Research Article

Otolith micro-structure analysis of rainbow trout alevins (*Oncorhynchus mykiss*) under rearing conditions

Guillermo Moyano[1,2], Guido Plaza[1] & María Isabel Toledo[1]
[1]Escuela de Ciencias del Mar, Pontificia Universidad Católica de Valparaíso,
P.O. Box 1020, Valparaíso, Chile
[2]Instituto de Fomento Pesquero, División de Investigación Pesquera
Blanco 839, Valparaíso, Chile

ABSTRACT. Otolith microstructure (MO) analysis was used to back-calculate growth patterns from hatching to the yolk-sac absorption in Rainbow trout alevins (*Oncorhynchus mykiss*), under experimental conditions, from winter to spring in Central-Chile. MO showed the following main features: (i) occurrences of multiple primordia in the central region of otolith (MP); (ii) several increments (22 ± 3 rings) before a prominent hatch check surrounding MP and (iii) existence of a very distinctive check associated to yolk-sac absorption. Further findings were the validation of daily periodicity of micro-increments and a significant linear relationship ($P <$ 0.001; $r^2 = 0.91$) between the maximum otolith length (MOL) and total length (TL) of alevins. The linear MOL-TL relationship validated the use of the biological intercept method to back-calculate daily growth rates. Growth rates profiles followed a decreasing trend from hatching to the end of yolk-sac period (16 ± 1 days), ranging from 0.34 mm d^{-1} to 0.22 mm d^{-1} (0.30 ± 0.037). MO analysis demonstrated to be a powerful tool in back-calculating growth patterns of alevins once they had absorbed their yolk-sac.

Keywords: *Sagittae*, otolith, growth rate, *Oncorhynchus mykiss*, Chile.

Análisis de la micro-estructura de otolitos en alevines de trucha arcoiris (*Oncorhynchus mykiss*) en cautiverio

RESUMEN. La micro-estructura de otolitos (MO) fue utilizada para retro-calcular los patrones de crecimiento, desde la eclosión hasta la absorción del saco vitelino, en alevines de trucha arcoiris (*Oncorhynchus mykiss*), en la zona central de Chile. La MO mostró tres características principales: (i) ocurrencia de múltiples primordios en la región central de los otolitos (MP), (ii) formación de varios micro-incrementos antes de la eclosión (22 ± 3 micro-incrementos) (iii) y existencia de una marca distintiva correspondiente a la absorción del saco vitelino. Otros resultados fueron la validación de la periodicidad diaria de micro-incrementos y la existencia de una relación lineal significativa ($P < 0,001$; $r^2 = 0,91$) entre la longitud máxima del otolito (LMO) y la longitud total de los alevines (L_T). La relación lineal L_{MO}-L_T validó el uso del método de intercepto biológico, para retro-calcular las tasas diarias de crecimiento (TDC). Las TDC mostraron una tendencia decreciente desde la eclosión hasta 6 días después de la absorción del saco vitelino (16 ± 1 días), las TDC variaron desde los 0,22 mm d^{-1} a los 0,34 mm d^{-1} (0,30 ± 0,037). La MO demostró ser una herramienta efectiva para el análisis retrospectivo de los patrones de crecimiento en alevins una vez absorbido su saco vitelino.

Palabras clave: *Sagitta*, otolitos, tasa de crecimiento, *Oncorhynchus mykiss,* Chile.

Corresponding author: Guillermo Moyano (guillermo.moyano@ifop.cl)

INTRODUCTION

Otolith microstructure analysis has allowed great progress in studies on growth dynamics in the early stages of teleost fishes in the last two decades (Cowen, 1991; Fowler & Short, 1996; Shafer, 2000; Hindell & Jenkins, 2004; John *et al*., 2010; Wu *et al*., 2011). In addition to age and growth, otoliths can record events

of the early life histories, such as hatching, yolk sac absorption, first feeding (Neilson & Geen, 1985; Mugiya & Oka, 1991; Carreño, 2010), ontogenetic transitions from pelagic to benthic environment, among others (Victor, 1982; Sponaugle & Cowen, 1994; Hindell & Jenkins, 2004; Kohn & Clements, 2010). The potential of otolith microstructure still remains largely unexplored in many species; therefore, developing detailed descriptions of the micro-structure on a target species is the first stage to subsequently lead to potential applications (Plaza *et al.,* 2001). This process must necessarily be accompanied by the validation of the periodicity of the formation of micro-increments before revealing the early life history of a given species (Geffen, 1992).

