UNIVERSITÉ DU QUÉBEC À RIMOUSKI

# CHANGEMENTS ONTOGÉNÉTIQUES CHEZ L'OMBLE DE FONTAINE (*Salvelinus fontinalis*) DANS DEUX HABITATS DISTINCTS

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## INTÉGRATION MORPHOLOGIQUE, VARIANCE DE LA FORME ET PROGRÈS DEVELOPPEMENTAL

MÉMOIRE
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PAR
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> “The process of learning is essential to our lives. All higher animals seek it deliberately. They are inquisitive and they experiment. An experiment is a sort of harmless trial run of some action which we shall have to make in the real world; and this, whether it is made in the laboratory by scientists or by fox-cubs outside their earth. The scientist experiments and the cub plays; both are learning to correct their errors of judgment in a setting in which errors are not fatal. Perhaps this is what gives them both their air of happiness and freedom in these activities.“
>
> - J., Bronowski. The Common Sense of Science (Vintage), p. 111.

# RESUME

L'évolution d'un phénotype complexe peut être contrainte ou facilitée par l'intégration morphologique. L'omble de fontaine (*Salvelinus fontinalis)* présente une divergence phénotypique subtile générée par la génétique, la locomotion et l'alimentation, ce qui en fait un excellent modèle pour l'étude de la plasticité développementale et autres processus évolutifs. La majorité des études ayant porté sur l'intégration morphologique ont trouvé que cette dernière est dynamique chez les organismes en développement. Lors de l'ontogénie, les changements morphologiques complexes et coordonnés qui se produisent dans les diverses parties du corps sont influencés par l'intégration. Pour déterminer de quelle façon les conditions environnementales locales influencent l'intégration morphologique et le développement chez l'omble de fontaine provenant de deux habitats distincts (lac et ruisseau), nous avons étudié le niveau et la structure de l'intégration, la variance de la forme ainsi que le déclenchement de l'ossification des éléments des nageoires pectorales et médianes. L'ossification de la majorité des éléments se produit avant que les jeunes ombles atteignent une longueur standard de 30 mm, et l'ossification de ces éléments a lieu à une taille significativement plus petite chez les ombles de ruisseau. Il est possible d'observer deux patrons ontogénétiques distincts en ce qui concerne le niveau d'intégration, la variance de la forme et la structure de la variation. Au cours de l'intervalle d'ossification rapide, l'intégration morphologique est relativement faible et en diminution, spécialement chez les ombles de ruisseau, et la variance diminue de moitié. Lorsque l'ossification est entamée dans presque tous les éléments des nageoires pectorales et médianes, le niveau d'intégration augmente et la variance se stabilise. Tout au long de ces changements parallèles de l'intégration et de la variance, on observe une modification graduelle et une convergence de la structure de la (co)variation entre les deux habitats. L'ossification plus hâtive des nageoires pectorales et médianes observée chez les ombles de ruisseau est probablement due à la plus importante demande locomotrice. La présence de deux patrons ontogénétiques distincts en ce qui concerne le niveau d'intégration, la variance de la forme et la structure de la variation met en lumière la complexité de ces réponses. Ces résultats soulignent l'importance de mieux comprendre les changements morphologiques complexes et coordonnés qui se produisent depuis le tout début de l'ontogénie et la façon dont ces derniers sont influencés par les conditions environnementales au cours du développement.

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| **CODE** | **SIGNIFICATION** |
|---|---|
| Apr2-12 | Anal proximal radials 2-12 |
| CS | Centroid size |
| darcPU3-5 | Dorsal arcocentra of preural centra 3-5 |
| Dpr1-13 | Dorsal proximal radials 1-13 |
| E1-2 | Epurals 1-2 |
| GLS | Generalized least squares procrustes superimposition |
| H1-6 | Hypurals 1-6 |
| HAPU1-5 | Haemal arch of preural centra 1-5 |
| HSPU4-5 | Haemal spine of preural centra 4-5 |
| LPpr1-4 | Left pectoral proximal radials 1-4 |
| NAPU1-5 | Neural arch of preural centra 1-5 |
| NSPU3-5 | Neural spine of preural centra 3-5 |
| PC | Principal component |
| PCA | Principal component analyses |
| PU1-5 | Preural centra 1-5 |
| RPpr1-4 | Right pectoral proximal radials 1-4 |
| SL | Standard Length |
| $SL_{50}$ | Standard Length at which 50% of the specimens are ossified for a given element. |

# INTRODUCTION

La variation présente à l'intérieur d'une population est un thème important en biologie évolutive parce qu'elle est fondamentale pour l'évolution. La variation phénotypique rend possible la sélection alors que la variation génétique lui permet de prendre effet. La variation est le matériel de base sur lequel la sélection peut agir et sa structure peut influencer le résultat de la sélection.

Les études sur la variation utilisent souvent la variance comme mesure alors que les comparaisons de la structure de cette variation peuvent être comparées à l'aide de différentes méthodes apparentées à l'analyse en composante principale à partir de matrices de variance-covariance (Zelditch et al., 2004). La structure de la variation peut aussi être analysée dans un contexte d'intégration morphologique. Cette dernière se manifeste par une corrélation entre les traits phénotypiques et c'est la correspondance entre les éléments du phénotype, générée par les interactions fonctionnelles et/ou développementales entre ces éléments, qui fait référence à l'intégration morphologique (e.g., Olson et Miller, 1958; Cheverud, 1982; Zelditch, 1988). L'évolution d'un phénotype complexe peut donc être modifiée par l'intégration morphologique puisque cette dernière influence la variation soumise à la sélection par le système de développement.

Puisque l'intégration résulte de la nature hiérarchique des processus de développement (West-Eberhard, 2003) et que ces processus varient lors de l'ontogénie, on s'attend à ce que l'intégration varie dans le temps. De fait, les études documentant la dynamique ontogénétique de l'intégration (e.g. Atchley et Rutledge, 1980; Zelditch, 1987, 1988; Zelditch et Carmichael, 1989; Badyaev et Martin, 2000; Ackerman, 2005; Ivanovic et al., 2005; Willmore et al., 2006; Young, 2006; Zelditch et al., 2006) ont majoritairement trouvé que la structure de la variation se modifie chez les organismes en développement.

La corrélation entre les traits phénotypiques peut non seulement être variable au cours de l'évolution et du développement, elle est aussi capable de répondre à certains changements des conditions environnementales. La plasticité phénotypique peut être considérée comme la propriété de traits individuels, par contre, en fonction des conditions environnementales, l'expression d'un trait peut être corrélée avec celle d'autres traits, ce qui modifie l'intégration morphologique (Schlichting et Pigliucci, 1998). L'intégration morphologique peut être stable si les traits présentent toujours le même patron de corrélation entre eux. Par contre, il est aussi possible qu'elle soit variable si les conditions environnementales modifient le patron de corrélation entre les traits. Les conditions environnementales locales pourraient donc influencer la structure de la variation. On s'attendrait donc à trouver plusieurs études documentant les différences entre des populations ou des espèces apparentées quant au niveau et à la structure de l'intégration. Par contre, la majorité de ces études ont plutôt trouvé une stabilité de l'intégration entre les populations ou les espèces apparentées (Merila et

Bjorklund, 1999; Ackerman et Chevrud, 2000; Marroig et Cheverud, 2001; Young et Badyaev, 2006).

L'objectif principal de ce projet est de fournir une description de la variation et de sa structure par la mesure de la variance de la forme ainsi que du niveau et de la structure de l'intégration au cours de l'ontogénie chez les jeunes de l'année de l'omble de fontaine (*Salvelinus fontinalis*) provenant de deux habitats distincts : Lac et ruisseau. Chez les poissons du genre *Salvelinus*, de la plasticité ainsi qu'une importante variabilité morphologique, écologique et génétique font de ces espèces un excellent modèle pour l'étude de la plasticité développementale et autres processus évolutifs (Magnan et al., 2002). L'omble de fontaine présente une divergence phénotypique et comportementale entre des formes alternatives limniques et benthiques, en lac (Venne and Magnan, 1995) et en ruisseau (McLaughlin et Grant, 1994), qui reproduit fidèlement la distinction limnique/benthique retrouvée de façon répétée chez les téléostéens en lac (Robinson et Parsons, 2002). Les études expérimentales ainsi que les observations en milieu naturel suggèrent que la locomotion et l'alimentation, jumelées ou non avec la divergence génétique, sont nécessaires à l'induction et au maintien du polymorphisme trophique (Proulx et Magnan, 2004).

Les études antérieures ayant porté plus spécifiquement sur les adaptations aux conditions hydrodynamiques chez les salmonidés ont documenté des changements de taille et de forme qui sont directionnels (e.g. Imre et al., 2001, 2002; Pakkasmaa et Piironen, 2001; Peres-Neto et Magnan, 2004; Grünbaum et al., 2007) ou non-directionnels (Grünbaum et al., 2007). De plus, les adaptations aux conditions hydrodynamiques incluent aussi des systèmes internes comme le squelette. La fonction mécanique du système musculo-squelettique est la création du mouvement et la transmission des charges (Prendergast, 2002). Les os sont des tissus vivants, dynamiques, qui croissent et changent de forme tout en constituant la charpente du corps chez les vertébrés et ils se remodèlent de façon à s'adapter aux charges mécaniques auxquels ils sont soumis (Wolf, 1892, Herring, 1993). Au cours de l'ontogénie, la différentiation des tissus est régie à la fois par des facteurs génétiques et par des facteurs épigénétiques comme les stimuli mécaniques (Herring, 1993). En particulier, chez l'omble chevalier (*Salvelinus alpinus*), une forte vélocité du courant accélère la formation des os et modifie les patrons d'ossification (R. Cloutier, comm. pers.).

Puisque la vélocité du courant influence à la fois la morphologie externe et interne, il est naturel qu'elle modifie aussi le niveau et la structure de l'intégration. Peres-Neto et Magnan (2004) se sont penchés sur cette question et ils ont trouvé une diminution du niveau d'intégration morphologique en fonction des conditions hydrodynamiques. À l'exception de Grünbaum et al. (2007), les études concernant les effets de la vélocité du courant sur la morphologie et l'intégration chez les poissons ont porté sur des spécimens juvéniles ou adultes et n'ont pas adressé l'ontogénie de ces changements. Au cours du développement, les changements morphologiques complexes et coordonnés qui se produisent dans les diverses parties du corps sont influencés par l'intégration (Zelditch et Fink, 1995). Une description précise de l'intégration est essentielle afin de trouver une explication épigénétique aux trajectoires ontogénétiques ainsi que pour explorer la relation entre le développement et l'évolution (Zelditch et al., 1992).

En plus d'analyser le niveau et la structure de l'intégration, cette étude estime le progrès développemental par la détermination du déclenchement de l'ossification des éléments des nageoires pectorales et médianes (dorsale, anale et caudale). Une ossification plus rapide est attendue chez les ombles de ruisseau à cause de l'effet de la vélocité du courant. En lac et en ruisseau, une diminution de la variance de la forme au cours de l'ontogénie est prévue puisque, avec le ralentissement du développement, même de larges différences dans le degré de maturité développementale n'auront qu'un faible impact sur le phénotype. Une plus grande coordination de la locomotion devrait générer de plus fortes corrélations entre les caractères fonctionnellement liés, ce qui élèverait le niveau d'intégration. L'intégration devrait donc augmenter au cours de l'ontogénie et les ombles de ruisseau devraient montrer une plus forte intégration à cause de la demande locomotrice.

