Population differences in the onset of cranial ossification in pumpkinseed (*Lepomis gibbosus*), a potential cost of rapid growth

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Abstract: Interspecific comparisons suggest that a trade-off exists between development and somatic growth rate. We provide evidence for a trade-off between cranial ossification and growth rate within a single species, the pumpkinseed sunfish (*Lepomis gibbosus*). We compare fish derived from two populations known to differ in their somatic growth rates. Fish were hatched from eggs and maintained under common conditions. Juveniles were sampled weekly through the first 8 weeks after hatching, preserved, and stained to determine the onset of ossification of superficial cranial bones. Fish from both populations showed a similar pattern of ossification in the cranial bones, but fish from the fast-growth population showed an average delay of 2 days in the onset of ossification for each bone. As a result, the fast-growth fish were both older and larger at each stage of ossification. We suggest that such a delay in ossification may have fitness effects through feeding ability, swimming ability, and (or) defense against predators.

Résumé : Des comparaisons entre espèces montrent qu'il y a compromis entre le développement et le taux de croissance somatique. Nous montrons l'existence d'un compromis entre l'ossification crânienne et le taux de croissance chez des individus d'une même espèce, le crapet-soleil (*Lepomis gibbosus*). Nous comparons des poissons issus de deux populations qui montrent des taux de croissance somatique différents. On a fait éclore de oeufs de chacune de ces populations et maintenu les poissons dans les mêmes conditions. On a recueilli des juvéniles à chaque semaine durant les huit semaines qui ont suivi l'éclosion, et on les a préservés et colorés pour déterminer le moment où débutait l'ossification des os crâniens superficiels. Les poissons des deux populations ont montré des profils d'ossification similaires, mais on a observé chez ceux de la population à croissance somatique rapide que l'ossification de chaque os débutait en moyenne deux jours plus tard. Ainsi, les poisson à croissance rapide étaient plus vieux et plus gros à chaque stade d'ossification. Nous pensons qu'un tel retard dans l'ossification peut avoir des effets sur la valeur adaptative en affectant la capacité de s'alimenter, la nage et (ou) la capacité de se déendre contre les prédateurs.

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Introduction

Growth rate is an important component of many fitness traits including vulnerability to predation, competitive ability, and fecundity. In fact growth rate is often used as a surrogate measure of fitness (e.g., Schluter 1995). However, genetic variation for growth rate is large both among species and among populations within a species (reviewed in Arendt 1997). Understanding what drives this variation is critical for understanding fitness in ecological studies as well as choos-ing appropriate populations for restocking lakes (e.g., Conover and Schultz 1995) and as a primary target of artificial selection in fisheries.

A recent concern of fisheries is that selection for rapid growth in hatchery stock may have resulted in competitively superior fish. When hatchery fish are released or escape, they may then outcompete local populations and drive wild

strains extinct. This scenario depends upon the assumption that rapid growth translates directly into greater fitness. However, a few recent studies have found costs of rapid growth including reduced starvation resistance in a butterfly (Gotthard et al. 1994), increased risk taking to obtain food in tadpoles (Skelly 1996), and reduced swimming performance in fish (e.g., Gregory and Wood 1998). Another likely cost of rapid growth is a decrease in development rate (reviewed in Ricklefs et al. 1994). In this case, "growth" is restricted to mean an increase in size, while "development" refers to in-creased function through cellular differentiation. The trade- off between growth and development occurs because most cells are no longer able to divide and thus contribute to growth after they differentiate and begin to take on their ma-ture function. Thus the optimal growth rate for an individual depends upon the balance between the advantages of in-creasing size rapidly and the need for either rapid or exten-sive development.

Development of a number of tissues, especially muscle, bone, and nerves, has been proposed to limit growth rate for the entire body (Ricklefs et al. 1994). Previous work has focused primarily on skeletal muscle, especially in birds where muscle is critical for thermoregulation as well as locomotion (Starck and Ricklefs 1998) and in fish where recruitment of new muscle fibers has been correlated with growth rate (Weatherley et al. 1988). Another likely rate-limiting tissue

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Table 1. Order in which cranial bones ossify in pumpkinseed.