Recently new applications of otolith microstructure analysis have contributed to reveal stock structure in a number of species by identifying population units showing an specific otolith growth pattern linked to a particular geographical area. Furthermore, patterns of otolith microstructure have also been useful in determining the contribution that juvenile growing in different nursery areas made to the adult population (Bolles & Bess, 2000; Blick, 2001; Zimmerman, 2003; Turan *et al.*, 2006; Clardy, 2008; John *et al.*, 2010); Quiñonez-Velásquez & López-Olmos, 2011). The otolith microstructure has also been used in evaluating the contribution of stock enhancement plans, particularly in anadromous fish, because the micro-structure of otolith from reared juveniles differ markedly from those juveniles spawned in natural conditions (Neilson & Geen, 1985; Mugiya & Oka, 1991; Larsen & Reisenbichler, 1993; Volk *et al.*, 1995). The current study attempted to develop an otolith microstructure analysis in juveniles of the rainbow trout (*Oncorhynchus mykiss*) in captivity, establishing firstly methodological procedures of preparation and interpretation of otolith micro-structure, followed by a validation process of primary micro-increments.

MATERIAL AND METHODS

Rearing protocols and otolith preparation proce-dures

This study was carried out from May to September 2007 in a pisciculture located at Rio Blanco, Central Chile, in the Saladillo's village (32º55'50''S, 70º16'54''W) about 35 km far from Los Andes city. A sub-sample of 384 specimens of rainbow trout alevins (*Oncorhynchus mykiss*) were taken from a large sample reared under uncontrolled biotic and abiotic factors. During the period of incubation, mean monthly temperature recorded twice a day (08.00 and 18.00 hrs), ranged from 4.0 to 12.0ºC. Temperature variations during the embryonic, larval development and yolk-sac absorption ranged from 4-8, 7-9 and 8-12°C, respectively. The development of individuals during the study period was normal, with low mortality rates of about 1.4% per month and a hatching rate of 97%.

Juveniles were collected from incubation trays and then preserved in 100 ml bottles with a 95% alcohol solution. Most individuals used for this study were sacrificed one week after the complete yolk-sac absorption. The total length of each specimen was measured to the nearest 0.1 mm and then sagitta otoliths were extracted using dissecting needles under a stereomicroscope (2S Luxeo Labomed©). Otoliths were then embedded with its distal face down in a drop of fingernail oil. To reveal primary increments otoliths were polished by hand using sandpapers ranging from 1500 and 2000 in grain size. The micro-increments were observed under a light microscope (Digi Digital February 1500©), identified as concentric units encompassing a light and dark zone. Each pair of these units was identified as primary micro-increment. An analysis of variance (One-way ANOVA) was used to test for significant differences in the numbers of micro-increments between right and left otolith.

Back-calculated daily growth rates

Daily growth rates were back-calculated using the biological intercept method (Campana, 1990), defined as:

$$L_i = L_c + (R_i - R_c) \times (L_c - L_0) \times (R_c - R_0)^{-1} \qquad (1)$$

Where L_i is the fish length at the time of formation of the i-th primary micro-increment, R_i is the otolith radius at the time of formation of the i-th micro-increment primary or other mark, L_C and R_C are the fish length and otolith radius at the time of capture. L_0 and R_0 are the fish length and otolith radius at the time of initial proportionality between somatic growth and otolith size (*i.e.*, the biological intercept). In this case it was assumed that this proportionality started at hatching time, in which alevins had an average size of 18.4 ± 0.5 mm. The daily growth rates of individual fish (DGR) were calculated subtracting a given length by its previous value using the formula *DGR = (Li-Li-1)*.

