or D and minerals such as manganese, phosphorus, magnesium and zinc, may be compromised in triploid fish due to differences in cellular morphology (reviewed by Benfey, 1999) and increased heterozygosity (Allendorf and Leary, 1984), thereby affecting skeletal development. Alternatively, depression of osteoblasts with insufficient or excessive growth and thyroid hormones may in part explain why a higher prevalence of skeletal deformity occurred in triploid and diploid Atlantic salmon smolt subject to higher growth rates (FW smolt) in the current study, since alteration of osteogenic activity could cause dissociation of development processes (Stockard, 1921) and contribute to skeletal abnormality. In addition, it is possible that the significant decrease in relative gill surface area observed in triploid fish in the current study may contribute to decreased rate of calcium uptake via the chloride cells of the gill lamellae (Wendelaar Bonga and Flik, 1991) and thereby affect osteogenic activity during an increased rate of development.

The relatively high contribution of GFD to the total deformity prevalence in triploid fish highlights the importance of this deformity in terms of the possible impact of deformity on the normal physiological function of triploid fish. In addition, acquiring suitable methods for the irradication of GFD would contribute greatly to the irradication of differential deformity prevalence in triploid fish. By comparison, short opercula was the most predominant deformity type observed in diploid fish, and although the prevalence of short opercula was comparatively higher in triploid fish, the magnitude of the difference between ploidy groups was small. If short opercula deformity impacts on the normal physiological function of afflicted fish, it is unlikely that it contributes greatly to the differential mortality between diploid and triploid fish under suboptimal conditions.

6.5.7 Summary

In summary, it has been shown that triploid Atlantic salmon are prone to a higher prevalence of skeletal deformity, particularly GFD and jaw deformity, than diploid salmon. Lower jaw deformity was occasionally detected in fish during early FW development, but was more prevalent in fish during the post-smolt stage both under FW and SW conditions, and could possibly be exascerbated by higher growth rates at this stage. The aetiology of skeletal deformity remains unclear, but it appears that the environmental requirements, particularly the nutritional requirements of triploid fish, or the ability of triploid fish to uptake and utilise nutrients, may differ to that of diploid fish and consequently may contribute to the higher incidence of skeletal deformity in triploid fish under standardised conditions. The reduced gill surface area of triploid fish may impact on either ionoregulation or respiratory efficiency under conditions of high oxygen demand and may contribute to high mortality under suboptimal culture conditions.

Chapter 7:

GENERAL DISCUSSION

7. GENERAL DISCUSSION:

7.1 BACKGROUND TO THIS STUDY

This study was instigated to address a number of concerns within the Tasmanian Atlantic salmon industry regarding the commercial production of triploid fish. The first of these concerns was the perceived susceptibility of triploid fish to stress, either during standard husbandry procedures which involve handling and crowding, or when the fish are exposed to suboptimal water conditions such as high temperature and low dissolved oxygen levels (*pers comm* Harry King, Operations manager, SALTAS Pty Ltd., Tasmania, 1996; Mick Hortle, Manager, Tassal Limited, Tasmania, 1999). To date, this perception has been based on records of high mortality and observation of altered behaviour in triploid fish. To verify the biological nature of this perception, we examined the physiological stress response of diploid and triploid Atlantic salmon subject to confinement, both during the FW and SW phases of the lifecycle. We examined both the primary endocrine stress response and the secondary respiratory haematology stress response, since it was possible that one or both of these aspects may have contributed to differential physiological function of triploid fish under stressful conditions.

The second concern which presents additional difficulty in the commercial production of triploid Atlantic salmon populations in Tasmania is the high incidence of lower jaw deformity observed during the SW phase of the life cycle (Jungawalla, 1991; Hughes, 1992). The incidence of this deformity does not appear to affect survival of afflicted fish but most likely results in ram ventilation to faciltate respiration in afflicted fish. Furthermore, the prevalence of LJD compromises the quality of harvested fish. The incidence of skeletal deformities in cultured fish is common and has been attributed to either genetic and/or environmental parameters, particularly during the period of organogenesis. Prior to this study, the incidence of LJD during early development in the FW phase of the lifecycle, had not been

recorded. Nor had there been a comparative study of skeletal development and the incidence of skeletal deformity between diploid and triploid fish under standardised conditions. In this study, we examined the time of onset of LJD and other deformities in different population types, and subsequently hypothesised possible mechanisms by which these deformities occur. The possible impact of skeletal deformities on the physiological function of afflicted fish was of particular interest, as we found that triploid fish suffer from reduced gill surface area compared to diploids.

The main premise of this study was that the differences in cellular morphology that exist between diploid and triploid fish may contribute to the differential physiological function of triploid fish, particularly with regard to stress responses, respiratory haematology and skeletal development, thereby accounting for the phenomenon observed in commercially produced triploid fish. By investigating potential differences in the physiology of diploid and triploid fish it was hoped that this study may provide suggestions as to how to improve production management procedures and the quality of triploid fish.

7.2 STRESS RESPONSES

The study of stress responses in triploid fish is novel. Prior to the commencement of the current study, the only other study which has examined the primary stress response of triploid fish is that of Biron and Benfey (1994), in which brook trout *Salvelinus fontinalis* were subject to acute handling stress under FW conditions. The current study expands upon this earlier work and examined the effect of confinement on the primary stress response in triploid salmonids under FW and SW conditions, both of which is pertinent to commercial husbandry practices (Chapter 3; Sadler *et al.*, 2000b).

In this study, changes in plasma cortisol and plasma lactate levels following confinement of diploid and triploid Atlantic salmon, both before (FW parr) or after (SW smolt) transfer to sea water did not differ significantly with ploidy status.