Ossification stage	Most recently ossified bone
0	No ossification
1	Premaxill, maxilla, and dentary
2	Cleithrum
3	Opercle
4	Quadrate
5	Preopercle
6	Angular
7	Interopercle or articular
8	Ineropercle and articular
9	Urohyal
10	Supracleithrum
11	Posttemporal
12	Branchiostegal rays
13	Subopercle
14	Ventral hypohyal
15	Pterotic
16	Frontal
17	First infrorbital or supraoccipital
18	First infraorbital and supraoccipital
19	Dermosphenotic
20	Supratemporal
21	Lateral ethmoid

Note: The order could not be determined for bones listed on the same line. Ossification stage used for statistical analysis.

is the skeleton of vertebrates. For example, Starck (1994) compared ossification in the long bones of the leg and wing for the buttonquail (*Turnix suscitator*), a slow-growing species of bird, and the budgerigar (*Melopsitacus undulatus*), a fast-growing species. Indicative of a trade-off between growth rate and skeletal development, the bones of the buttonquail contained a greater proportion of ossified tissue relative to cartilage than the budgerigar at any given developmental stage, i.e., ossification occurred at a faster rate in the slower growing species. It is, however, difficult to generalize from only two species especially if size, age, and developmental stage do not show the same relationship in each species. For example, the budgerigar has more time to develop a smaller skeleton than the buttonquail (Starck 1994; but see Starck 1998). Moreover, for a trade-off between growth and development to have evolutionary significance, it must exist at the intraspecific level (Futuyma and Moreno 1988). A stronger test for this trade-off would be to compare two conspecific populations known to have different growth rates.

Populations of pumpkinseed sunfish (*Lepomis gibbosus*) are known to differ predictably in intrinsic growth rate depending upon the presence or absence of bluegill sunfish (*Lepomis macrochirus*) (Arendt and Wilson 1997). Juveniles of both species are confined by predators to the shallow, weedy zones of lakes where they compete for littoral invertebrates (Mittelbach 1984). However, when they exceed the gape limit of most predators (approximately 70 mm standard length), bluegill move into the open water where they specialize on zooplankton. At about the same size, pumpkinseed become large enough to effectively crush gastropods,

their primary adult diet. This means that there is an advantage for pumpkinseed in lakes with bluegill to grow quickly in order to reach a size-refuge from competition. Pumpkinseed in lakes with bluegill have evolved faster growth rates than in lakes without bluegill (Arendt and Wilson 1997). This study uses the known differences in growth rate to examine the trade-off between growth rate and skeletal development in pumpkinseed.
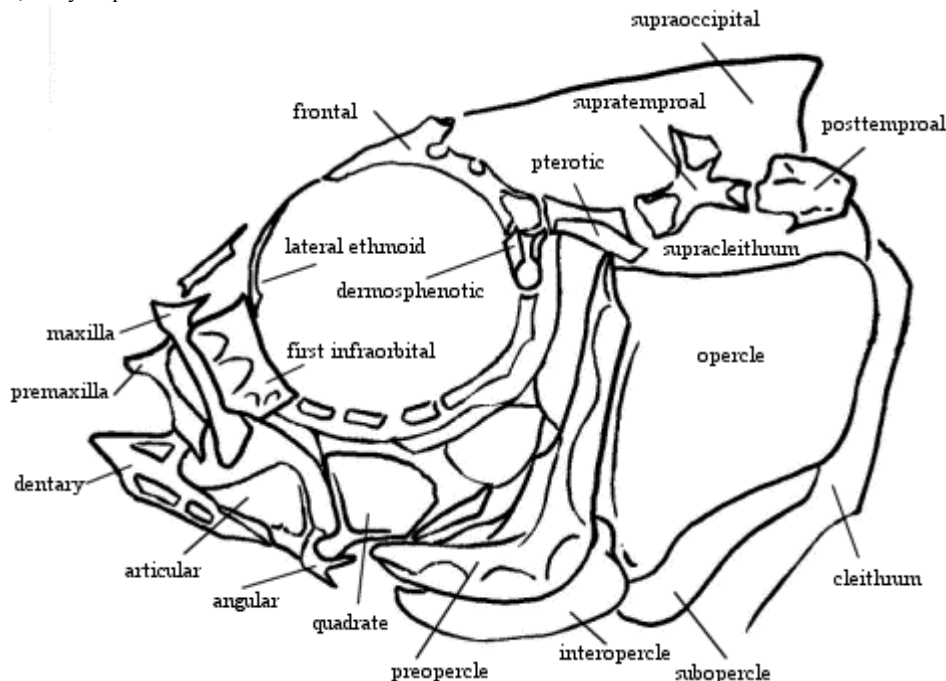
Methods