# CHAPITRE PREMIER

## ONTOGENETIC CHANGES IN BROOK CHARR (*SALVELINUS FONTINALIS*) FROM TWO CONSTRASTING HABITATS

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## MORPHOLOGICAL INTEGRATION, VARIANCE OF SHAPE AND DEVELOPMENTAL PROGRESS

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## 1.1 SUMMARY

Evolutionary change in complex phenotypes can be both facilitated and constrained by morphological integration. Brook charrs (*Salvelinus fontinalis*) present a trophic polymorphism generated by swimming demands for feeding and locomotion that makes them a good model system for studies of developmental plasticity and other evolutionary processes. Previous studies have shown that integration is a dynamic feature in developing organisms and that the complex and congruent ontogenetic morphological changes arising in diverse parts of the body are integrated. To determine whether local environmental factors affect integration and developmental rate in brook charr yearlings from two distinct habitats (lake and stream), we examine timing of ossification of pectoral and median fin elements, as well as measure the level and structure of morphological integration and the variance of body shape. Most ossification events occur before yearlings reach 30 mm, and those occur at a significantly smaller size in the stream habitat. During the interval in which those events occur rapidly, morphological integration tends to be relatively low and decreasing, especially in the stream samples, while variance of shape decreases by half. When the onset of ossification is nearly complete, integration increases and variance stabilizes. Throughout these parallel changes in variance and integration, there is a gradual and convergent change in the structure of (co)variation between habitats. The existence of two distinct ontogenetic patterns related to the level and structure of integration as well as to variance highlights the complexity of these responses. The first pattern is evident during the period of intense onset of ossification events while the second pattern can be observed when the onset of ossification is nearly complete. We interpret these patterns as being a result of both a change in the rate of developmental progress as well as in the effect of locomotion on the muscular and the skeletal systems. Stream fish ossify at a smaller size and this could be related to a more important swimming demand. These results highlight the importance of better understanding the complex and congruent morphological changes arising from early ontogeny and in which way these are influenced by environmental conditions during growth.

## 1.2 INTRODUCTION

Phenotypic variation within populations provides the opportunity for selection to act, and genetic variation enables natural selection to effect change. Therefore, variation is the raw material on which selection acts, and its structure can influence the outcome of selection.

Studies of variation use variance as a metric while the comparison of the structures of variation can be compared by various methods related to principal component analysis of variance–covariance matrices (Zelditch et al. 2004). The structure of integration can also be explored in the context of morphological integration. Morphological integration is manifested by the correlation among traits and is signified by the interdependence among traits caused by developmental and/or functional interactions among them (e.g., Olson and Miller 1958; Cheverud 1982; Zelditch 1988). Evolutionary change in complex phenotypes can be biased by morphological integration because that integration influences whether selectively useful variation can be generated by the developmental system.

Integration results from the hierarchical structure of developmental processes, meaning that each trait is a decomposable mosaic of parts that are semi-independent in their regulation and function (West-Eberhard 2003), and is likely to be temporally dynamic because processes responsible for integrating the phenotype change over time. Studies documenting ontogenetic dynamics of integration have found that the structure of variation is not a static feature in developing organisms (Atchley and Rutledge 1980; Zelditch 1987, 1988; Zelditch and Carmichael 1989; Cane 1993; Badyaev and Martin 2000; Ackerman 2005; Ivanovic et al. 2005; Willmore et al. 2006b; Young 2006; Zelditch et al. 2006). The structure of variation could also be influenced by adaptation to local environmental factors. Therefore, we might expect to find differences among ecologically diversified populations but most studies have found that the structure of integration differs little if at all (e.g. Merila and Bjorklund 1999; Ackerman and Chevrud 2000; Marroig and Cheverud 2001; Young and Badyaev 2006).

The main objective of this study is to describe variation by measuring variance of body shape as well as overall level and structure of integration found through ontogeny in brook charr (*Salvelinus fontinalis*) yearlings from two contrasting habitats (i.e. Lake and Stream). The morphological, ecological and genetic variability and plasticity found within species of the charrs genus *Salvelinus* make them a good model system for studies of developmental plasticity and other evolutionary processes (Magnan et al. 2002). Brook charrs present a behavioral and phenotypic divergence between limnetic and benthic alternatives phenotypes for lake (Venne and Magnan 1995) and stream-dwelling fishes (McLaughlin and Grant 1994) that closely mimics the trophic polymorphism repeatedly found in various lacustrine teleosts (Robinson and Parsons 2002). Experimental and field observations suggest that swimming demands for both feeding and locomotion and/or a different genetic background are necessary in the induction and maintenance of this trophic polymorphism (Proulx and Magnan 2004).

Previous studies focusing on adaptations to swimming demand in salmonids have documented directional (e.g., Imre et al. 2001, 2002; Pakkasmaa and Piironen 2001; Peres-Neto and Magnan 2004; Grünbaum et al. 2007) and non-directional (Grünbaum et al. 2007) changes in body size and shape. In addition, swimming demand influences the development of internal structures such as the skeletal system (R.Coutier, pers. comm.). The musculo-skeletal system serves the mechanical function of creating motion and transmitting loads (Prendergast 2002). Bone provides the framework for the vertebrate body and is a living, dynamic tissue that is constantly in flux by growing and undergoing remodeling. It remodels adaptively in response to the mechanical loading regime it faces (Wolf 1892, Herring 1993). During ontogeny, tissue differentiation is regulated by both genetic factors and by epigenetic factors, such as mechanical stimuli. The effect of swimming demand on patterns and timing of ossification has been examined for Arctic charr (*Salvelinus alpinus*) and ossification events occur earlier at high water velocities (pers. obs., R.Cloutier).

Swimming demand generates external and internal morphological changes so we would anticipate that it should also affect the overall level and structure of integration. Peres-Neto and Magnan (2004) reared brook charr and Arctic charr (*Salvelinus alpinus*) yearlings for a period of six months under four water velocities, finding that the level of integration was negatively associated with water velocity for Arctic charr but for brook charr, the highest level of integration was found in fishes reared at medium water velocity. With the exception of Grünbaum et al. (2007), studies on morphological integration as well as on size and shape changes in reaction to swimming demand have all focused on juvenile or adult specimens and have not addressed the ontogeny of these changes. The complex and congruent internal and external morphological changes arising in diverse parts of the body are influenced by integration (Zelditch and Fink 1995) and a precise description of integration is crucial both for finding appropriate epigenetic explanations for ontogenetic trajectories and for exploring the relationship between development and evolution (Zelditch et al. 1992).

In addition to analyzing variance and integration of body shape, we also evaluate developmental progress, measured using ossification events of pectoral and median fins supports (anal, dorsal and caudal). We expect earlier ossification for stream-dwelling fishes because of the effect of swimming demand. Variance of shape should decrease through ontogeny in both habitats because, as developmental progress slows down, even large differences in degree of maturity will have small phenotypic effects due to a gradual deceleration in overall rates of development. Greater co-ordination in locomotion is expected to generate stronger correlations among characters that are functionally related, increasing the level of integration. This implies that integration is expected to increase through ontogeny but also that Stream fish should display stronger integration because of the effect of swimming demand.

## 1.3 MATERIALS AND METHODS

### 1.3.1 SAMPLE

Wild-caught brook charr (*Salvelinus fontinalis*) yearlings were sampled from two separate sites located in the Matapedia Valley, eastern Québec, Canada. The first site, North Gunn Creek is a tributary of the Causapscal River (48° 32'N, 67° 07'W). The second study site, Casault Lake (48° 29'N, 67° 09'W; 414 ha) is a highly productive lake emptying in to the Causapscal River; sampling was restricted to the littoral zone. Both Casault Lake and North Gunn Creek are connected to the Causapscal River (approximate distance of 14 km). ). It is clear that Gunn Creek and Casault Lake differ in more that just water velocity but for the sake of simplicity, Casault Lake will be referred to as "Lake" while Gunn Creek will be referred to as "Stream". Data collection started May 18th 2005 in the Lake habitat and May 23rd 2005 in the Stream habitat and ended July 21st 2005 at both sites. Sampling was carried out daily before June 21st (10 specimens per day) and at weekly intervals thereafter (25 specimens per week) in both habitats.

Specimens were euthanized (saturated carbon dioxide solution), fixed in 5% neutral formaldehyde for 48 hours and conserved in 70% ethanol.

Immediately following each capture, the site was marked and a multiparameter sampling system (150 MDS, 600QS YSI®) was used for measurement of temperature (C°), dissolved oxygen (mg $l^{-1}$) and pH. Water velocities (cm/s) were measured using a SONTEK handheld acoustic Doppler velocimeter (SonTek Handheld FlowTracker ADV, San Diego, CA) which provide reliable and accurate water velocity measurements for low velocity values (e.g., <3 cm/s). Additional measurement of water depth (cm) and characterization of substrate type were done.

### 1.3.2 OSSIFICATION SEQUENCE

A total of 198 specimens were cleared and doubled stained with Alcian blue for cartilage and Alizarine Red S for bone (Dingerkus and Uhler 1977). A structure was considered to be cartilaginous when it uptaked Alcian blue and ossified when it uptaked Alizarine Red S. These specimens were used to validate 5-mm size classes in terms of their representation of skeletal development as well as to quantify developmental progress with respect to the pectoral, anal, dorsal and caudal fins. Age being unknown for these wild-caught brook charrs, we use standard length (SL) as a *proxy* for biological time (Adriaens and Verraes 2002). SL was measured using the distance between the tip of snout and posterior end of hypurals landmarks. There were three possible developmental states for each endoskeletal element; 0: absence, 1: cartilage and 2: bone. Only the transition between cartilage and bone was used because the appearance of cartilage was heavily influenced by the size of smaller specimens, whereas ossification was well represented in our sampling range.

We used a logistic regression to describe the relationship between the continuous predictor variable (SL) and the random component (developmental states for each element) in order to estimate the SL at which 50% of the specimens was ossified for a given element in each habitat ($SL_{50}$). The significance of logistic regressions was tested using the $G^2$ statistic, also termed the likelihood ratio $\chi^2$ statistics, which is more robust than the Wald *t* (Quinn and Keough 2002). Out of the 142 skeletal elements of the pectoral and median fins initially included, only 66 elements (figures 3, 4) for which the logistic model was significant ($P \leq 0.05$) were considered for analysis of developmental progress. The main effect of habitat type was tested using a permutation paired *t*-test in which the $SL_{50}$ was randomly reassigned 9999 times within each of the 66 elements (block) and for which the *t* statistic was computed each time. The *p*-value of the two-tailed test is two times the proportion of randomized samples exterior to the *t* value of the sample. Logistic regression and permutation paired *t*-test were programmed on SYSTAT (Version 11, SYSTAT Software Inc., Richmond, California).

### 1.3.3 MORPHOMETRIC ANALYSIS

To minimize digitizing error, the left side of each individual was digitized by the same observer (L.F.R.) and the camera was adjusted so that each fish took up about the same amount of the frame, regardless of its size. For specimens smaller than 30mm, images were acquired through a Qicam digital camera with CCD sensor (Meyer Instruments, TX, USA) mounted on a Leica MZ16A binocular microscope, whereas images of larger specimens where taken with an Olympus Camedia C5060 digital camera mounted on a stand with two lateral lights. We used the Northern Eclipse Software (6.0, Empix Imaging Inc., Ontario, Canada) connected to a graphic pad for scaling the images. A total of 12 landmarks were digitized (figure 1). Only specimens with intact fins and minimal body bending were included for a final sample size of 751 individuals (394 from Casault Lake and 357 from Gunn Creek). Total samples were divided in six successive 5-mm size classes. Sample sizes for the Lake habitat were 70, 133, 81, 62, 34 and 14 for <20 mm, 20-25 mm, 25-30 mm, 30-35 mm, 35-40 mm and > 40 mm, respectively. Sample sizes for the Stream habitat were 27, 72, 110, 69, 43 and 36 for <20 mm, 20-25 mm, 25-30 mm, 30-35 mm, 35-40 mm and > 40 mm, respectively. Scatterplots of landmarks after procrustes superimposition were used to visually detect gross outliers resulting from mislabeling of landmarks. Data used for all analyses were geometrically scaled to unit centroid size (CS) and superimposed using generalized least squares (GLS) procrustes superimposition; this procedure preserves all information about shape differences among specimens, removing only the information unrelated to shape (i.e., scale, position, and orientation). The GLS procrustes superimposition (Dryden and Mardia 1998) first aligns the centroids of each configuration on a common origin. Each specimen is then scaled to unit its centroid size (CS), a measure of size independent of shape in the absence of allometry (Bookstein 1991). Next, specimens are rotated optimally to minimize the Procrustes distance (square root of the sum of squared differences between corresponding landmarks), a measure also used to quantify the amount of difference between any two shapes (Bookstein 1991). Configurations are projected to a linear space (Kendall tangent space) appropriate for multivariate analyses (Slice 2001).