Confinement elevated plasma cortisol and plasma lactate from pre-stress control levels irrespective of ploidy status, at both the FW parr and SW smolt stages. The duration of confinement (1,3 or 6 hours) affected the magnitude of the plasma cortisol and plasma lactate responses in SW smolt, but within each treatment these levels did not vary with ploidy status. Furthermore, plasma cortisol levels in both diploid and triploid SW smolt subject to 2 hours of confinement, decreased to pre-stress levels within 6 hours post-confinement, although plasma lactate levels did not fully recover to pre-stress levels for the duration of the experiment (> 48 hours). The values recorded in the current study were similar to those previously recorded for diploid salmonids. The results indicated there was no significant difference in the primary stress response of Atlantic salmon with ploidy status (Chapter 3; Sadler *et al.*, 2000b), which is in agreement with the findings of Biron and Benfey (1994). Furthermore, the extent of anaerobic metabolism under the current experimental conditions was similar between diploid and triploid Atlantic salmon (Chapter 3; Sadler *et al.*, 2000b). These results were supported by those of the more detailed examination of the secondary haematological responses in diploid and triploid Atlantic salmon (Chapter 4; Sadler *et al.*, 2000a).

The findings of this study were surprising given the differential mortality of triploid fish under suboptimal commercial culture conditions and the ubiquitous perception among salmon farmers that triploids are more susceptible to stress. Furthermore, we expected there to be a differential stress response, as indicated by a difference in the magnitude or the time of onset of the plasma cortisol response due to differences in cell morphology within the endocrine and vascular tissues between diploid and triploid fish. We found a trend of delayed secretion of plasma cortisol in triploid fish compared to diploid fish; however, this was not statistically significant. Biron and Benfey (1994) reported a similar trend in an unpublished study of triploid rainbow trout *Oncorhynchus mykiss*.

7.3 RESPIRATORY HAEMATOLOGY

In the absence of a difference in the primary stress response of diploid and triploid Atlantic salmon, it was hypothesised that differences in the cellular morphology of erythrocytes may contribute to compromise aerobic capacity or respiratory efficiency in triploid Atlantic salmon under suboptimal conditions. The study of the haematology of triploids is not novel. Other authors have examined various haematological parameters, including Hct, RBCC, Hb, plasma glucose, plasma lactate and blood oxygen affinity at a single level of blood plasma pH (Graham *et al.*, 1985) in pre-stress diploid and triploid salmonids. These previous works have however provided conflicting results (reviewed by Benfey, 1999). The current study expanded upon previous studies by examining the effects of confinement on haematological parameters in diploid and triploid Atlantic salmon. In addition, we examined blood rheology and changes in blood oxygen affinity and saturation with decreased plasma pH, the latter occuring under conditions of exercise and stress (Chapter 4; Sadler *et al.*, 2000a). Despite having larger, fewer erythrocytes, under the current experimental conditions triploid Atlantic salmon SW smolt had similar blood oxygen carrying capacity, blood viscosity and haematological stress responses to diploid SW smolt.

It is possible that under extreme commercial culture conditions such as those experienced at harvest, a combination of exhaustive exercise, handling and confinement stress and hypoxia, have an additive effect such that triploid fish are physiologically compromised. Thomas (1999) found that a combined exercise and stress treatment resulted in significantly higher plasma cortisol levels in diploid Atlantic salmon, whereas an exercise or stress treatment alone, did not. Furthermore, although this study did not find any statistically significant differences in physiological parameters of stress and respiratory haematology with ploidy status, it may be that a combination of the subtle differences in various haematological parameters, and/or the significantly decreased gill surface area in triploid fish (Chapter 6) contribute to the compromised respiratory function of triploid fish under extreme culture conditions. The effects of chronic stress, hypoxia, exhaustive

exercise and reduced gill surface area on stress responses and respiration in triploid Atlantic salmon have yet to be investigated.

Alternatively, the parentage of the different experimental populations may have masked possible differences in the stress response between diploid and triploid individuals. A recent study of the immunological response of triploid rainbow trout showed that there was variation in the differential response of diploid and triploid fish according to family groups (*pers comm.* Dr. Anne Bowden, University of Glasgow, Scotland, 1999, unpublished data). Within some families produced by single male and female crosses, there were significant differences between diploid and triploid siblings, whereas in other families there was no difference between diploid and triploid siblings (*pers comm.* Dr. Anne Bowden, University of Glasgow, Scotland, 1999, unpublished data). In this study, each population consisted of progeny from an outcrossed group of broodfish (gametes from a number of males and females were pooled), and therefore there is the possibility that any potential differences between different diploid and triploid fish could be masked by infra-family variation.

Not-with-standing these possibilities, the weight of evidence in the current study suggests that whatever the cause of high mortality rates in triploid populations under suboptimal husbandry conditions, it most likely is not due to differential primary endocrine or secondary haematological stress mechanisms. Nor is it likely to be associated with blood oxygen carrying capacity or blood viscosity. The reason for differential mortality in triploid fish lies in some other physiological mechanism.

7.4 SKELETAL DEVELOPMENT AND DEFORMITY

The high incidence of skeletal deformity in triploid fish compared to diploid fish following first feeding (Chapter 6) is a strong indication that triploid fish respond to their environment in a different way to diploid fish. However, whether this is due to epigenetic factors (environmental/physiological factors which influence phenotype) or morphogenetic factors (morphological genotype) is unclear. The types of

deformity observed in fish, including short opercula, gill deformity, jaw deformity and spinal deformity, have all been reported to occur in salmonids and other species, under conditions of vitamin C deficiency, or perterbations in culture parameters during embryological development, such as water temperature. The salmon growout feed (GIBSONS) used in the current study, is formulated to provide sufficient vitamins and minerals, according to the nutritional requirements of salmonids (*pers comm.* Rhys Hawler, Researcher, Gibsons, Tasmania, 1999) and, given that environmental conditions were standardised between populations throughout development, it is likely that the physiological function of triploids, such as vitamin and mineral absorption, and/or the genetic make-up of triploid fish contributes to the higher incidence of deformity (Chapter 6). Hughes (1993) found that although there can be symptoms of vitamin C deficiency in both diploid and triploid fish, only the triploid fish will show high incidence of skeletal deformity.