Breeding stock

Under common conditions, pumpkinseed from Lake Opinicon grow more quickly than pumkinseed from Warrens Pond (Arendt and Wilson 1997). Adults from these populations were stocked into separate breeding ponds at the Cornell Experimental Pond Facility in Ithaca, N.Y., during the Spring of 1995 (details of lakes in Arendt and Wilson 1997). Eggs were collected from June 1-10, 1996, by placing clay tiles in nests of male pumpkinseed. Tiles were checked daily for presence of eggs. We chose to compare two populations rather than all six because of the intensive labor needed to rear pumpkinseed and to minimize variation in the timing of hatching. However, because differences in growth rate are due to evolutionary history in the presence or absence of bluegill rather than among populations (Arendt and Wilson 1997), the effect detected here is probably general.

Eggs were transferred in water-filled coolers to our laboratory in Binghamton (travel time approximately 1 h). They were placed in 76-L aquaria in their native pond water with a 0.01% solution of Malachite green to reduce fungal growth. When approximately 90% of a brood had hatched (designated day 0), the entire brood was transferred to a 38-L aquarium filled with conditioned tap water, one brood per tank. Opinicon eggs were derived from four nests, and Warrens eggs were derived from six nests. Each aquarium had an initial density of 180-200 fry. Fish were fed newly hatched *Artemia* (approximately $600L^{-1}$) once a day immediately following the onset of exogenous feeding. This density was sufficient to ensure that food was continuously available. Fish were collected daily for the first week after hatching, and then at weekly intervals for 8 weeks (day 56 after hatching). Collections were spread evenly over each brood to ensure maximum genetic diversity in each sample, with approximately 15 fish collected from each population except the final week when only 10 fish were available. Fish were fixed in 10% neutral buffered formalin for 1 month.

Ossification rate

Fish were stained for cartilage using Alcian blue and bone using Alizarin red following Song and Parenti (1995), and stored in 90% ethanol. To allow us to qualitatively compare the degree of ossification for a given bone, we stained for both cartilage and bone. However, because the Alcian blue stain takes place in an acidic solution, this method has the potential of decalcifying bone. As a result, we probably did not detect the earliest point at which ossification begins. Because all fish of a given age were handled in like manner, this should not alter the relative onset of ossification for any given fish. Bones were scored for the appearance of any red, a sign that ossification had begun, using a dissecting microscope. The bones used are described in Table 1 and Fig. 1. Only superficial bones of the cranium that could be scored unambiguously for the onset of ossification were used. Although easily identifiable, a number of bones (e.g., ceratohyal, nasal, and supraethmoid) showed no ossification by 8 weeks when collection was terminated and thus were not included.

Fig. 1. Cranial skeleton of pumpkinseed. Two 10-month-old pumpkinseed whose crania were nearly fully ossified were dissected to determine size and poisition of bones. Bones of the suspensorium are visible only from the ventral and are not shown here. In addition, only superficial bones are shown.

Statistics

Each fish was assigned an ossification stage equivalent to the most recently ossified bone (Table 1). Populations were then compared for the relative degree of ossification among fish of a given age. The distributions were clearly not normally distributed, so populations were compared using a Mann-Whitney U test for non-parametric data (Sokal and Rohlf 1981).