Preliminary exploration of our data using principal component analyses (PCA) on GLS superimposed data for the complete set of specimens in each habitat showed fish bending to cause the most important proportion of the total variance (Casault Lake: 45.42%; Gunn Creek: 42.05%). The PCA on GLS superimposed data shows that, in both habitats, a first principal component score value of zero corresponds to a straight fish while both downward and upward bent specimens are located in the right and left quadrant, respectively (figure 2). To adjust for the fact that some fish tend to curve when euthanized, we used a regression of shape (partial warp and uniform deformation scores) on a first principal component score value of zero to which we added the residuals from the regression. Visual inspection of the resulting standardized data using PCA showed this procedure was successful in removing most fish bending without altering the ontogenetic signal contained in our data (figure 2).

Although geometric scaling removes variation in size, it does not eliminate the impact of that variation in size on shape (Zelditch et al. 2004b). We first checked the assumption that shape is linearly related to size within each size class. The Wilks lambda value is interpreted as the ratio of the generalized variance of the residuals from regression to the generalized variance based on the total variation in the data (simply deviations from the mean). Then, one minus the Wilks lambda value represents the proportion of the total generalized variance "explained" by the regression (Rohlf 2003). Additionally, we used a generalized form of Goodall's F-statistic to test the significance of the regression of geometric shape data on size, testing the null hypothesis that growth is isometric (Zelditch et al. 2004b). The amount of shape variance not related to size was high, ranging from 92.35 to 99.07%, but significant P values for the Generalized Goodall's F-test lead to the rejection of the null hypothesis for some size classes. Allometric corrections were done for all size classes by estimating the expected shape at the average CS for each size class. This was done by regressing shape (partial warp and uniform deformation scores) on an independent variable (CS). To the expected shape at the average CS for each size class, we added the residuals from the regression. The resulting landmark data files, standardized for both bending and allometry, were used to examine the variance of body shape as well as the level and structure of morphological integration.

Landmarks were digitized using tpsDig version 2.10 while regressions and tests of allometry were performed using tpsRegr version 1.34., freely available at: http://life.bio.sunysb.edu/ee/rohlf/software.html. GLS procrustes superimposition and the calculation of CS were performed in CoordGen6 (Sheets 2000), PCA were performed using PCAGen6 (Sheets 2001) and allometric corrections were performed in Standard6 (Sheets 2001); all programs part of the IMP series available at: http://www3.canisius.edu/~sheets/morphsoft.html.

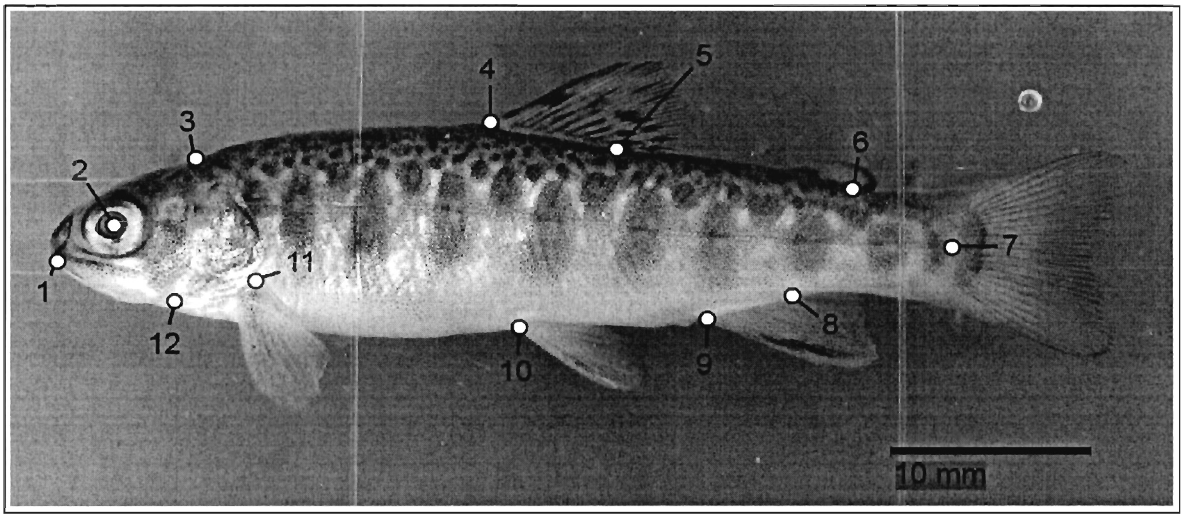

Figure 1. Twelve biologically relevant landmarks on the body of brook charr (*Salvelinus fontinalis*). 1. Tip of snout; 2. Center of the eye; 3. Posterior limit of skull roof; 4. Anterior insertion of dorsal fin base; 5. Posterior insertion of dorsal fin base; 6. Posterior insertion of adipose fin base; 7. Posterior end of hypurals; 8. Posterior insertion of anal fin base; 9. Anterior insertion of anal fin base; 10. Pelvic fin insertion; 11. Pectoral fin insertion. 12. Posterior limit of lower jaw.

### 1.3.1.1 Variance of shape

To estimate the variance of shape for each size-class in both habitats, we used the standard formula for a variance:

$$V = \frac{\sum_{j=1}^{j=n} d_j^2}{(n-1)} \qquad (1)$$

where *dj* is the Procrustes distance of individual *j* from the mean shape for its size and *n* is the sample size for a size class. This distance metric is Euclidean so V is also the trace of the variance-covariance matrix of shape variables (i.e., the sum of their univariate variances) and, in these calculations, it does not matter whether the variances are computed from the shape coordinates or any other geometric shape variables, such as the partial warp scores, because all give the same estimate of the distance. To determine whether size-classes or habitats differ in the variance of shape, we used a t-test, with standard errors of shape variance estimated by bootstrapping with 999 bootstrap resamples (calculations were done in Disparity Box6, part of the IMP series).

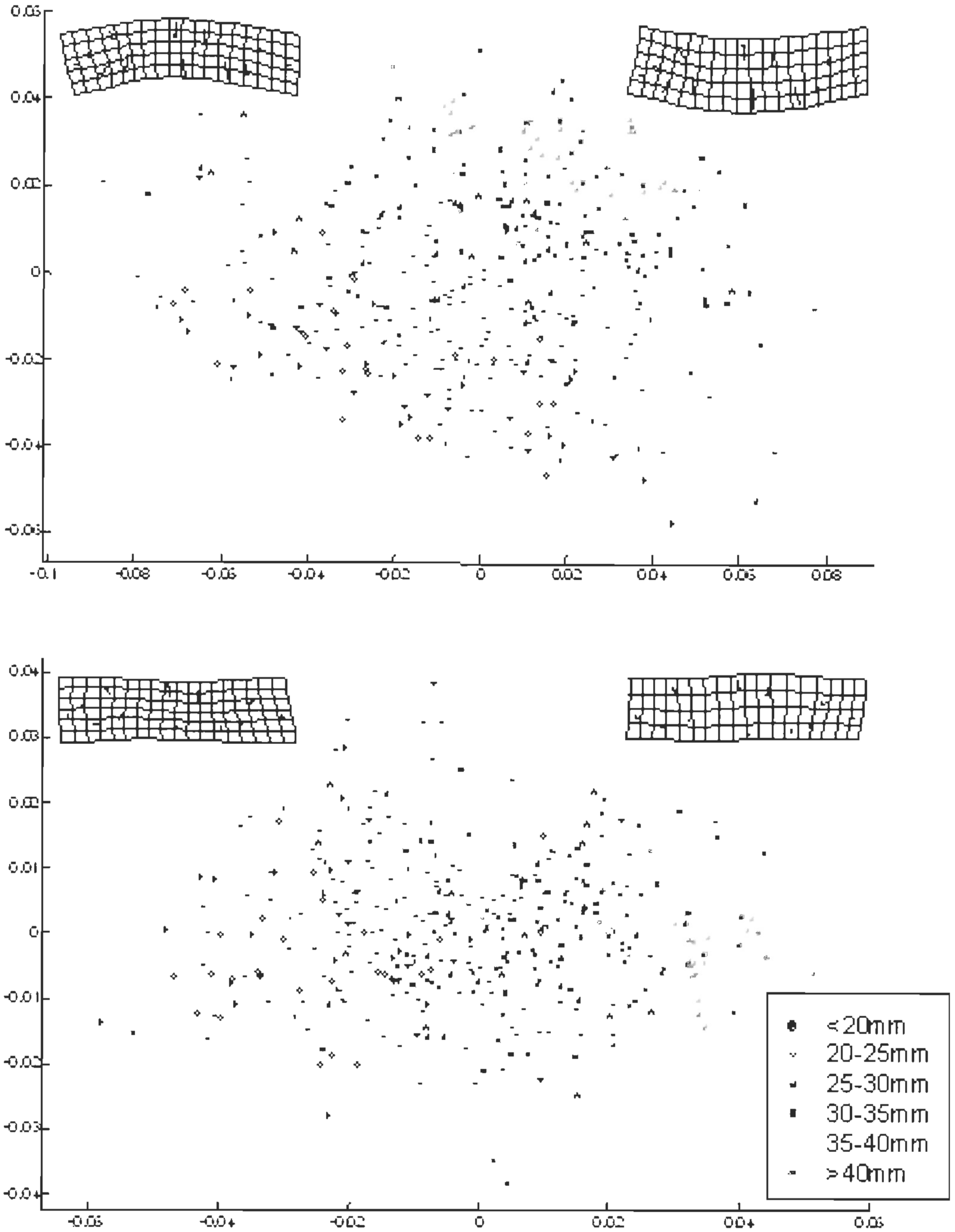

Figure 2. Scatterplots of the first two principal components for all brook charr yearlings from the Lake habitat showing mean shape differences. Each point represents a specimen. The first PC is depicted as a combination of the deformed grid and vectors of relative displacements of landmarks. A) Original GLS superimposed data; B) GLS superimposed data after standardization on a PC score of zero.

### 1.3.1.2 Overall integration level

We addressed the overall level of morphological integration ($I$) by using the variance of eigenvalues (Wagner 1984) from the covariance matrix (Young 2006). The rationale for this metric is that when covariances are high, most of the variance lies along the first few eigenvectors, whereas the remaining eigenvalues are small, producing a large variance in eigenvalues. Conversely, when the variables are nearly independent of each other, all eigenvalues are similar to each other and their variance is low (Wagner 1984). In Wagner (1984) eigenvalue variance was derived from a correlation matrix that makes $I$ directly comparable across populations or species. This is not true of eigenvalue variance calculated from covariance matrices (i.e. the statistic is scale dependent) so eigenvalues were standardized by the total shape variance in the sample (the trace of the variance–covariance matrix) (Willmore et al. 2006; Young 2006).

To determine whether the level of integration is any greater than expected by chance, we compared the observed variance of standardized eigenvalues to that expected from random data using a randomization procedure which was conducted as follows: (1) randomize the values within each variable independently in the data matrix; (2) superimpose the randomized data matrix using GLS; (3) conduct a PCA on the superimposed and permuted data matrix and (4) repeat steps 1-3 a total of 999 times. The standardized eigenvalue variances were calculated from these 999 simulations of random variation. The random values, minus the observed value, form the distribution of the values of $I$ under the null hypothesis (E.Marquez, pers. comm). If the confidence interval of the bootstrapped difference includes zero then differences in morphological integration are considered non-significant. To determine whether samples differ from each other by more than expected by chance alone, the same randomization protocol was used except that, in this case, the difference in $I$ was calculated for two consecutive size classes or habitats. The calculation of variance of standardized eigenvalues and bootstrapping of data matrix were performed using Mint (E.Marquez, pers. comm).