If vitamin deficiency contributes to the high incidence of deformity in triploid fish, it remains unclear as to why triploid fish would absorb or utilise vitamins and minerals in a different fashion to diploids. It has previously been suggested that stress can affect vitamin C requirements (reviewed by Li *et al.*, 1998), and in view of the previously held belief that triploid fish are more susceptible to stress than diploid fish, it follows that differential stress levels could possibly contribute to vitamin and mineral deficiency in triploid fish. However, the results of the current study (Chapters 3 & 4; Sadler *et al.*, 2000a, 2000b) and those of Biron and Benfey (1994) show there is little difference in the primary physiological stress response of triploid salmonids compared to that of diploid salmonids and therefore it is unlikely that stress levels are related to the incidence of deformity. Conversely, there is the possibility that deformity contributes to differential stress levels in deformed individuals.

The high incidence of skeletal deformities in triploid populations may also contribute to the high mortality observed in these fish. Other studies have indicated that skeletal deformity, such as short opercula, jaw deformity and spinal deformity can affect the growth and survival of afflicted individuals, presumably due to the

effects on feeding and on the buccal pump used for respiration. In particular, the significantly reduced gill surface area of normal triploid fish and triploid fish affected by gill filament deformity, compared to that of normal diploid fish (Chapter 6), may compromise ionoregulation and/or respiratory gas and metabolite exchange in these fish, under conditions of high oxygen demand or low oxygen availability. This may explain previous reports that triploid populations suffer high mortality under conditions of high temperature or low dissolved oxygen levels; however, further examination of differential gill function and the possible effects of reduced gill surface area in diploid and triploid fish is required.

Comparative study of skeletal development between diploid and triploid Atlantic salmon is completely novel and offers the unique opportunity to examine the possible effect of the intraspecific difference in cell size on skeletal differentiation, morphology and ossification. Although there are studies which describe the development of various parts of the skeleton in salmonids, the synchronous development of the complete skeleton of Atlantic salmon, particularly for triploid Atlantic salmon, does not exist. This study describes the skeletal ontogeny of different populations of Atlantic salmon, which was found to be similar between populations under standardised conditions, regardless of sex or ploidy status (Chapter 5). This is surprising, since it was thought that the differences in cellular morphology between diploid and triploid fish could potentially change the temporal onset of cell condensation, chondrification and ossification, due to the potentially different cellular energetics and inter-cellular communication (Chapter 5). This investigation highlights the capacity for normal skeletal development in triploids and suggests that environmental factors may influence the phenotypic expression of skeletal deformity in triploid Atlantic salmon. In addition, the current study provides a sound reference for aquaculturalists interested in the relative development of different parts of the skeleton in Atlantic salmon.

7.5 CONCLUSIONS

The primary and secondary stress responses of triploid Atlantic salmon following confinement were not different to diploid Atlantic salmon, both under FW and SW conditions, despite differences in cellular morphology with ploidy status. Furthermore, there were few significant differences in the blood oxygen carrying capacity and blood viscosity of diploid and triploid Atlantic salmon. It is unlikely that the primary and secondary physiological stress responses and respiratory haematology contribute to any difference in the performance or mortality of triploids during management practices.

Triploid Atlantic salmon were susceptible to a higher incidence of skeletal deformity post-first feeding, which appears to be due to a differential phenotypic response to environmental factors rather than the capacity for normal skeletal development in triploid fish, since initial skeletal ontogeny and larval deformity rates were similar between diploid and triploid populations.

Triploid Atlantic salmon with either normal gills or gills affected by GFD had a reduced gill surface area index compared to that of diploid salmon and the repercussions of this phenomenon on respiration under conditions of exhaustive exercise or on ionoregulation is unknown, but may contribute to a potential difference in the performance of triploids, particularly during stressful management procedures.

Appendices

APPENDIX A

Milt extender solution

36.0 g Potasium Chloride (KCl)

9.6 g Sodium Chloride (NaCl)

2.0 g Sodium Dihydrogen Orthophosphate ($NaH_2PO_4.H_2O$)

1.2 g Magnesium Sulphate ($MgSO_4.7H_2O$)

1.2 g Calcium Chloride ($CaCl_2$)

5.0 g Sodium Hydrogen Carbonate ($NaHCO_3$)

5.0 g Glucose ($C_6H_{12}O_6$)

Dissolve in de-ionised water and make up to 5 litres.

Milt Activator solution

45.0 g Sodium Chloride (Na Cl)

6.0 g Tris (Hydroxymethyl) Methylamine

4.0 g Glycine (Aminoacetic acid)

Dissolve in de-ionised water and make up to 9 litres.

APPENDIX B

Table A. Mean (± SE) total wet weight (TWWt.) of four Atlantic salmon populations; all-female diploids (FD), mixed sex diploids (MD), all-female triploids (FT) and mixed sex triploids (MT), at different stages of development. Accumulated temperature units (ATU = ° days) represent development post-fertilisation. * denotes significant difference between diploid and triploid populations at each stage, ^ denotes significant difference between all-female and mixed sex populations at each stage, "4810SW" indicates SW smolt, "4928FW" indicates smolt maintained in FW, n = 20 - 200 according to Table 2 (Chapter 2).