Results

We were able to order the ossification of each bone with the exception of the first three: the premaxilla, maxilla, and dentary (Table 1). We found some variation in the order in which certain bones ossified, a situation also noted by Mabee and Trendler (1996) in *Betta splendens* and Cubbage and Mabee (1996) in *Danio rerio*. In pumpkinseed, ambiguity in order occurred for two bone pairs: the interopercle and articular and the first infraorbital and supraoccipital. Because of the small sample size, we could not determine which order was more typical for either pair.

We did not detect any ossification in either population until week 4. At week 4, only 21% of Lake Opinicon fish showed any ossification, while 75% of the Warrens Pond fish had at least the premaxilla, maxilla, and dentary beginning to ossify. Warrens fish were at a significantly greater stage of ossification during weeks 4 ($U = 159$; $p = 0.01$), 5 ($U = 181.5$; $p = 0.003$), and 7 ($U = 160.5$; $p = 0.03$). Although they tended to be more advanced during week 6, this difference was not significant ($U = 119$; $p = 0.5$) (Fig. 2). Only 10 fish were collected from each population in week 8. Of these, all Warrens fish were at stage 21 while nine of 10 Opinicon were at stage 21 and the remaining fish was at stage 20. This difference was not significant ($U = 55$; $p = 0.3$).

To help visualize the differences in ossification on a by-the-bone basis, we also calculated the average age at which each ossification stage was reached. These were estimated as follows. For Opinicon fish, 21% of the 28-day-old fish showed ossification of the premaxilla, maxilla, and dentary bones while 100% of the 35-day-old fish showed ossification of these bones. The mean age was thus estimated as $(28 \times 0.21) + (35 \times 0.79) = 33.5$ days (we use 79% for the 35-day-old fish because presumably, 21% of these fish would have shown ossification before age 28 if we had tested them then). Visualized this way, it is clear that ossification in Warrens fish occurs about 2 days earlier than in Opinicon fish for nearly all bones (Fig. 3). Because of the low resolution of sampling and the potential for decalcification of bone while staining for cartilage, these estimates probably overestimate absolute ages of ossification. These results should be interpreted in terms of relative timing between populations and not as an estimate of the actual age of ossification for each bone.

Discussion

Our data indicate that Lake Opinicon fish showed a delay in the onset of cranial ossification relative to the Warrens Pond fish. As a result, Opinicon fish are both older and larger than Warrens fish when they are at the same ossification state. The genetic propensity for faster growth in Opinicon fish should exaggerate the size effect. Comparing size at age (Fig. 4), a 2-day delay in ossification for Opinicon fish would translate into a size difference of about 0.5 mm total length on average greater than a Warrens fish.