Otolith size-fish size relationships

Back-calculation of previous length depends upon the existence of a linear relationship between otolith size and fish size (Campana, 1990). This relationship was evaluated using linear regression analysis of total fish length (TL) on maximum otolith length (MOL). A second requirement is to validate the periodicity of the

formation of micro-increments, in order to reconstruct the patterns of growth to a known previous period (Francis, 1990; Jones, 1992). For this purpose, the relationship between the numbers of days after hatching and the number of micro-increments formed after hatch check was evaluated by testing if the slope was significantly $\neq 1$ using a t-student test, as $t_0 = (b_1 - 1)/(se \times (b_1))$, where, t_o is the statistical test, b_1 is the slope of the regression, (b_1) is the standard error of the slope. For further evaluate the proportionality between the otolith size-fish size relationship the logarithmic method described by Fuiman (1983) was used as $Y = b X^a$, where Y is the maximum otolith size, X is the total fish length, a is growth coefficient, and b is the is the intercept. In this procedure $a = 1$ denotes that the relationship between two variables is isometric, $a > 1$ the otolith growth is faster in proportion to the fish size, and when $a < 1$ the fish growth is faster in proportion to the otolith growth.

RESULTS

Overview of the otolith microstructure of rainbow trout alevins

Sagitta otoliths of rainbow trout alevins showed an oval structure with a sharped rostrum and a post-rostrum with a circular shape and irregular contour (Fig. 1a). A characteristic feature was the presence of several primordiums in a single otolith (Fig. 1b). A primary micro-increments showed the characteristics L and D-zones formed on regular concentric pattern from the primordiums to the otolith edge. Primary micro-increments throughout the post-rostrum were distinctive (Fig. 2a) and regular unlike the rostrum where they showed lower resolution. Primary increments counted through post-rostrum did not show significant differences between the left and right sagiitae (ANOVA, $P = 0.416$, n = 22). A distinctive feature was the presence of two prominent micro-increments "checks" clearly visible in all sagitta otoliths. In a sub-sample of 50 otoliths analyzed the first marks was observed on average at 22 micro-increments from the primordium (Fig. 2b). It was assumed that the first check was formed at hatching time, while the second check, characterized by a depression in the structure of micro-increment, was associated to yolk-sac absorption (Fig. 2c) These inferences were validated by observing larvae otoliths of newly hatched alevins from a single cohort (n = 16), which showed a mean of 22 micro-increments (range 21-25, n = 16) from the primordiums to the last ring in the otolith edge. The formation of the second mark (yolk-sac absorption; (Fig. 2d) occurred at a mean of 17 primary increments after the first check.

Otolith size-fish size relationships

The relationship between TL and MOL of alevins was significantly linear (TL = 36.35 + 4.4MOL; $P < 0.0001$; $r^2 = 0.91$; n = 384), demonstrating the proportionality between both variables (Fig. 3). This fact was confirmed by the allometric relationships between somatic growth and otolith growth (TL = b MOL^a; TL= 0.073 OL$^{1.05}$ ($r^2 = 0.91$, $P < 0.05$ and n = 314) where the growth coefficient (a) resulted to be equal to 1.

Validation of the periodicity of formation of primary increments

The t-student statistic showed that the frequency of formation of micro-increments was 1.03 days ($b1$), (SE = 0.008, $t = 3.70$). The relationship between the number of micro-increments and elapsed days after hatching was described by a highly significant linear model (Fig. 4), Y = 1.0054 X. Intercept (0), $r^2 = 0.99$).

Back-calculated daily growth rates

Figure 5 shows the average back-calculated daily growth rates (DGR) derived from the analysis of 384 alevins, from the formation of the first micro-increment up to 6th days after the yolk-ac absorption.
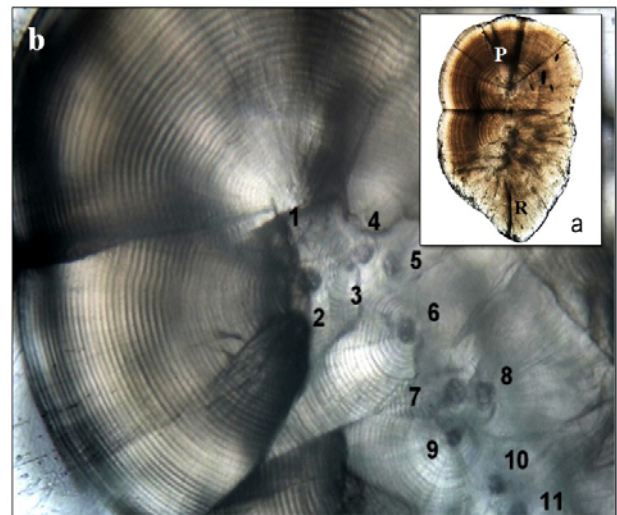

Figure 1. a) Sagittae otolith of a *Oncorhynchus mykiss* alevin illustrating its main parts. P = post-rostrum, R = rostrum; b) presence of multiple primordium (denoted with numbers) in the central region of otoliths of rainbow trout. Zoom: 400x, alevin length: 26.6 mm.