### 1.3.1.3 Structure of integration

To compare the structure of variation, we asked whether samples occupy the same morphological space, meaning that they do not differ in eigenvectors by more than expected by chance. Such comparisons are often done using Common Principal Components Analysis (CPCA, Flury 1988), but that approach presupposes that principal components are well-defined. Most of the variance-covariance matrices analyzed herein do not have distinct eigenvalues which rendered the use of common subspace analysis more appropriate. This method tests whether samples differ significantly in a set of eigenvectors spanning a given number of dimensions. The difference between those sets of eigenvectors is measured by the minimum angle through which one subspace must be rotated to align it with the other (Zelditch et al. 2006). This value is then compared to the distribution of angles obtained by drawing two random samples (with replacement, 999 iterations) from each data set, and measuring the minimum angle through which the subspace of one sample must be rotated to align it with the other. Thus, the angle between the two samples is compared to the distribution of angles obtained within each sample (for technical details, see Zelditch et al. 2006). We compare successive and non-successive size classes as well as corresponding size classes of both habitats. We compare just enough dimensional subspaces to account for 80% of the variance; typically this is between six and eight components. We consider only 80% because PCs accounting for the last 20% are individually trivial (each accounts for <5% of the variance). PCA were performed using PCAGen6, common subspace analysis was done using SpaceAngle, programs part of the IMP series.

#### 1.3.1.4 Variation patterns

Informal comparisons of variation patterns were performed by visual inspection of the principal components (PCs) of variation for all size classes from both habitats; these components represent statistically independent dimensions of variation. The shape changes associated with the PCs can be visualized and interpreted as patterns of variation, but they should not be expected to correspond to particular morphological changes. Geometrically, they can be interpreted as those directions of the dimensional shape space that account for the most scatter among data points. The first few PCs can therefore be used as a summary of the main features in shape variation. To test whether two successive PCs have distinct eigenvalues, we used a test developed by Anderson (1958) (Anderson 1963; Morrison 1967; Zelditch et al. 2004b). This test determines if a pair of PC axis are significantly different from one another. The pairwise comparison statistic starts with the first and second PC, then second and third and so on, until a pair of components is not statistically different at 0.05, using the Chi-square statistic. The first null hypothesis that the first eigenvalue is equal to the second was only rejected once (20-25 mm Lake yearlings). It is therefore a plane or large space of variation that will be interpreted rather than an axis.

## 1.4 RESULTS

### 1.4.1 SAMPLE

All of the Lake fish were caught at null or near null water velocities while only 15.88% of Stream fishes were caught at these water velocities. A total of 7.58% of the Stream specimens were caught at water velocities between 0.0 and 0.5 body length per second (bl/s), 3.97% at water velocities between 0.5 and 1.0 bl/s and the majority our Stream specimens (72%) were caught at water velocities higher than 1.0 bl/s (figure 1).

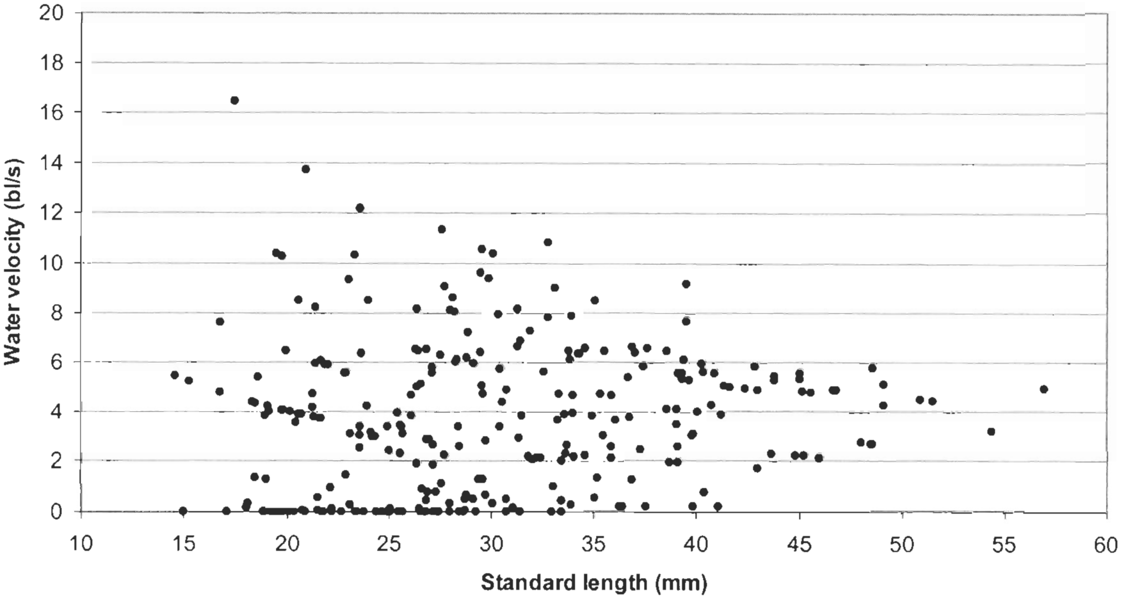

Figure 3. Relative water velocity, in body length per second (bl/s), measured immediately following each capture using a SONTEK handheld acoustic Doppler velocimeter. Data for Stream fishes only.

### 1.4.2 OSSIFICATION SEQUENCE

The majority of ossification events occur before yearlings reach 30 mm (figures 4, 5, 6). Proximal radials at the center of the dorsal fin (PRD3 to PRD11) and the anal fin (PRA3 to PRA10) ossify before yearlings reach 20 mm. Elements ossifying later include the ones at the margins of the dorsal and anal fins. Proximal radials from the left and right pectoral fins ossify around 25 mm. In the caudal fin, the majority of elements ossify before yearlings reach 20 mm. These include arches, as well as haemal and neural spines of the caudal vertebra, hypurals, epurals and uroneural 1. Elements of the caudal fin ossifying later include the dorsal and ventral arcocentra, preural centra and ural centra 1 and 2 and uroneural

2. A permutation paired *t*-test shows ossification events in Stream fish occur at a significantly smaller $SL_{50}$ ($p = 0.0003$) with a mean difference of 0.719 mm.

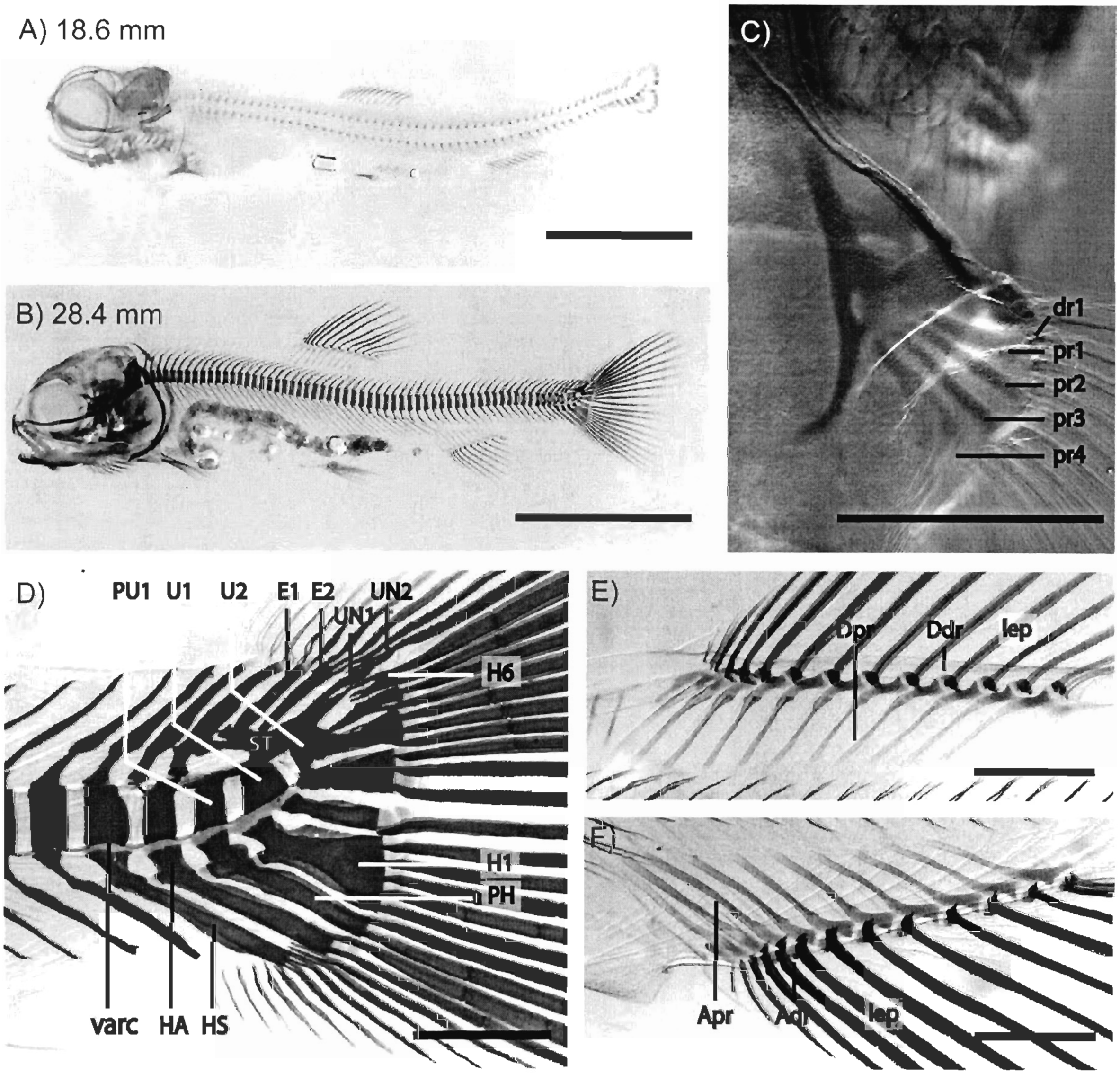

Figure 4. Cleared and stained brook charr (*Salvelinus fontinalis*) showing bone in red and cartilage in blue. A) Complete specimen, standard length 18.6mm, scale bar 5 mm. B) Complete specimen, standard length 28.4mm, scale bar 10 mm. C) Pectoral fin; scale bar 1 mm. dr, distal radials; pr, proximal radials. D) Caudal fin, scale bar 1 mm. E1-2, epurals 1-2; HA, haemal arch; HS, haemal spine, H1-6, hypural 1-6; PH, parhypural or haemal spine of preural centrum 1; PU1, preural centra 1; UN1-2, uroneural 1-2; U1-2, ural centra 1-2 ; varc, ventral arcocentra. E) Dorsal fin; scale bar 1 mm. Ddr, dorsal distal radials; Dpr, dorsal proximal radials; lep, lepidotrichia. F) Anal fin, scale bar 1 mm. Adr, anal distal radials; Apr, anal proximal radials; lep, lepidotrichia.