Fig. 2. Distribution of pumpkinseed ranked by ossification stage (Table 1) at each sampling age. Solid bars, Warrens fish; open bars, Opinicon fish. Populations were compared at each age using a Mann-Whitney U test (*$p < 0.05$; **$p < 0.01$).
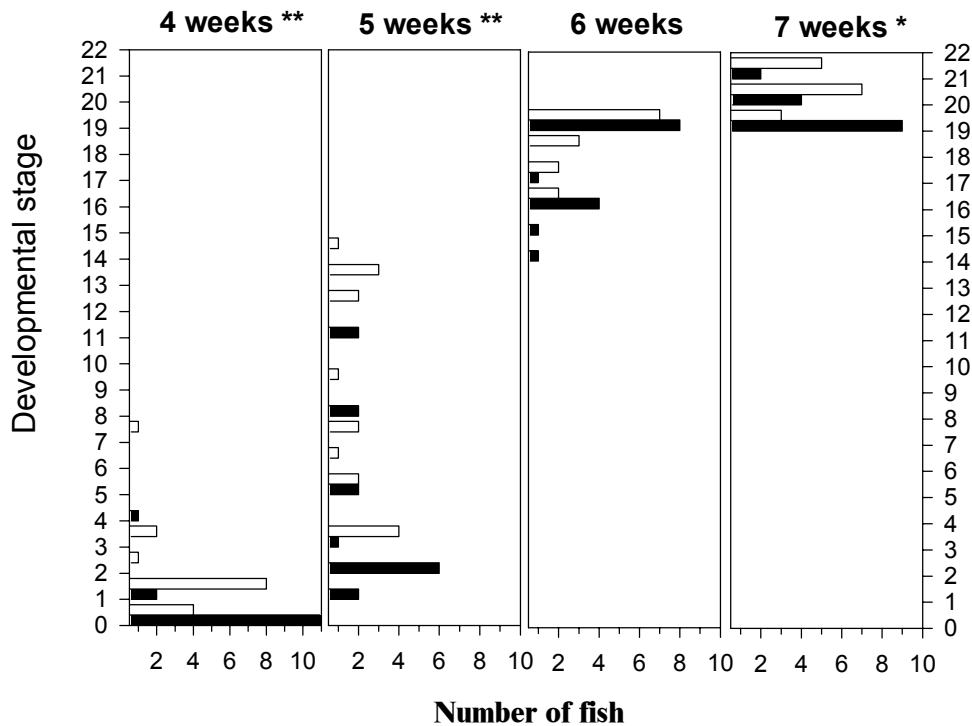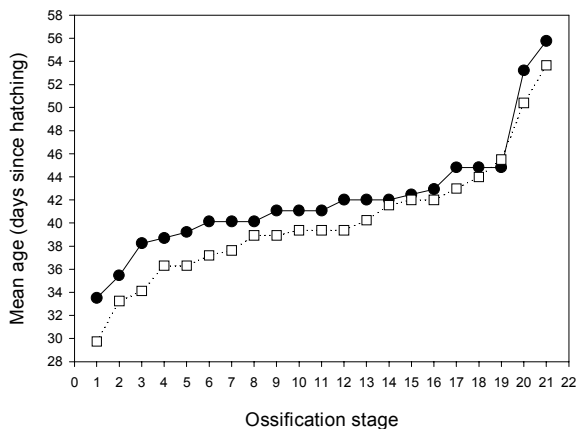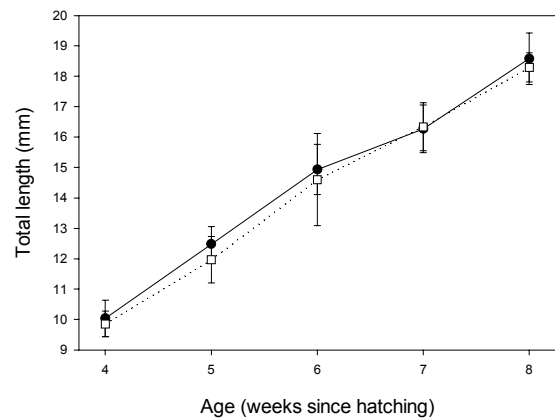

Fig. 3. Mean age at which each ossification stage (estimated from data in Fig. 2) is reached for Warrens pumpkinseed (squares, broken line) and Opinicon pumpkinseed (circles, solid line). Note that Warrens fish ossify at a younger age for all bones except the dermosphenotic, stage 19.

Fig. 4. Mean and total lengths and standard deviations for Warrens and Opinicon pumpkinseed at each age (symbols as in Fig. 3). Taken with Fig. 3, a 2-day delay in ossification results in Opinicon fish being approximately 0.5 mm larger than Warrens fish at each stage.

This difference is dramatic considering that fish grew about 10 mm over the 5 weeks of this study.

The differences in timing of ossification may be due to a delay in the onset of ossification for Opinicon fish, a slower rate of ossification, or both. Starck (1994) described a difference in the rate of ossification, with a fast-growing species of bird ossifying more slowly than a slow-growing species. Alternatively, Palmer (1981) has suggested that mineralization occurs at a nearly constant rate in all organisms regard-

less of growth rate. Although Palmer (1981) originally suggested this only for the calcium carbonate skeleton of mollusks, the calcium phosphate skeleton of vertebrates appears to be produced in a like manner (Anderson 1985). If mineralization occurs at a constant rate, then the difference between Opinicon and Warrens fish we describe probably does reflect a difference in the onset of ossification. The current data do not allow us to distinguish between rate or the onset of ossification.