Figura 1. a) Otolito sagital de un alevín de *Oncorhynchus mykiss* ilustrando sus partes principales. P = cauda; R = rostro; b) presencia de primordios múltiples (indicados por números) en los otolitos de truchas arcoiris. Aumento: 400x, talla alevín: 26,6 mm.
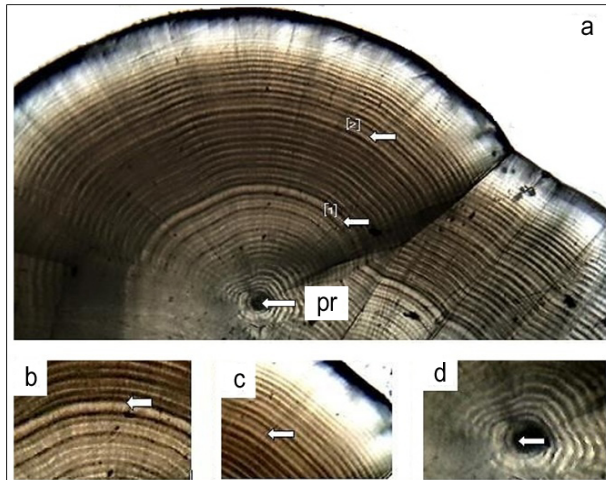
Figure 2. Otolith micro-structure of *Sagitta*e from alevins *Oncorhynchus mykiss* illustrating the formation of hatch check at the 21th micro-increment (1) and first-feeding check at 34th micro-increment (2). The number of increments were counted from the primordium (pr) nearest to the post-rostrum. Enlarged views near to the yolk-sac check (b), first-feeding check (c) and primordium (d). Zoom: 400x, alevin length = 26.4 (mm), maximum otolith length: 0.49 (mm).

Figura 2. (a) Micro-estructura de un otolito sagital de un alevin de *Oncorhynchus mykiss*. Ilustración de la marca de eclosión en el anillo nº21 (1), y la marca de absorción del saco vitelino (2) al micro-incremento nº34. El número de micro-incrementos fueron contados desde el primordio (pr) más cercano al cauda. Fotografías aumentadas cerca de la marca de eclosión (b) primera alimentación (c) y del primordio (d). Aumento: 400x, talla alevín: 26,4 mm; longitud máxima del otolito: 0,49 mm.

DGRs ranged from 0.22 to 0.34 mm d^{-1} (mean: 0.30 ± 0.037 mm d^{-1}). The average curve of the back-calculated total length showed that juveniles rainbow trout completely absorbed their yolk-sac at a total length of 24 mm.

DISCUSSION

Characterization of otolith microstructure

The microstructure of sagittal otoliths of *Oncorhynchus mykiss* alevins was characterized by the following five distinctive features: (i) absence of significant differences in the amount of micro-increments between pairs of sagittae, (ii) occurrence of micro-increments before hatching, (iii) a linear relationship between the otolith size and fish size, (iv) primary micro-increments very distinctives from the primordium to the otolith edge, and (v) occurrence of
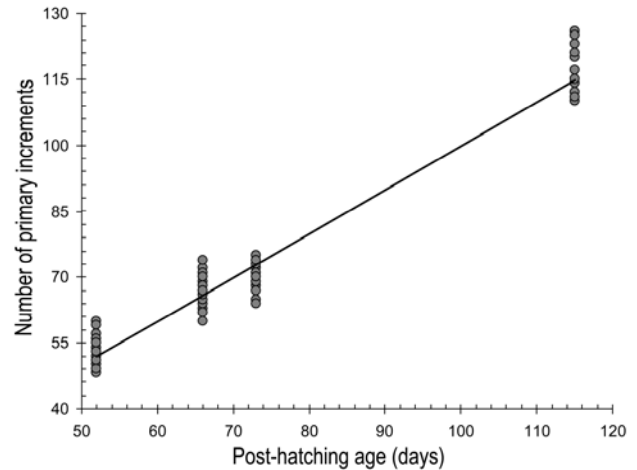
Figure 3. Linear regression between the number of post-hatching days and number of micro-increments in otoliths (s*agittae*) formed after hatch check in *Oncorhynchus mykiss* alevins. Equation: Y = 1.0054 X Intercept (0) (r² = 0.98).