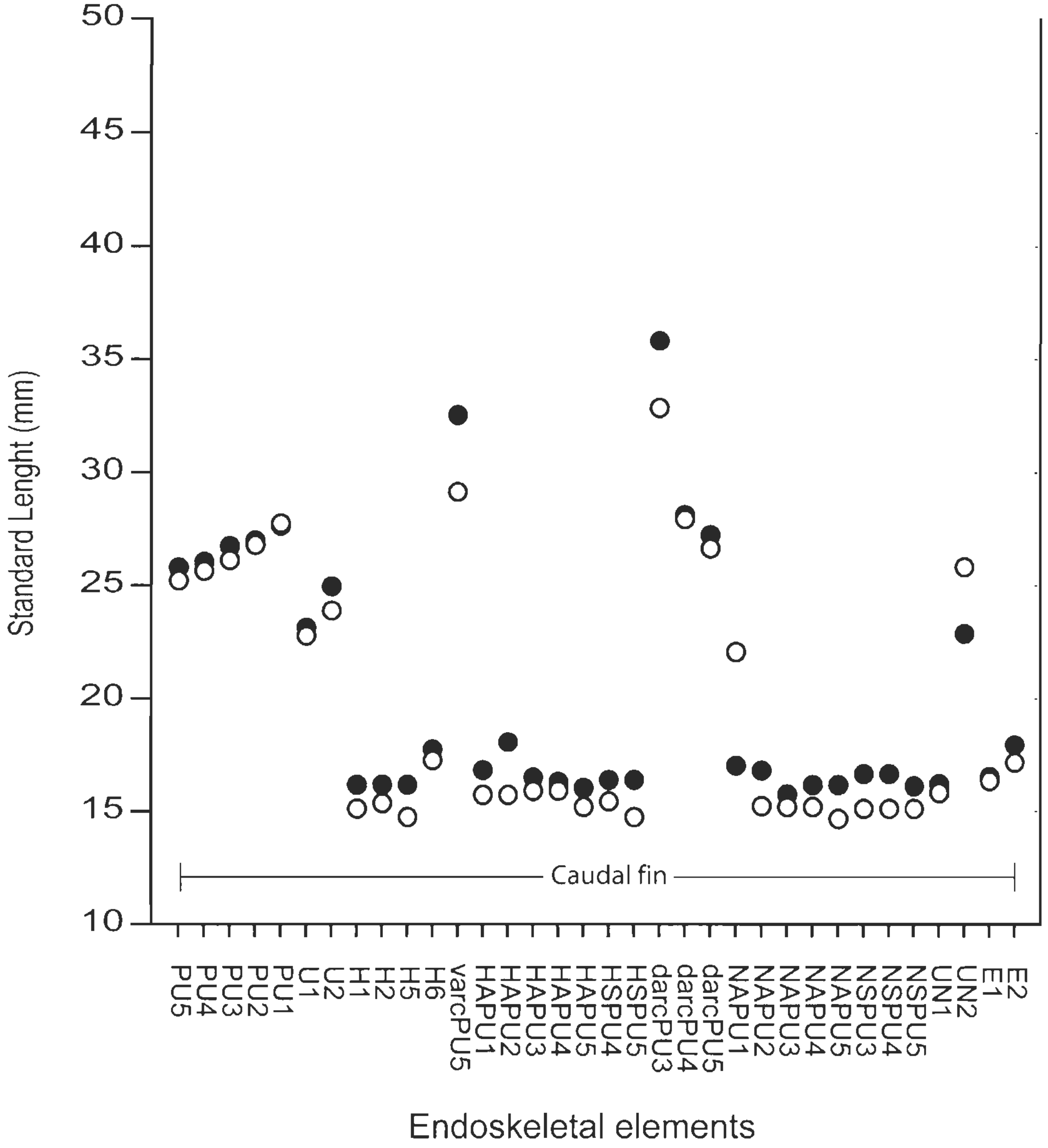

Figure 5. SL$_{50}$ determined by logistic regression for the caudal fin of brook charr yearlings from Lake and Stream, indicated by filled and empty symbols, respectively. darcPU3-5, dorsal arcocentra of preural centra 3-5; E1-2, epurals 1-2; H1-6, hypurals 1-6; HAPU1-5, hemal arch of preural centra 1-5; HSPU4-5, haemal spine of preural centra 4-5; NAPU1-5, neural arch of preural centra 1-5; NSPU3-5, neural spine of preural centra 3-5; PU1-5, preural centra 1-5; U1-2, ural centra 1-2; UN1-2, uroneural 1-2; varcPU1-5, ventral arcocentra of preural centra 1-5.

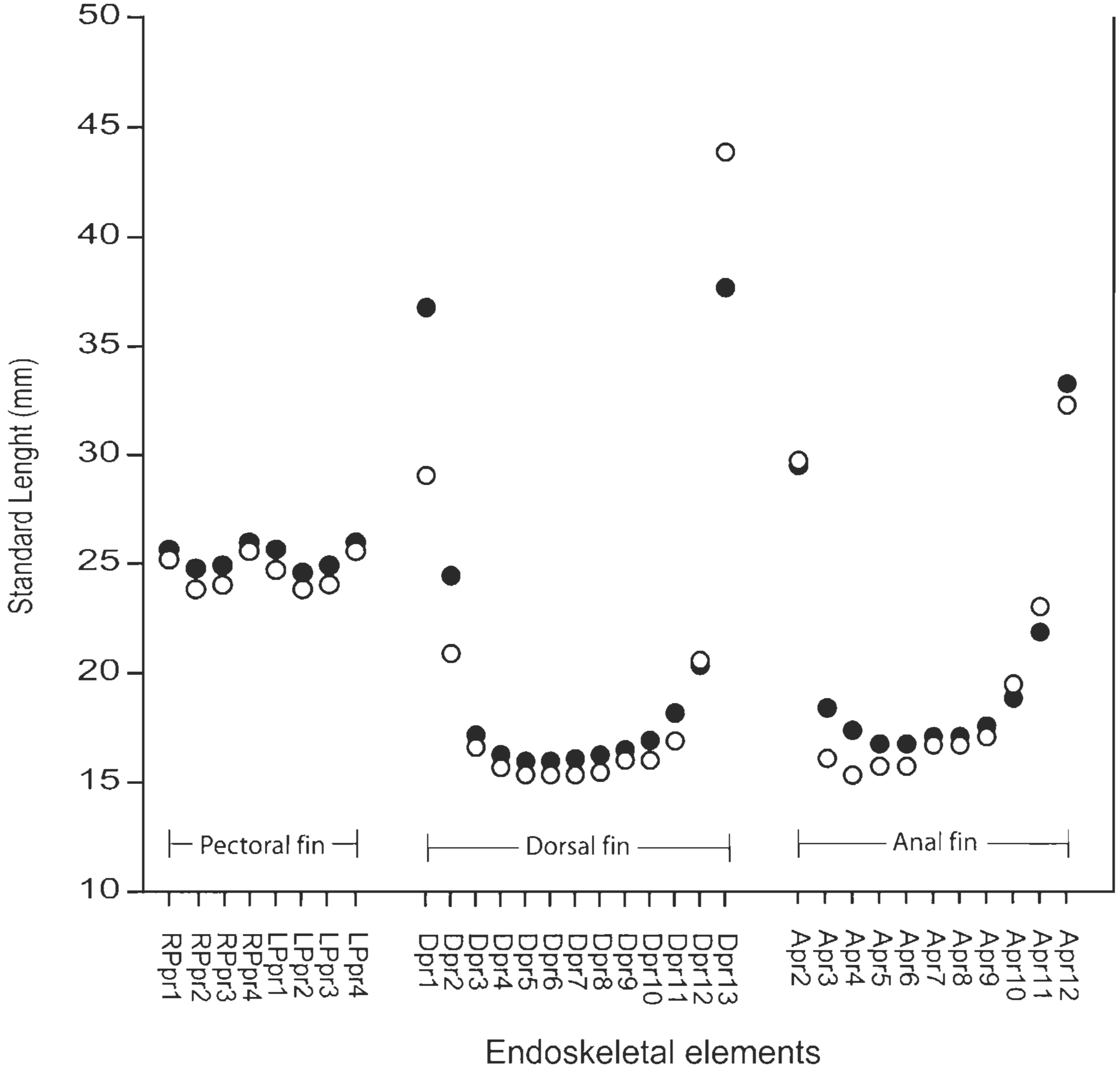

Figure 6. $SL_{50}$ determined by logistic regression for the pectoral, dorsal and anal fins of brook charr yearlings from Lake and Stream, indicated by filled and empty symbols, respectively. Apr2-12, Anal proximal radials 2-12; Dpr1-13, dorsal proximal radials 1-13; LPpr1-4, left pectoral proximal radials 1-4; RPpr1-4, right pectoral proximal radials 1-4.

## 1.4.3 MORPHOMETRIC ANALYSIS

### 1.4.3.1 Variance of shape

In both habitats, variance is approximately halved between the smallest size class and 35 mm yearlings and remains constant thereafter (figure 7). This decrease is statistically significant. Between habitats, only one size class (25-30 mm) shows a significantly different level of shape variance.

### 1.4.3.2 Overall integration level

For all size classes, we can reject the null hypotheses that the samples are no more highly integrated than expected by chance ($P \leq 0.001$ for all samples). The Lake habitat was more highly integrated in the 20-25 mm, 25-30 mm and 30-35 mm classes whereas other size classes do not differ between habitats (table 1). The higher level of integration seen in yearlings larger than 40 mm from the Lake habitat is most likely an effect of small sample size and is not significantly different from the level of integration found for >40 mm Stream yearlings (figure 7).

In both habitats, there is a significant decrease of integration early in ontogeny, before 25 mm in Lake and 30 mm in Stream (table 1, figure 7). There is a tendency for increasing integration strength later in ontogeny even if differences in the level of integration between consecutive size classes were not always significant (table 1).

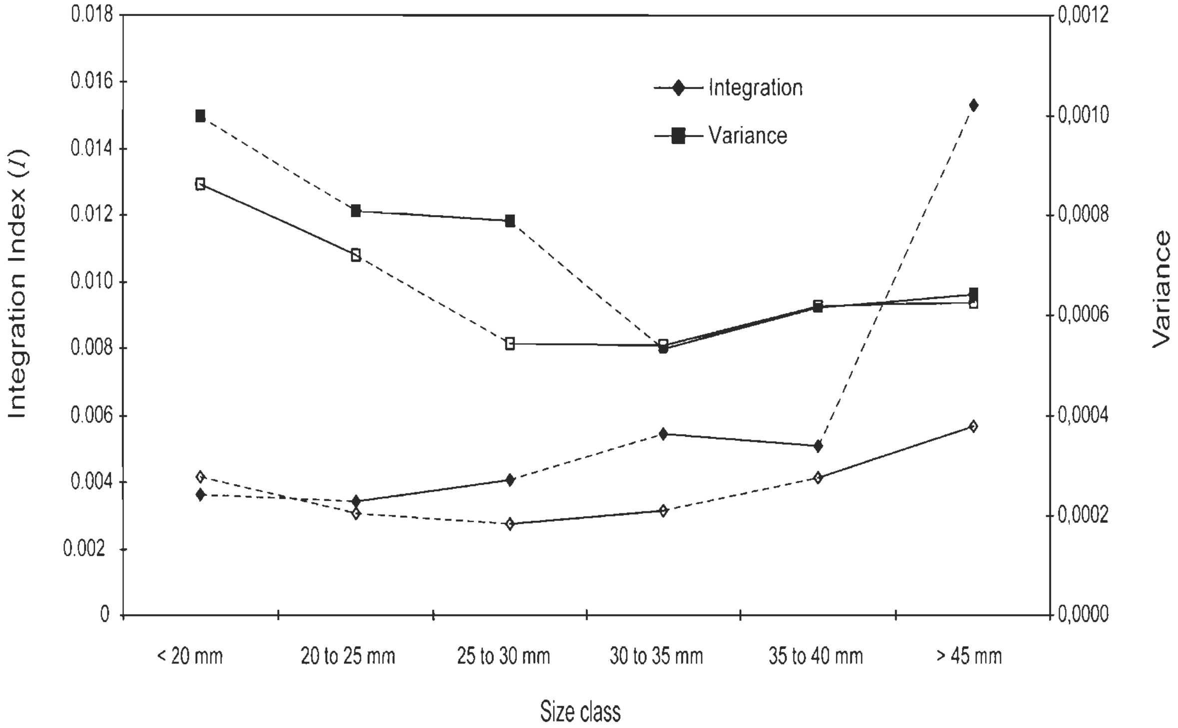

Figure 7. Index of integration (*I*) and variance through ontogeny for brook charrs from Lake and Stream, indicated by filled and empty symbols, respectively. Index of integration (diamond) is estimated by the variance of their standardized eigenvalue from their covariance matrix and variance (square) is the trace of the variance–covariance matrix of shape variables. Significant changes between successive size classes are indicated by a dotted line. Value for *I* is larger than expected by chance for all samples.