We interpret the population differences in relative timing

355

Can. J. Fish Aquat. Sci. Vol. 57, 2000

of ossification in terms of a trade-off between growth rate and cranial ossification, but because only two populations were compared this conclusion remains tentative given the following alternative explanations. The difference in ossification may also reflect an adaptation to water pressure, assuming that the Warrens fish habitually occupy greater depths than the Opinicon fish. We think this unlikely, as sunfish of this age are typically found in very shallow water (Mittelbach 1984), and because Warrens Pond and Lake Opinicon have similar average depth (Fox 1994). Ossification rate may also reflect adaptation to differences in calcium availability. Loss of skeletal elements in some populations of threespine stickleback (*Gasterosteus aculeatus*) has been attributed to low calcium level (Bell et al. 1993). We think that this explanation is unlikely because our measures indicate that Warrens Pond and Opinicon Lake have similar levels of free calcium (38 and 35 mg·L^{-1}, respectively). Finally, timing of ossification may have no fitness consequences, the differences between populations being due to random drift rather than to a trade-off with growth rate. We suspect, however, that timing of ossification does have important fitness consequences. In most species of fish, as in the present study, bones associated with feeding and respiration often ossify first. This pattern has often been interpreted as adaptive (e.g., Mabee and Trendler 1996), although to our knowledge this has never been tested empirically. It is also possible that this pattern of ossification is a developmental constraint on the ossification of vertebrates in general, as Smith (1997) has recently shown that these bones are also the first to ossify in a broad phylogenetic survey of mammals. Another likely cost of delayed skeletal development relates not to the cranial skeleton but to the post-cranial skeleton. A delay in ossification of the pectoral and pelvic girdle as well as of fin rays may compromise maneuverability. Likewise, delayed ossification of vertebrae probably decreases swimming efficiency. In fact, several recent studies have shown that rapid growth reduces a fish's ability to maintain position in a current (Gregory and Wood 1998 and references therein). Whether this is due to differences in muscle development or skeletal strength is not known. A third potential advantage of early ossification may be protection from predators. Several species of fish are known to show scars indicative of failed predation attempts (Smith and Lemly 1986; Reist et al. 1987; Reimchen 1992), and highly ossified bones, scales, and fin spines would probably contribute to the likelihood of an individual surviving such an event. The adaptive value of an ossified skeleton is greatly in need of empirical testing. Pumpkinseed may be ideal for such testing because it provides individuals at the same age that differ in the degree of ossification. The effects of ossification on performance can be tested on pumpkinseed without the confounding factor of body size or the problems inherent in comparing different species.

We tentatively interpret the delay in ossification rate as a cost that is compensated for in Opinicon pumpkinseed by the benefit of rapid growth. Ecologically, rapid growth is advantageous under competition with bluegill because faster growth rate is most apparent when food is limited. On the other hand, slower growth is advantageous in lakes where bluegill are absent and food is abundant. This is because the cost of slower intrinsic growth rate is minimized, but any potential benefit of rapid skeletal development should be maximized. This hypothesis requires testing at multiple food levels before it can be confirmed. Finally, if a delay in skeletal development is typical of selection for rapid growth, then the competitive advantages of rapid growth assumed for escaped hatchery fish may not be as great as is often expected.

Acknowledgements

References

Anderson, H.C. 1985. Normal biological mineralization. *In* Calcium in Biological Systems. *Edited by* R.P. Rubin, G.B. Weiss and J.J.W. Putney. Plenum Press, New York. pp. 599-606.

Arendt, J.D. 1997. Adaptive intrinsic growth rates: an integration across taxa. Q. Rev. Biol. **72:** 1-29.

Arendt, J.D., and Wilson, D.S. 1997. Optimistic growth: competition and an ontogenetic niche-shift select for rapid growth in pumpkinseed sunfish (Lepomis gibbosus). Evolution, 5**1:** 1946-1954.

Bell, M.A., Orti, G., Walker, J.A., and Koenings, J.P. 1993. Evolution of pelvic reduction in threespine stickleback fish: a test of competing hypotheses. Evolution, **47:** 906-914.