Figura 3. Regresión lineal entre el número de días pos-eclosión y el número de micro-incrementos en los otolitos (s*agittae*) formados posterior a la marca de eclosión en *Oncorhynchus mykiss*. Ecuación: Y = 1,0054 X Intercepto (0) (r² = 0,98).
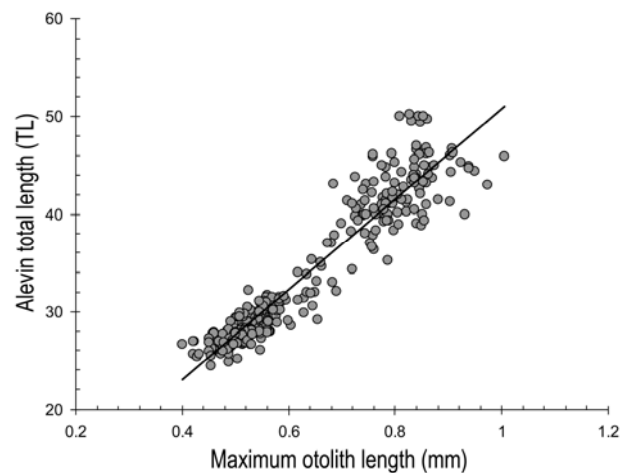

Figure 4. Linear regression between maximum length of the otolith (*sagittae*) and alevin total fry length of *Oncorhynchus mykiss*. Line was defined by the equation LT = 46.35 MOL + 4.3973 (r² = 0.90).

Figura 4. Regresión lineal entre longitud máxima del otolito (*sagittae*) y longitud total del alevín *Oncorhynchus mykiss*. La recta estuvo definida por la ecuación LT = 46,35 MOL + 4,3973 (r² = 0,90).

multiple primordia. Each of these five aspects are discussed in detail below.

At the beginning of this research one-way ANOVA did not show significant differences in the number of
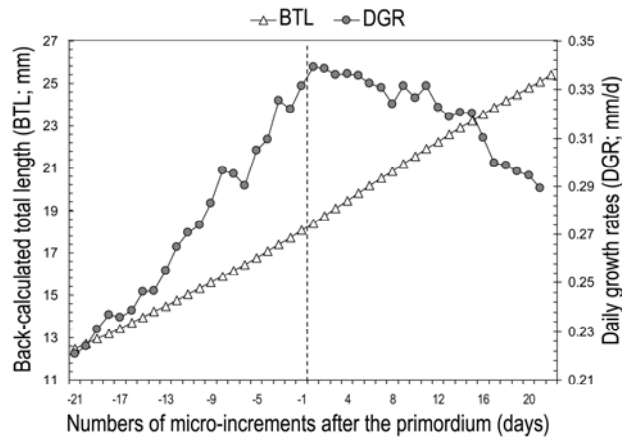
Figure 5. Changes of daily growth rate (mm day^{-1}) and back-calculated total length over age during the incubation stage of rainbow trout alevins (*Oncorhynchus mykiss*). Dashed line denotes the separation bewteen micro-increments formed before and after hatching.

Figura 5. Cambios en la tasa de crecimiento diaria (mm día^{-1}) y la longitud total retro-calculada (mm) en función de la edad, durante la etapa de incubación de alevines de trucha arcoíris (*Oncorhynchus mykiss*). La línea segmentada denota la separación entre los micro-incrementos formados antes y después de la eclosión.

daily increments between the left and right otolith; hence, in further analysis the otolith pair having the higher resolution of their micro-increments was used. This result is consistent with studies in other species, *i.e., Austromenidia regia* (Peñailillo & Araya, 1996), *Salmo salar* (Geffen, 1983; Carreño, 2010), *Sebastes inermis* (Plaza *et al*., 2001). This finding allowed us to reduce and optimize the working time associated with the extraction, handling and preparing otoliths for examination of their daily increments.