ATU (° days)	FD	MD	FT	MT
772	0.16 ± 0.004	0.16 ± 0.005	0.15 ± 0.003	0.15 ± 0.004
843	0.17 ± 0.003	0.17 ± 0.005	0.17 ± 0.005	0.17 ± 0.004
913	0.20 ± 0.002*	0.20 ± 0.003*	0.18 ± 0.0025	0.17 ± 0.003
1510	1.05 ± 0.027*	0.99 ± 0.023*	0.85 ± 0.02	0.82 ± 0.025
2350	6.23 ± 0.16 *	6.53 ± 0.15*	5.55 ± 1.41	5.45 ± 0.124
3118	27.49 ± 0.56	29.36 ± 0.53^	30.58 ± 0.55*	31.57 ± 0.58*^
4026	57.99 ± 3.36	55.55 ± 1.09	57.71 ± 1.4	63.02 ± 2.08
4202	67.83 ± 1.25	66.56 ± 1.03^	65.04 ± 1.17	72.07 ± 1.16^
4810SW	91.0 ± 1.94*	87.3 ± 1.81*	78.69 ± 1.97	86.66 ± 1.98
4928FW	157.21 ± 2.17*	153.54 ± 2.1*	135.41 ± 1.82	141.7 ± 2.39

Table B. Mean (± SE) fork length (FL) of four Atlantic salmon populations; all-female diploids (FD), mixed sex diploids (MD), all-female triploids (FT) and mixed sex triploids (MT), at different stages of development. Accumulated temperature units (ATU = ° days) represent development post-fertilisation. * denotes significant difference between diploid and triploid populations at each stage, ^ denotes significant difference between all-female and mixed sex populations at each stage, "4810SW" indicates SW smolt, "4928FW" indicates smolt maintained in FW, n = 20 - 200 according to Table 2 (Chapter 2).

ATU (°days)	FD	MD	FT	MT
772		2.75 ± 0.02		2.76 ± 0.02
843	2.77 ± 0.02	2.78 ± 0.03	2.82 ± 0.02	2.79 ± 0.02
913	2.9 ± 0.01^	2.86 ± 0.01	2.87 ± 0.01^	2.86 ± 0.01
1510	4.61 ± 0.04*	4.6 ± 0.03*	4.36 ± 0.04	4.37 ± 0.05
2350	8.05 ± 0.07*	8.14 ± 0.06*	7.83 ± 0.06	7.7 ± 0.06
3118	12.86 ± 0.09	13.3 ± 0.10^	13.36 ± 0.09*	13.53 ± 0.09*^
4026	16.87 ± 0.33	16.76 ± 0.11	17.07 ± 0.13*	17.52 ± 0.16*
4202	17.94 ± 0.14	18.04 ± 0.12^	18 ± 0.12*	18.49 ± 0.10*^
4810SW	20.63 ± 0.12*	21.24 ± 1.0*	20.4 ± 0.13	20.86 ± 0.12
4928FW	24.39 ± 0.10*	24.38 ± 0.11*	23.91 ± 0.09	24.1 ± 0.13

APPENDIX C

Cortland's physiological saline solution (Wolf, 1963)

Stock solution A (1 L)

72.5 g Sodium chloride (NaCl)

2.3 g Calcium chloride ($CaCl_2$)

3.8 g Potassium chloride (KCl)

4.1 g Sodium dihydrogen orthophosphate ($NaH_2PO_4.H_2O$)

2.03 g Magnesium chloride ($MgCl_2.6H_2O$)

2.3 g Magnesium sulphate ($MgSO_4.7H_2O$)

Stock solution B (1 L)

10.0 g Sodium carbonate ($NaCO_3$)

Cortland's Solution (1L)

100 ml Solution A

100 ml Solution B

800 ml Distilled water (DW)

1 g Glucose

1 g Bovine Serum Albumin

APPENDIX D

Taylor's double staining and clearing technique (Taylor and Van Dyle, 1985) with modifications

1) Fixation:

- Fix fresh material in 10% buffered neutral formalin (10 × volume of sample for 2 days or longer).

2) Dehydration:

- Transfer specimens to 30%, 50%, then 70% ethanol (specimens can be stored in 70% Ethanol)
- Further dehydrate in 95% ethanol, then 2 changes of 100% ethanol for 1 - 4 hours each. Leave in 100% ethanol for 1 - 2 days, depending on the specimen size.
- Note: Eviscerate specimens as much as possible. Ensure complete dehydration for effective alcian blue staining.

3) Staining with Alcian blue:

- Add 0.3g alcian blue to mixture of 400ml glacial acetic acid : 600ml ethanol (Solution has shelf life of 3 - 4 weeks) OR Acid free method is advised if calcium loss is to be avoided (Scott, 1985): 1% alcian blue in 0.15 M sodium acetate with 0.05 M magnesium chloride.
- Place specimens directly in alcian blue solution (10 × volume of specimen for 1 - 2 days or until specimen fully stained)

4) Neutralisation:

- Transfer specimens to 1% potassium hydroxide (KOH) (50 × volume of specimen) for 1 - 2 days. Change solution at end of 1st day if specimens are large.

5) Bleaching:

- Bleach in a solution of 1 part 3% hydrogen peroxide (H_2O_2) and 9 parts 1% KOH solution (10 × volume of specimen) OR 1 part 30% H_2O_2 and 9 parts 4% KOH (for large specimens). Make solution fresh from stock solutions.
- Place specimens (in bleach solution) in sun-light until heavily pigmented areas are pale. Small specimens take ≥ 2 hours.

6) Clearing:

- Enzyme buffer solution = 3 parts saturated sodium borate ($Na_2B_4O_7.H_2O$) and 7 parts DW.
- Add 1g trypsin (≈ 0.5 teaspoon) to 1 L enzyme buffer solution. Stir gently to dissolve.
- Immerse bleached specimens in enzyme solution (10 - 40 × volumeof specimens) at 20 - 30°C. Change solution every 2 days until clear. Small specimens take ≥ 2 days.

7) Staining with Alizarin Red:

- Add Alizarin red to 1% KOH until KOH turns a deep purple.
- Transfer enzyme treated specimens to Alizarin red solution until bone structures are stained (1 - 2 days).

8) Final clearing:

- Rinse Alizarin red specimens in DW to clear OR, soak in enzyme buffer solution if there is still protein to be digested (step 6).

9) Glycerin storage:

- Work specimens through a glycerin series (40% glycerin in 1% KOH, 70% glycerin in 1% KOH, 100% glycerin)
- Store specimens in pure glycerin to which a few crystals of thymol have been added (Thymol inhibits growth of moulds and bacteria).