Table 1. Difference in the level of integration for brook charr yearlings from Lake and Stream, as estimated by the confidence interval from 1000 bootstrapped differences of the variance of standardized eigenvalues. Direction of significant changes ($p < 0.0001$) are provided for habitat comparisons in the diagonal, for successive size classes in Lake in the lower half of the matrix and for Stream in the upper half of the matrix.

| | < 20 mm | 20-25 mm | 25-30 mm | 30-35 mm | 35-40 mm | >40 mm |
|---|---|---|---|---|---|---|
| < 20 mm | **NS** | ↓ | ↓ | ↓ | ↓ | ↑ |
| 20-25 mm | ↓ | ≠ | ↓ | ↑ | **NS** | ↑ |
| 25-30 mm | ↑ | **NS** | ≠ | **NS** | **NS** | ↑ |
| 30-35 mm | ↑ | ↑ | ↑ | ≠ | ↑ | ↑ |
| 35-40 mm | ↑ | ↑ | ↑ | **NS** | **NS** | **NS** |
| >40 mm | **NS** | **NS** | **NS** | ↑ | ↑ | **NS** |

### 1.4.3.3 Structure of variation

There is a gradual change in structure of (co)variation throughout ontogeny. Only one comparison reveals a statistically significant difference between successive size classes: 20-25 mm vs 25-30 mm samples from the Stream habitat (table 1). However, comparisons to the smallest size class (<20 mm) shows angles between samples are increasing as ontogeny proceeds in both habitats (Table 2B). The structure of (co)variation differs significantly between the smallest size class and larger ones in both habitats (i.e. 30-35 mm and 35-40 mm in Lake and 35-40 and >40 mm in Stream). The two habitats differ statistically significantly in structure of (co)variation only at smaller sizes (i.e., <30 mm) (Table 3).

### 1.4.3.4 Variation patterns

For all size classes in both habitats, with the exception of 20-25 mm from the Lake habitat, variation is equally distributed over the first few dimensions, as expected in light of the general inability of Anderson's test (1958) to reject the null hypothesis. The different PCs presented in figures 8 and 9 therefore represent a plane or space of variation and no single PC can be considered a major axis of variation.

For most Lake size classes, proportion of body height relative to body length is highly variable (Figure 8), as shown mostly by the vertical variation of landmarks at the anterior and posterior insertion of the dorsal fin versus the pelvic fin (PC1: <20 mm, 20-25 mm, 25-30 mm, 30-35 mm; PC2: 20-25 mm, 25-30 mm, >40 mm; PC3: <20 mm, 35-40 mm, PC4: 35-40 mm). Another feature shared by several sizes implies variability of head size relative to body length, as shown by the opposed variation of landmarks at the mouth and pectoral fin insertion (PC1: 35-40 mm and >40 mm; PC2: <20 mm; PC4: 20-25 mm and 25-30 mm). Finally, several PCs show that posterior body proportions are variable, indicated alternatively by the opposed variation of the anterior and posterior dorsal fin base relative to the adipose fin and caudal peduncle or by a variation of the anterior pelvic fin insertion opposed to the caudal fin (PC2: 30-35 mm and 35-40 mm; PC3: 20-25 mm, 25-30 mm, >40 mm; PC4: 30-35 mm and >40 mm).

Variation patterns seen in Stream samples are less clear (figure 9). A variation in body height relative to body length seems important but dispersed on different PCs. In addition to involving landmarks at the anterior and posterior dorsal fin base and pelvic fin, variability in height includes the posterior limit of skull roof landmark as well as caudal peduncle height (PC1: 20-25 mm, 25-30 mm, 30-35 mm; PC2: 35-40 mm and > 40 mm; PC3: 20-25 mm, 30-35 mm, 35-40 mm; PC4: 20-25 mm and >40 mm). Variability of head size relative to body length, is shown by the opposed scatter of landmarks at the mouth and pectoral fin insertion (PC1: 35-40 mm and >40 mm; PC2: <20 mm and 20-25 mm). Some PCs suggest posterior body proportions are variable, mostly indicated by the simultaneous variation of the anterior and posterior dorsal fin base insertion relative to a scatter of the adipose and caudal peduncle but also seen in the variation of the adipose fin relative to the caudal peduncle (PC1: <20 mm; PC2: 30-35 mm; PC3: 25-30 mm and >40 mm, PC4: <20 mm, 25-30 mm, 30-35 mm, 35-40 mm).

These three patterns of variation seen in Lake and Stream : (1)body height relative to body length, (2) head size relative to body length and (3) posterior body proportions show no ontogenetic trend as all PCs are equivalent.

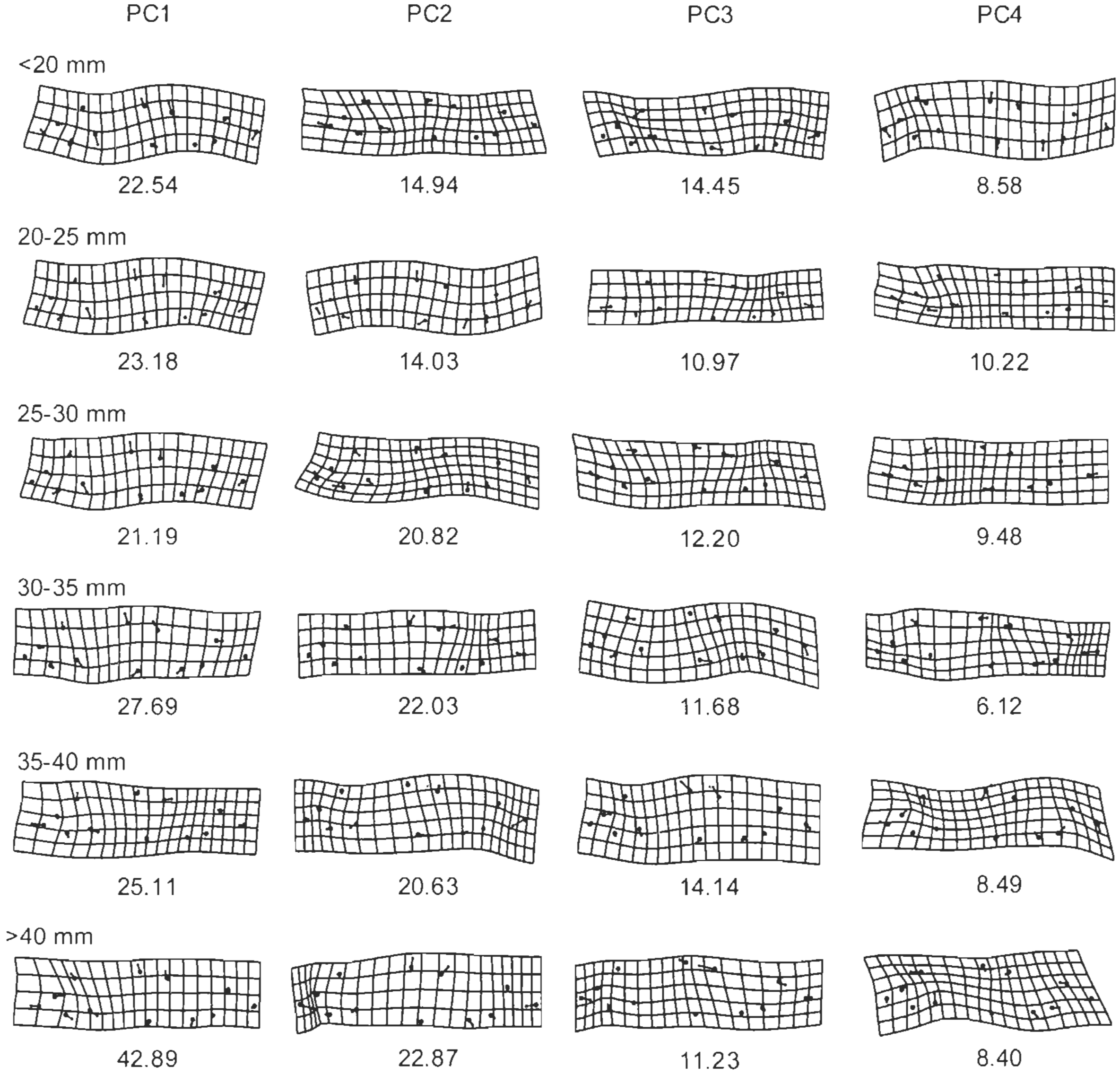

Figure 8. The structure of variation for each size sample of Lake brook charrs. Shown are the four Principal Components (PCs) of variation for shape variables after removing bending and allometry. Each PC is depicted as a combination of the deformed grid and vectors of relative displacements of landmarks. Size range of each sample is indicated on the left; the percentage of variation explained by each PC is indicated below.

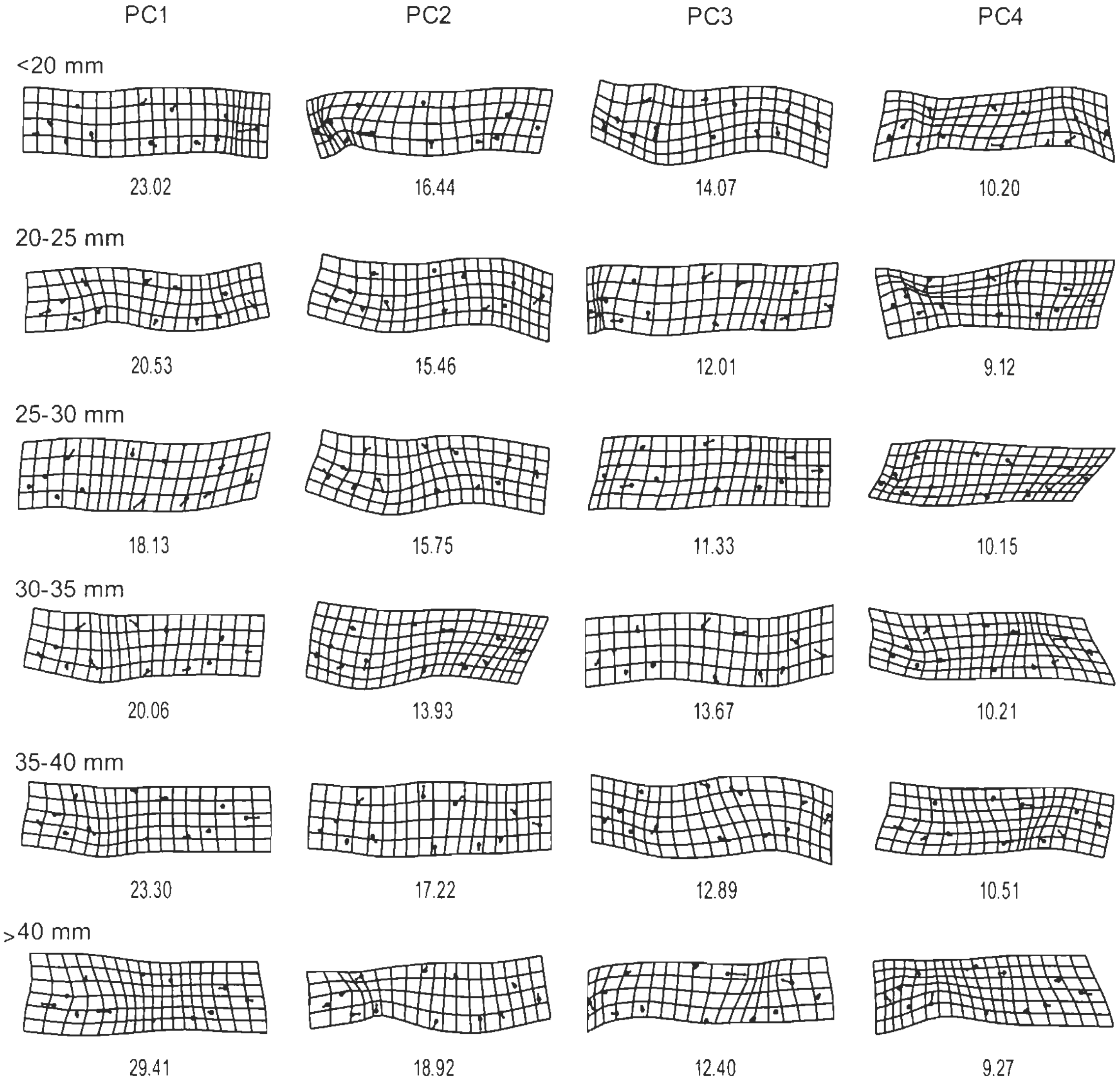

Figure 9. The structure of variation for each size sample of Stream brook charrs. Shown are the four Principal Components (PCs) of variation for shape variables after removing bending and allometry. Each PC is depicted as a combination of the deformed grid and vectors of relative displacements of landmarks. Size range of each sample is indicated on the left; the percentage of variation explained by each PC is indicated below.