The otolith length-fish length relationship was significantly linear, demonstrating that otolith growth was proportional to the growth of fish for *Oncorhynchus mykiss*. This result showed that fish size can be estimated using the otolith size and also that individual growth patterns of juveniles back-calculated using the biological intercept method were reliable. Proportional relationships between fish size and otolith size have also been reported in different species of salmon, *e.g., Salmo salar* (Wright *et al*., 1990; Carreño, 2010), *Oncorhynchus keta* (Fukuwaka, 1998), *Oncorhynchus tshawytscha* (Neilson & Geen, 1982) and other teleost species, *i.e., Fundulus heteroclitus* (Radtke & Dean, 1982), *Engraulis ringens* (Hernandez & Castro, 2000); *Sebastes inermis* (Plaza *et al*., 2001), *Sardinops melanostictus* (Plaza *et al*., 2008). In some cases absence of proportionality

have also been observed (decoupling), but mainly in extreme conditions of both feeding and temperature (Mosegaard *et al*., 1988; Hare & Cowen, 1995; Baumann *et al*., 2005). Therefore, it is reasonable to hypothesize that proportionality in the otolith size-fish size relationship found in the present study are an indirect evidence that experimental conditions were suitable. In parallel, the allometric analysis performed between both variables confirmed the existence of an isometric proportionality between fish and otolith growth. Fuiman (1983) stated that isometry between two growth variables continues through adulthood; hence, a similar proportionality between otilith size and fish size could be expected in later stages of this species.

One of the most distinctive findings of this study was the presence of primary micro-increments very distinctive, which were visible after polishing, unlike those reported in yolk-sac larvae in other salmonids where the resolution was lower (Neilson & Geen, 1985; Van Der Walt & Faragher, 2002; Carreño, 2010). The high resolution of micro-increments could be a result of the uncontrolled conditions of light and water temperatures. Under these conditions, the alevins were exposed to natural fluctuations or photoperiod and daily temperature. Such variations might be reflected in the marked difference between the rich zone (L) and poor (D) in calcium carbonate, which led to easy identification of primary micro-increments. In this context, it should be noted that temperature changes alone have been successfully used experimentally for massive tagging salmon (Neilson & Genn, 1982, 1985; Ojanguren *et al*., 1999, Van Der Walt & Faragher, 2002), where temperature changes resulted in the deposition of dark and light bands in the otolith. These studies have demonstrated that fluctuations in tempe-ratures produce very distinctive micro-increments.

The formation of several primordia was another distinctive feature of the microstructure *Oncorhynchus mykiss* alevins. This micro-structural feature seems to be common in salmonids (Neilson & Geen, 1985; Fukuwaka, 1996; Van Der Walt & Faragher, 2002; Carreño, 2010), but it is rarely present in other teleost fish (Brother *et al*., 1983, Atherinidae). Although to date there is not a physiological explanation for these findings, Radtke & Dean (1982) proposed that the existence of multiple primordia may be a result of the long incubation period in these species as well as due to the large egg size. This inference is reasonable since, according to Breton (2007), the duration of the incubation stage in rainbow trout is relatively long, encompassing the period elapsed from spawning to the yolk-sac absorption, in approximately 710 degrees days.

Validation of the periodicity of formation of primary increments

In this study the number of micro-increments formed from hatching to the collection date of alevins matched with the number of days elapsed during the same period, enabling reliable comparison of juvenile growth rates on a daily basis. This finding is consistent with those reported by other studies of formation of micro-increments on a daily basis in salmon, *Oncorhynchus tshawytscha* (Neilson & Geen, 1982), *Salmo salar* (Geffen, 1983; Carreño, 2010), *Oncorhynchus keta* (Fukuwaka, 1996). Conversely, the frequency of micro-increments before hatching could not be validated in the present research. However, the occurrence of these micro-increments have also been reported in other salmonids (Radtke & Dean, 1982; Carreño, 2010), which seems to be a result of the long incubation period in these species. Additionally, it should be noted that these micro-increments were easily identifiable and measurable because they were very distinctive. Therefore, it is highly recommended to perform a study addressed to validate the periodicity of formation of this micro-increments during the embryonary stage, because growth during this period can be used to evaluate the maternal effect on the of offspring quality.

Back-calculated growth patterns

The average growth curve showed similar trends with three stages: (i) embryonary stage, (ii) the period of yolk-sac absorption, and (iii) the post-absorption period. During the first stage the estimated growth rates were based on the assumption that otolith size and embryo size were directly related. The increasing trend observed, irrespective of periodicity of formation of this micro-increments, suggest an increasing growth, presumably as a result of the embryo development. Unfortunately, there is no study based on a similar methodology so as to compare this results. From the second stage and throughout the post yolk sac absorption growth grates gradually decreased likely due to the diminution of yolk-sac and the transition to exogenous feeding.