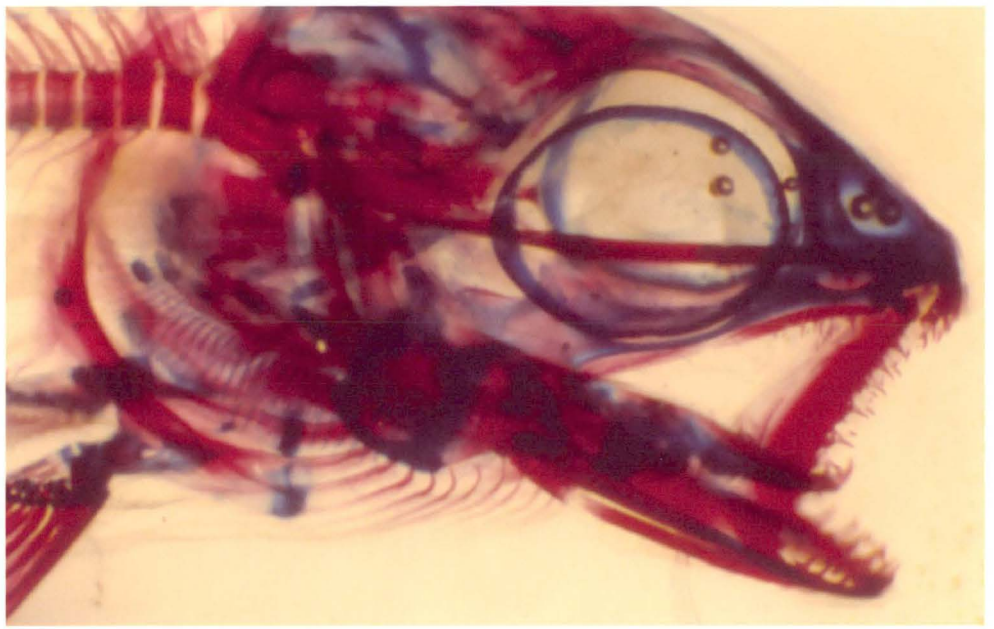
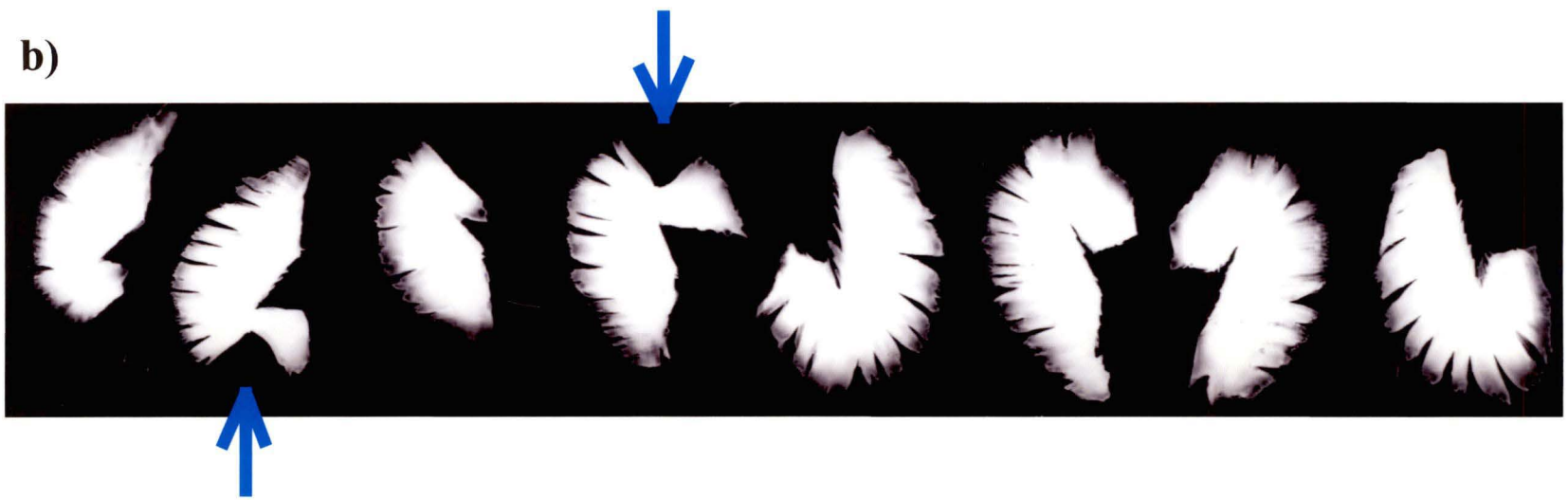


Figure A. Lateral view of the cranium of a stained and cleared (Taylor and Van Dyke, 1985) Atlantic salmon fry.

Figure A. Photographic contact print of the full complement of branchial arches dissected from (a) a normal Atlantic salmon SW smolt and (b) an Atlantic salmon smolt afflicted with gill filament deformity syndrome (GFD). Arrows denote regions of missing primary gill filaments.

a)

b)