Conover, D.O., and Schultz, E.T. 1995. Phenotypic similarity and the evolutionary significance of countergradient selection. Trends Ecol. Evol. **10**: 248-252.

Cubbage, C.C., and Mabee, P.M. 1996. Development of the cranium and paired fins in the zebrafish *Danio rerio* (Ostariophysi, Cyprinidae). J. Morphol. **229:** 121-160.

Fox, M.G. 1994. Growth, density, and interspecific influences on pumpkinseed sunfish life-histories. Ecology, **75:** 1157-1171.

Futuyma, D.J., and Moreno, G. 1988. The evolution of ecological specialization. Annu. Rev. Ecol. Syst. **19:** 207-233.

Gotthard, K., Nylin, S., and Wiklund. C. 1994. Adaptive variation in growth rate: life history costs and consequences in the speckled wood butterfly, *Pararge aegeria*. Oecologia, **99**: 281-289.

Gregory, T.R., and Wood, C.M. 1998. Individual variation and the interrelationships between swimming performance, growth rate, and feeding in juvenile rainbow trout (*Oncorhynchus mykiss*). Can. J. Fish. Aquat. Sci. **55:** 1583-1590.

Mabee, P.M., and Trendler, T.A. 1996. Development of the cranium and paired fins in *Betta splendens* (Teleostei: Percomorpha): intraspecific variation and interspecific comparison. J. Morphol. **227:** 249-287.

Mittelbach, G.G. 1984. Predation and resource partitioning in two sunfishes (Centrarchidae). Ecology, **65:** 499-513.

Palmer, A.R. 1981. Do carbonate skeletons limit the rate of body growth? Nature (Lond.), **292:** 150-152.

Reimchen, T.E. 1992. Injuries on stickleback from attacks by a toothed predator (*Oncorhynchus*) and implications for the evolution of lateral plates. Evolution, **46:** 1224-1230.

356

Can. J. Fish. Aquat. Sci. Vol. 57, 2000

Reist, J.D., Bodaly, R.A., Fudge, R.J.P., Cash, K.J., and Stevens, T.V. 1987. External scarring of whitefish, *Coregonus nasus* and *C. clupeaformis* complex, from western Northwest Territories, Canada. Can. J. Zool. **65:** 1230-1239.

Ricklefs, R.E., Shea, R.E., and Choi, I. 1994. Inverse relationship between functional aturity and exponential growth rate of avian skeletal muscle: a constraint on evolutionary response. Evolution, **48:** 1080-1088.

Schluter, D. 1995. Adaptive radiation in sticklebacks: trade-offs in feeding performance and growth. Ecology, **76**: 82-90.

Skelly, D.K. 1996. Tadpole Communities. Am. Sci. **85**: 36-45.

Smith, K.K. 1997. Comparative patterns of craniofacial development in eutherian and metatherian mammals. Evolution, **51:** 1663-1678.

Smith, R.J., and Lemly, A.D. 1986. Survival of fathead minnows after injury by predators and its possible role in the evolution of alarm substance. Environ. Biol. Fishes, **15:** 147-149.

Sokal, R.R., and Rohlf, F.J. 1981. Biometry. W. H. Freeman and Company, New York.

Song, J., and Parenti, L.R. 1995. Clearing and staining whole fish specimens for simultaneous demonstration of bone, cartilage, and nerves. Copeia, **1995:** 114-118.

Starck, J.M. 1994. Quantitative design of the skeleton in bird hatchlings: does tissue compartmentalization limit posthatching growth rates? J. Morphol. **222:** 113-131.

Starck, J.M. 1998. Structural variants and invariants in avian embryonic and postnatal development. *In* Avian growth and development. *Edited by* J.M. Starck and R.E. Ricklefs. Oxford University Press, New York. pp. 59-88.

Starck, J.M., and Ricklefs, R.E. 1998. Avian growth and development. Oxford University Press, New York.

Weatherley, A.H., Gill, H.S., and Lobo, A.F. 1988. Recruitment and maximal diameter of axial fibres in teleosts and their relationship to somatic growth and ultimate size. J. Fish Biol. **33:** 851-859.