Table 2. Differences in structure of variation A) for successive size classes; B) from the smallest size class (<20 mm). Measured by the angles (in degrees) between the subspaces encompassing 80% of the variation for brook charrs from Lake and Stream. Given are the number of PCs compared (Nb. of PCs), between-size angle (B), within-size angles (*W*(1)/*W*(2)). When B exceeds both *W*(1) and *W*(2), the difference between the two subspaces is statistically significant (indicated in bold face).

A)

| Size Classes | | Nb. of PCs | B | *W*(1) | *W*(2) |
|---|---|---|---|---|---|
| Lake | | | | | |
| <20 mm | 20-25 mm | 8 | 60.05 | 107.58 | 94.79 |
| 20-25 mm | 25-30 mm | 8 | 73.12 | 89.92 | 98.03 |
| 25-30 mm | 30-35 mm | 7 | 92.08 | 101.88 | 108.74 |
| 30-35 mm | 35-40 mm | 7 | 107.31 | 106.47 | 110.27 |
| 35-40 mm | >40 mm | 6 | 91.52 | 101.9 | 116.46 |
| Stream | | | | | |
| <20 mm | 20-25 mm | 8 | 109.14 | 114.96 | 112.74 |
| 20-25 mm | 25-30 mm | 8 | **102.41** | 98.87 | 97.35 |
| 25-30 mm | 30-35 mm | 8 | 84.63 | 93.04 | 104.25 |
| 30-35 mm | 35-40 mm | 8 | 101.27 | 106.63 | 110.89 |
| 35-40 mm | >40 mm | 7 | 79.56 | 110.97 | 106.59 |

B)

| Size Classes | Nb. of PCs | B | *W*(1) | *W*(2) |
|---|---|---|---|---|
| Lake | | | | |
| 20-25 mm | 8 | 60,05 | 107,58 | 94,79 |
| 25-30 mm | 8 | 107,82 | 109,54 | 106,73 |
| 30-35 mm | 8 | **127,73** | 108,14 | 117,07 |
| 35-40 mm | 8 | **122,05** | 108,31 | 112,53 |
| >40 mm | 8 | 126,02 | 108,86 | 132,83 |
| Stream | | | | |
| 20-25 mm | 8 | 109,14 | 114,96 | 112,74 |
| 25-30 mm | 8 | 124,04 | 125,57 | 120,14 |
| 30-35 mm | 8 | 110,04 | 123,33 | 120,35 |
| 35-40 mm | 8 | **139,51** | 121,55 | 119,51 |
| >40 mm | 8 | **137,07** | 124,65 | 121,13 |

Table 3. Differences in structure of variation between Lake and Steam, measured by the angles (in degrees) between the subspaces encompassing 80% of the variation. Given are the number of PCs compared (Nb. of PCs), between-size angle (B), within-size angles (*W*(1)/*W*(2)). When B exceeds both *W*(1) and *W*(2), the difference between the two subspaces is statistically significant (indicated in bold face).

| Size Classes | Nb of PCs | B | *W*(1) | *W*(2) |
|---|---|---|---|---|
| <20 mm | 8 | **136,46** | 100,5 | 117,65 |
| 20-25 mm | 8 | **111,1** | 85,94 | 99,58 |
| 25-30 mm | 8 | **109,03** | 98,58 | 96,87 |
| 30-35 mm | 8 | 93,8 | 104,26 | 106,17 |
| 35-40 mm | 7 | 108,69 | 108,88 | 109,85 |
| >40 mm | 6 | 120,68 | 132,22 | 122,7 |

## 1.5 DISCUSSION

Ontogenetic and habitat differences in timing of ossification, level and structure of integration as well as variance of body shape are evident for brook charr yearlings from Lake and Stream. The onset of most ossification events occur before yearlings reach 30 mm in standard length, and those take place at a significantly smaller size in Stream fishes. During the interval in which those events occur rapidly, overall level of integration tends to be relatively low and decreasing, especially in Stream samples while variance of shape decreases by half. During this period, fish go from having high and random body shape variations to displaying a lower but more random variation in body shape at 30 mm. When the onset of ossification is nearly complete, overall level of integration increases and variance of shape stabilizes. This means that, from this point on, the amount of body shape variation is constant but that this variation becomes less and less random as ontogeny proceeds. Throughout these parallel changes in variance and integration, there is a gradual and convergent change in the structure of (co)variation between habitats.

Fish from our Stream habitat have fins that ossify at a significantly smaller size, although we cannot determine whether that is due to genetic or environmental factors. Yet, during post-hatch development, higher water velocities do generate earlier ossification of endoskeletal elements in median fins of Arctic charrs (pers. obs., R. Cloutier) and brook charrs reared at higher temperatures reach a more ossified state at an earlier size/age than those reared at lower temperatures (Balon 1980). The smaller size at which Stream fish ossify could thus represent either an adaptation or a plastic response to its rearing temperatures and/or water velocity. In most species of fish, there is an early ossification of bones associated with feeding and respiration (Arendt and Wilson 2000). This pattern is generally interpreted as adaptive (e.g., Mabee and Trendler 1996), although, to our knowledge, this has never been tested empirically. Of more interest to this study, another likely benefit of early ossification relates not to the cranial skeleton but to the post-cranial skeleton. A delay in ossification of the elements in the pectoral and pelvic fins and their fin rays may compromise maneuverability. Pectoral fins of rainbow trout (*Oncorhynchus mykiss*) are completely inactive during fast steady swimming in microturbulent flow while their recruitment is restricted to slow velocity activities such as hovering, turning and braking (Drucker and Lauder, 2003). Likewise, delayed ossification of vertebrae probably decreases swimming efficiency. Vertebrae being mineralized elements much stiffer than the notochord, the ossification of the vertebrae restricts bending to the joints and affects body curvature (Long et al. 2002). Last, delayed ossification of endoskeletal elements and rays of the median fins may diminish swimming performances. In rainbow trout, the anal and dorsal fins are actively recruited in several swimming activities including steady swimming, hovering, turning and braking (Drucker and Lauder 2003; 2005) while the principal function of the caudal fin is found in fast acceleration rather than in steady swimming (Webb, 1977). Therefore, we tentatively interpret the smaller size at which the onset of ossification events occur in Gunn Creek as a reaction to swimming demand. Whether these changes in timing of ossification positively affect performance and whether they represent adaptations (i.e., evolved by natural selection for that function) has not been resolved.

The idea of functional adaptation of bone, its remodeling response to physical usage, stems from Woff's (1892) work hypothesizing that the maintenance and transformation of the skeleton is greatly influenced by the mechanical forces acting on it during its use. If mechanical loading have a similar effect on the growing skeleton, bone growth modeling likely reflects the complex interplay of not only epigenetic, hormonal, and biochemical factors during ontogeny but mechanical factors as well (Biewener and Bertram 1993). Even *in utero*, muscle loading is required for normal development. The fetuses of mutant mice that lack skeletal muscle (MyoD–/–/Myf5–/– (dd/ff)) develop without any active movement. Long bones of these mutant mice show altered morphological features and increased bone resorption (Gomez et al. 2007). Such studies documenting the effect of mutations and mechanical loading on skeletal development have never been conducted on fish. Nevertheless, during post-hatch development, higher water velocities generate earlier ossification of endoskeletal elements in median fins of Arctic charrs (pers. obs. R. Cloutier).

The overall level of integration tends to be relatively low, especially during the period of rapid onset of ossification events. This low level of integration is illustrated mostly by the equal distribution of eigenvalues, indicated by the results of Anderson's test, as the index of integration used here has no theoretical maximum. This interpretation is in agreement with Peres-Neto and Magnan (2004) who found relatively low levels of integration in brook charr. For a period of six months, charr yearlings were exposed to four differential water velocities and the variance of the eigenvalues from a correlation matrix of linear distances was used as an overall measure of integration. Despite differences in method and age of their specimens, we agree with Peres-Neto and Magnan's (2004) conclusion of a low level of overall integration in brook charr yearlings.

The early differences in morphological integration between habitats could originate from numerous factors. Such factors include intrinsic genetics (e.g. Cheverud 1982; Cheverud et al. 2004), maternal effects such as yolk sac reserves (Perry et al. 2004) and neuromuscular activity before hatching (Drapeau et al. 2007) and early swimming demand (Grunbaum et al. 2007) that co-ordinate epigenetic effects on muscles and bones. Concerning intrinsic genetic factors, alternative phenotypes can be confused with characteristics of populations that are divergent owing to reduced gene flow (West-Eberhard 2003). The differences we observe in morphological integration could be the result of genetic differentiation. Both Casault Lake and Gunn Creek are connected to the Causapscal River and gene flow between the two habitats could be tested by common garden experiments or lab rearing and molecular genetic tests. Regarding maternal effects or neuromuscular activity before hatching, there are no *a priori* reasons to presume they would differ between habitats. Consequently, we concentrate on potential effects of local environmental factors on early swimming demand that could alter morphological integration.

The fact that variation patterns differ before 30 mm in length, but only this early, suggests that, for yearlings that have just emerged from the substrate, external body shape could be influenced by the response of the developmental system to swimming demand. An early response of body length and body weight to swimming demand has been observed in Arctic charr as soon as 35 day post-hatching (Grünbaum et al. 2008). The negative association between the level of integration and water velocity found by Peres-Neto and Magnan (2004) is less clear for brook charr than for Arctic charr. For brook charr, the highest level of integration was found in fishes reared at medium water velocity. Our analysis suggests that Lake fish tend to be more highly integrated than the ones from the Stream habitat (i.e., 20-25 mm, 25-30 mm and 30-35 mm). The majority our Stream specimens (72%) were caught at water velocities faster than 1.0 body length per second, corresponding roughly to the "fast" treatment which generated the lowest integration level in Peres-Neto and Magnan (2004). The lower integration found in our fish reared in Stream habitat thus agrees with results obtained by Peres-Neto and Magnan (2004).