APPENDIX F

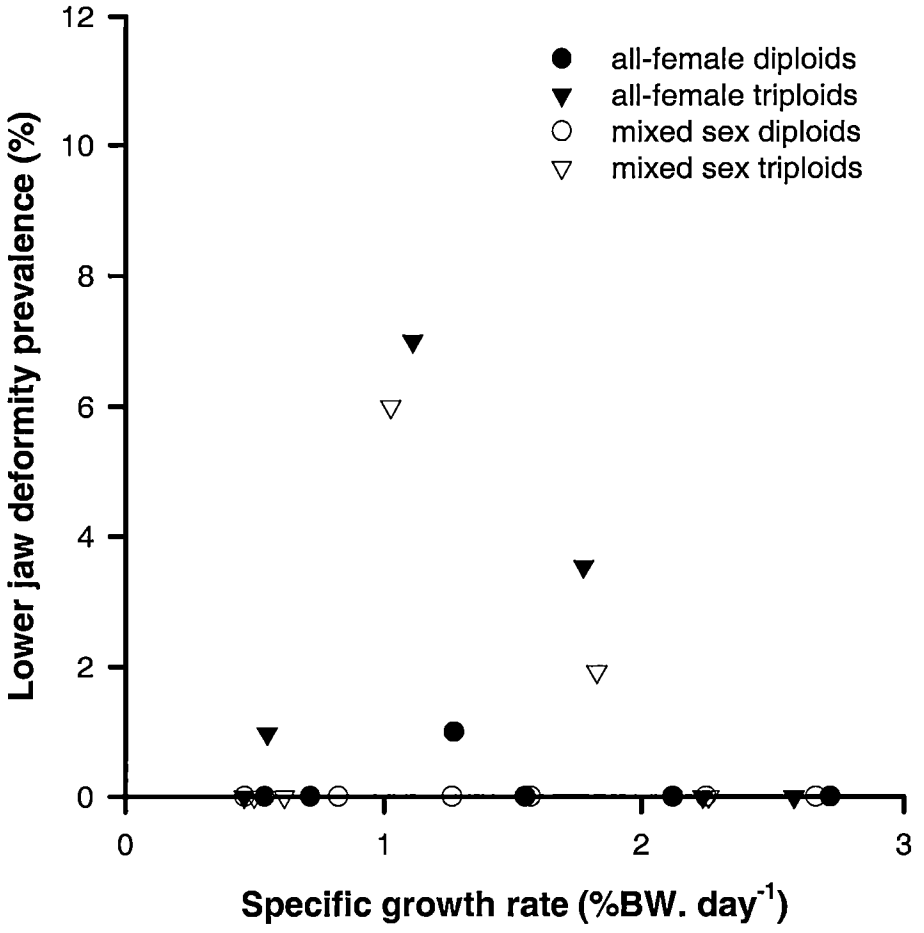


Figure A . Variation in prevalence of lower jaw deformity at each stage of development with specific growth rate of each population at each stage.

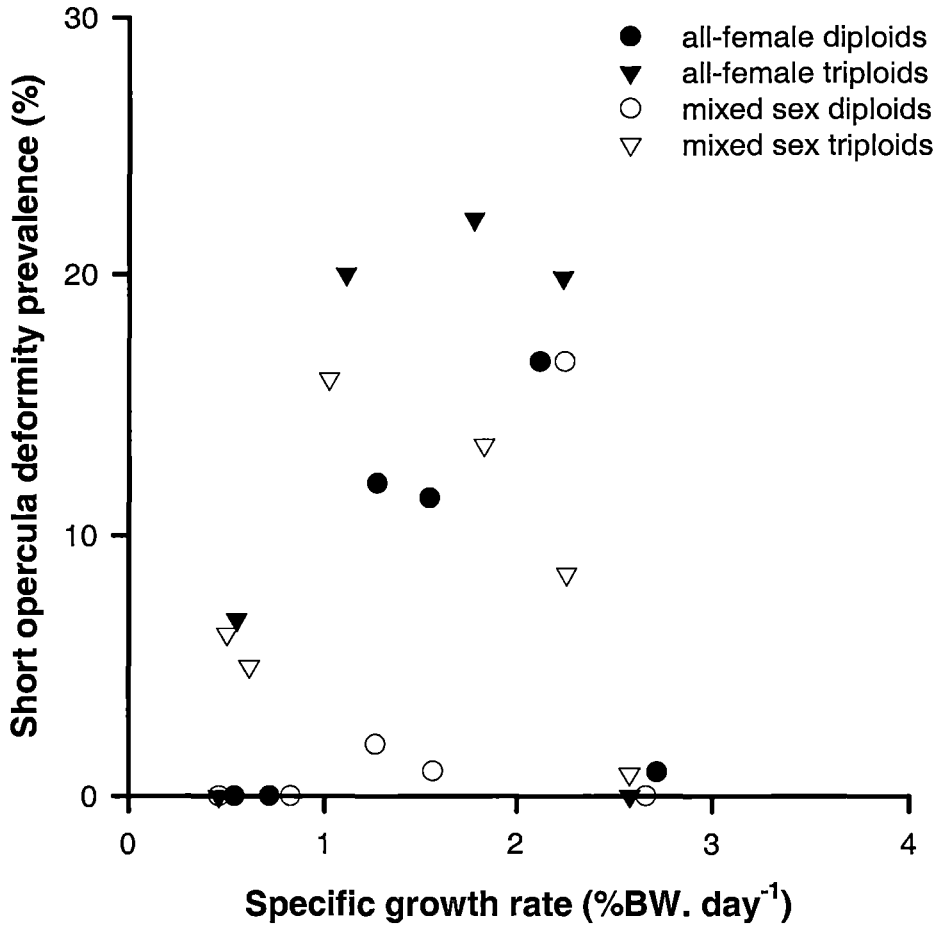


Figure B. Variation in prevalence of short opercula at each stage of development with specific growth rate of each population at each stage.

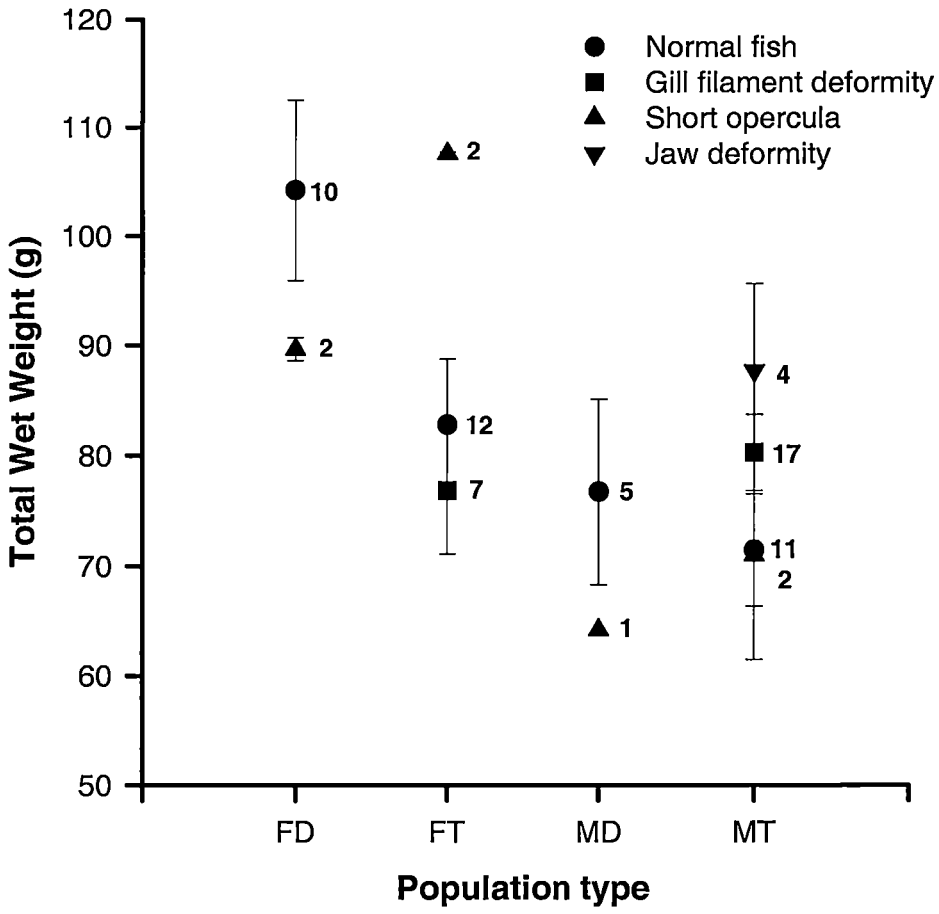


Figure C. Mean total wet weight (± SE) of SW smolt (4894° days) affected by gill filament deformity (GFD), short opercula or jaw deformity and normal SW smolt from four populations, all-female diploids (FD), all-female triploids (FT), mixed sex diploids (MD) and mixed sex triploids (MT). Superscripts denote sample numbers.

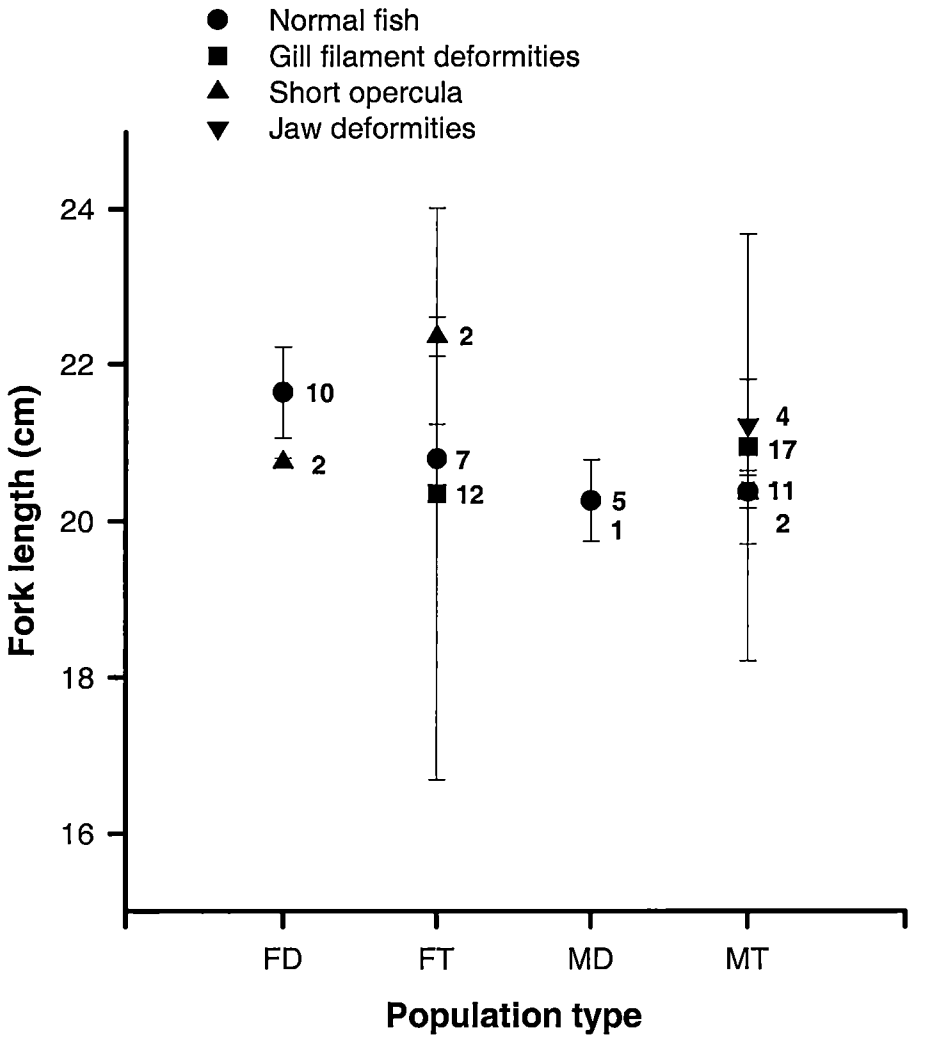


Figure D. Mean fork length (± SE) of SW smolt (4894° days) affected by gill filament deformity (GFD), short opercula or jaw deformity and normal SW smolt from four populations, all-female diploids (FD), all-female triploids (FT), mixed sex diploids (MD) and mixed sex triploids (MT). Superscripts denote sample numbers.