Between the smallest size class and 35mm yearlings, variance of shape decreases by half. A trend of early ontogenetic decrease of variance has been observed in skull size of laboratory rats (Nonaka and Nokata 1984), and skull shape of *Calomys expulsus* (Hingst-Zaher et al. 2000), *Sigmodon fulviventer* (Zelditch et al. 1993) and *Mus musculus domesticus* (Zelditch et al. 2004). It has been suggested by Zelditch et al. (2004) that, based on a general model for bone growth, variance is generated when bone grows under the direction of disorganized muscular movements and decreases with increasing neuromuscular control. Albeit very different from the mammalian skull system, the initially high level of variance and its early decrease seen in smaller sized brook charr could reflect the variance in body shape generated during the pre-hatch period. Disorganized muscular movements are also seen in embryonic motor behaviors of fishes. In the zebrafish (*Danio rerio*), movements appear in sequence and consist of an early period of transient spontaneous coiling contractions, followed by the emergence of twitching responses to touch, and later on, by the ability to swim (reviewed by Drapeau et al. 2002). Both the nervous and muscular systems must develop and function in concert to bring about proper locomotion as the appearance of each of these motor behaviors is related to changes in the cellular mechanisms generating locomotion (Drapeau et al. 2002).

During the period of rapid onset of ossification events, small fish go from having high and random body shape variations to displaying lower but more random variation in body shape at 30 mm. When the onset of ossification is nearly complete, integration increases and variance of shape stabilizes. Although this is not a constant increase in integration, comparisons of non-subsequent size classes do indicate an increasing trend. This means that, from this point on, the amount of body shape variation is constant but that this variation becomes less and less random as ontogeny proceeds. This direction in body shape variation is also suggested by fact the structure of variation in constant through ontogeny. There are three patterns of shape variation that are easily identifiable; (1) body height relative to body length, (2) head size relative to body length and (3) posterior body proportions. These patterns remain constant through ontogeny as none of the first principal components differs from the second within each size class (except for 20-25 mm in Lake). The greater co-ordination in locomotion and the adaptation of muscles and bones to each other could be responsible for creating direction in body shape variations and increasing the level of integration. The early loss of variance could be a result of selective deaths in the population or could indicate that variation is developmentally regulated. The later stability of the variance could also suggest canalization because we would expect continued production of variation by the ongoing process of development.

While the transition from pre-hatch movement to coordinated locomotion might possibly explain the low initial level of integration and its subsequent increase, it does not explain the early decrease in the level of integration observed before 25 mm and 30 mm for Lake and Stream fishes, respectively. The early decrease in the level of integration co-occurs with the period of intense onset of post-cranial ossification events. During this early period, specimens go from lacking ossified elements except some bones in the cranium to having an almost completely ossified post-cranial skeleton. The most likely explanation for the decrease in the level of integration before yearlings reach 30 mm is the importance of muscular and skeletal development during this period. The intense development of bones before yearling reach 30 mm is likely to also include the muscular system. Vertebrate striated muscles and bones belong to the same functional system and cannot function independently from one another. The muscles provide the mechanical forces, while the bones provide the support. The interconnecting signals that exist between these tissues are poorly understood. However, they both share a response to mechanical stimuli (reviewed by Herring 1994). A recent study by Steinbacher (2007) demonstrates that, in brown trout (*Salmo trutta lacustris*), contrary to what is found in smaller or slow growing species, stratified hyperplasia and mosaic hyperplasia begin simultaneously and progress vigorously. This may provide an efficient strategy to establish a large potential for post-hatching body growth. Stratified hyperplasia is the main mechanism responsible for the increase in the number of slow muscle fibres after hatching (Rowlerson et al. 1995; Barresi et al. 2001). Mosaic hyperplasia is responsible for expanding fast fibre number in the juvenile and adult stages of the vast majority of species (Weatherley et al. 1988). Intense development of both the muscular and the skeletal systems could produce greater variation among individuals in degree of maturity, therefore decreasing the level of integration.

With the exception of Grünbaum et al. (2007), previous studies on the effect of swimming demand on morphological integration or body size and shape changes have focused on juvenile and adult specimens and have not addressed the ontogeny of these changes. Whether early changes in morphology reflect adaptations to immediate environmental conditions or represent a by-product of the development of the propulsive musculature and the skeletal framework related to function remains to be explored. The hypothesis that ontogenetic changes in fish are not well suited to the immediate functional demands of each stage of growth is supported by recent findings on the ontogeny of form and function in the zebrafish. McHenry and Lauder (2006) conclude that drag production rises during larval and juvenile growth in contrast to suggestions that they should be optimal for immediate functional demands (e.g., Weihs, 1980; Müller and Videler, 1996; Müller and van Leeuwen, 2004). During ontogeny, large morphological changes could have few biological consequences while it is equally possible that small changes affecting just a few morphological details could have profound consequences for function. Whether changes in timing of ossification or morphological integration level affect swimming performance and whether these changes represent adaptations has not been resolved. Therefore, data on the role of muscular and skeletal development in determining body shape, as well as information on the influence of body shape on swimming cost of early developmental stages in fish are necessary to better understand the origin and consequences of ontogenetic shape changes.

## 1.6 CONCLUSION

Stream fishes ossify at a smaller size and this could be related to a more important swimming demand. The existence of two distinct ontogetetic patterns related to the level and structure of integration as well as to variance highlights the complexity of these responses. The first pattern is evident during the period of intense onset of ossification events while the second pattern can be observed when the onset of ossification is nearly complete. Before yearling reach 30 mm, the onset of most ossification events occur, overall level of integration tends to be relatively low and decreasing while variance of shape decreases by half. During this period, fish go from having high and random body shape variations to displaying a lower but more random variation in body shape at 30 mm. When the onset of ossification is nearly complete, overall level of integration increases and variance of shape stabilizes. This means that, from this point on, the amount of body shape variation is constant but that this variation becomes less and less random as ontogeny proceeds. We interpret these changes as being a result of both a change in the rate of developmental progress as well as the effect of locomotion on the muscular and the skeletal systems.

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# CONCLUSION

Cette étude met en lumière un faible niveau d'intégration morphologique chez les jeunes de l'année de l'Omble de fontaine, résultats qui est en accord avec une autre étude portant sur la l'intégration morphologique chez les salmonidés (Peres-Neto et Magnan, 2004). Ce faible niveau d'intégration est bien illustré par la variabilité morphologique et écologique trouvée chez les espèces du genre *Salvelinus*, souvent représenté par différents niveaux de divergence adaptative ou polymorphisme trophique (Magnan et al., 2002).

L'ossification de la majorité des éléments associés aux nageoires se produit avant que les jeunes ombles atteignent une longueur standard de 30 mm et l'ossification de ces éléments a lieu à une taille significativement plus petite chez la population de ruisseau. Cette différence dans le déclenchement de l'ossification des nageoires pectorales et médianes est probablement due à la plus importante demande locomotrice nécessaire en ruisseau. La vélocité du courant déclenche aussi une ossification plus rapide des nageoires médianes chez l'omble chevalier (Cloutier, communication personnelle). Il serait pertinent de déterminer si une ossification plus rapide du squelette post-crânien améliore les performances de nage, ce qui n'a jamais été testé. Dans le contexte des différences observées entre lac et ruisseau, une ossification plus rapide pourrait aussi être le résultat de la sélection naturelle et représenter une adaptation de l'omble de fontaine aux conditions qui prévalent en ruisseau.

On trouve deux patrons ontogénétiques distincts en ce qui concerne le niveau d'intégration, la variance de la forme et la structure de la variation. Le premier patron est observé au cours de l'intervalle d'ossification soutenue alors que le second patron se produit une fois que le rythme de l'ossification ralenti. Au cours de l'intervalle d'ossification rapide ayant lieu avant 30 mm, le niveau d'intégration morphologique est relativement faible et en diminution alors que la variance diminue de moitié. La structure de la variation diffère entre les habitats au cours de cette période. Au début de l'intervalle d'ossification soutenue, les poissons montrent donc une variabilité forte et relativement aléatoire de la forme du corps alors que lorsqu'ils atteignent 30 mm, cette variabilité est devenue plus faible mais encore plus aléatoire. Lorsque l'ossification est entamée dans presque tous les éléments des nageoires pectorales et médianes, le niveau d'intégration augmente et la variance se stabilise. À partir de ce moment, la quantité de variabilité dans la forme du corps reste donc constante mais devient de moins en moins aléatoire. Malgré les changements ontogénétiques dans le niveau d'intégration et de variance, la structure de la variation reste stable tout au long du développement.

Chez les plus petites classes de taille, la différence significative dans la structure de la variation retrouvée entre les habitats pourrait indiquer une sensibilité du système de développement dès le moment où les ombles de fontaine émergent du substrat. Cette sensibilité précoce est possible puisqu'un effet de la vélocité du courant sur la taille et la masse a pu être observé dès 35 jours après l'éclosion chez l'omble chevalier (Grünbaum et al. 2008). Le plus faible niveau d'intégration trouvé entre 20 et 35 mm chez les ombles de ruisseau concorde avec l'effet de la vélocité du courant sur le niveau d'intégration observé chez des l'omble de fontaine (Peres-Neto et Magnan, 2004).

Une diminution de la variance au cours de l'ontogénie a été observée dans la taille du crâne chez les rats de laboratoire (Nonaka et Nokata, 1984) et dans la forme du crâne chez *Calomys expulsus* (Hingst-Zaher et al., 2000), *Sigmodon fulviventer* (Zelditch et al., 1993) et *Mus musculus domesticus* (Zelditch et al. 2004). Cette diminution de la variance est attribuée à l'augmentation du contrôle neuromusculaire ayant lieu au cours du développement (Zelditch et al., 2004). La diminution de la variance observée chez l'omble de fontaine pourrait aussi être due à l'augmentation de la coordination locomotrice. Une augmentation du niveau d'intégration morphologique est observée lorsque l'ossification est entamée dans presque tous les éléments des nageoires pectorales et médianes. Ce résultat pourrait indiquer que l'augmentation de la coordination locomotrice et l'adaptation des muscles et des os les uns aux autres génèrent de plus fortes corrélations entre les traits liés par la fonction et ainsi donne une direction à la variabilité de la forme du corps. Cette interprétation est appuyée par la stabilité observée dans la structure de la variation au cours du développement.

Si l'augmentation du contrôle neuromusculaire au cours du développement explique la hausse du niveau d'intégration, elle n'explique en rien la diminution initiale du niveau d'intégration pendant l'intervalle d'ossification soutenue. L'explication la plus plausible pour cette baisse du niveau d'intégration concerne l'importance du développement musculaire et squelettique au cours de cette période. Chez les vertébrés, les muscles striés et les os forment un système fonctionnel indissociable (Herring, 1994). Chez les ombles de lac et de ruisseau, les plus petits poissons capturés ne possèdent que quelques éléments crâniens ossifiés alors qu'à la fin de l'intervalle d'ossification soutenue, le squelette post-crânien s'est presque ossifié en entier. De plus, à partir de l'éclosion, les salmonidés possèderaient un potentiel de développement musculaire exceptionnel (Steinbacher, 2007). Ce potentiel important de développement musculaire et squelettique pourrait produire une grande variabilité du degré de maturité developpemental entre les spécimens et ainsi réduire le niveau d'intégration mesuré pendant l'intervalle de d'ossification soutenue.

La présence de deux patrons ontogénétiques distincts en ce qui concerne le niveau d'intégration, la variance de la forme et la structure de la variation met en lumière la complexité de ces réponses. Le premier patron est observé au cours de l'intervalle d'ossification soutenue alors que le second patron se produit une fois que le rythme de l'ossification ralentit. Ces résultats soulignent l'importance de mieux comprendre les changements morphologiques complexes et coordonnés qui se produisent depuis le tout début de l'ontogénie et de quelle façon ces derniers sont influencés par les conditions environnementales au cours du développement. L'ossification plus hâtive observée chez les ombles de ruisseau est interprétée comme une réaction à la demande locomotrice mais il serait pertinent de déterminer si une ossification plus rapide du squelette post-crânien améliore les performances de nage. En effet, les changements dans le développement musculaire et osseux induits par la vélocité du courant ainsi que les conséquences de la plasticité du système musculo-squelettique sur la performance de nage au cours du développement restent à explorer.

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