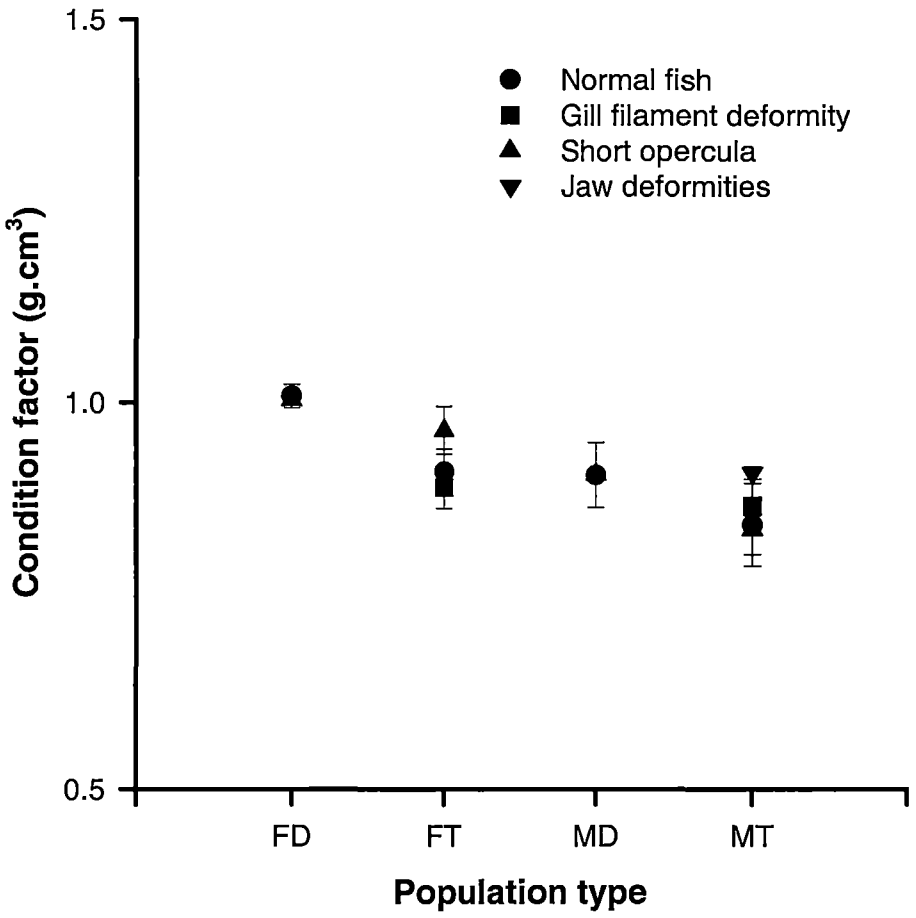


Figure E. Mean condition factor (± SE) of SW smolt (4894° days) affected by gill filament deformity (GFD), short opercula or jaw deformity and normal SW smolt from four populations, all-female diploids (FD), all-female triploids (FT), mixed sex diploids (MD) and mixed sex triploids (MT). Sample numbers as per Figs C & D.

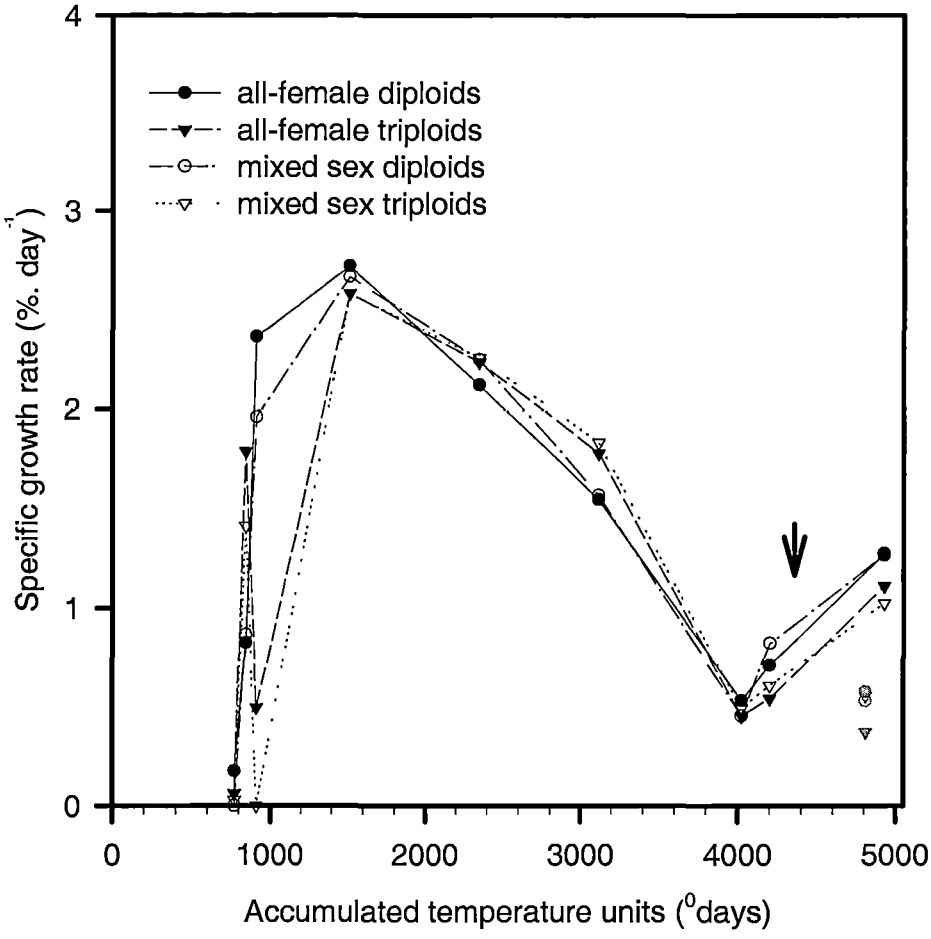


Figure F. Specific growth rates of four Atlantic salmon populations; all-female diploids, all-female triploids, mixed sex diploids and mixed sex triploids, during development in freshwater (FW) and seawater (SW). Accumulated temperature units (ATU = ° days) represent development post-fertilisation. Black symbols denote parr and smolt held in FW. Grey symbols denote smolt held in SW. Arrow indicates time of SW transfer.

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