As a corollary, otolith microstructure analysis proved to be an effective tool to back-calculate growth patterns of rainbow trout alevins once they had absorbed their yolk sac. This technique can be used to evaluate the successfulness of plans adressed to release reared fry rainbow trouts in rivers and tributaries in Chile. This applicability is based on a simple principle, that is, "growth of fry reared under relatively stable environmental conditions and with homogenous diets is expected to differ significantly from their wild counterparts, and the growth differences are recorded in the otoliths". Indeed, to date differences in otoliths microstructure between hatched-reared and wild fry have been documented in juvenile of rainbow trout *Oncorhynchus mykiss* (Hayes, 1995), chinook salmon *O. tshawytscha* (Zhang *et al.*, 1995; Volk *et al.*, 1996) and sockeye salmon *O. nerka* (Finn *et al.*, 1997) in northern hemisphere. As the current study showed micro-increments very distinctive in reared fry of *Oncorhynchus mykiss* this approach can be applied in Chile and worth of further research.

REFERENCES

Baumann, H., M.A. Peck & J.P. Herrmann. 2005. Short-term decoupling of otolith and somatic growth induced by food level changes in post-larval Baltic sprat *Sprattus sprattus*. Can. J. Fish. Aquat. Sci., 56: 539-547.

Blick, D. & P. Hagen. 2001. The use of agreement measures and latent class models to assess the reliability of classifying thermally marked otoliths. Fish. Bull., 101: 1-10.

Bolles, K. & G. Begg. 2000. Distinction between silver hake (*Merluccius bilinearis*) stocks in U.S. waters of the northwest Atlantic based on whole otolith morphometric. Fish. Bull., 98: 451-462.

Breton, B. 2007. El cultivo de la trucha. Ediciones Omega, Barcelona, 338 pp.

Brothers, E.B., D. Williams & P.F. Sale. 1983. Length of larval life in twelve families of fishes at "One Tree Lagoon," Great Barrier Reef, Australia. Mar. Biol., 76: 319-324.

Campana, S.E. 1990. How reliable are growth back-calculation based on otoliths? Can. J. Fish. Aquat. Sci., 47: 2219-2227.

Carreño, D. 2010. Análisis retrospectivo del crecimiento diario durante la etapa de incubación de salmón del Atlántico, S*almo salar*, utilizando micro-incrementos de otolitos. Tesis de Ingeniería en Acuicultura, Pontificia Universidad Católica de Valparaíso, Valparaíso, 33 pp.

Clardy, T., W. Patterson, D. De Vries & C. Palmer. 2008. Spatial and temporal variability in the relative contribution of king mackerel (*Scomberomorus cavalla*) stocks to winter mixed fisheries off South Florida. Fish Bull., 106: 152-160.

Cowen, R.K. 1991. Variation in the planktonic larval duration of the temperate wrasse *Semicossyphus-pulcher*. Mar. Ecol. Prog. Ser., 69: 9-15.

Fowler, A.J. & D.A. Short. 1996. Temporal variation in the early life-history characteristics of the King George whiting (*Sillaginodes punctata*) from analysis of otolith microstructure. Mar. Fresh. Res., 47: 809-818.

Finn, J.E., C.V. Burger & L. Holland-Bartelsa. 1997. Discrimination among populations of sockeye salmon fry with fourier analysis of otolith banding patterns formed during Incubation. Trans. Am. Fish. Soc., 126: 559-578.

Francis, R. 1990. Back-calculation of fish length: a critical review. J. Fish Biol., 36: 883-902.

Fuiman, L.A. 1983. Growth gradients in fish larvae. J. Fish Biol., 23: 117-123.

Fukuwaka, M. 1996. Allometric back-calculation of individual growth for chum salmon otolith during early life. Sci. Hokk. Salm. Hatch., 50: 113-116.

Fukuwaka, M. 1998. Scale and otolith patterns prove growth history of Pacific salmon. N. Pac. Anadr. Fish Comm. Bull., 1: 190-198.

Geffen, A.J. 1983. The deposition of otolith rings in Atlantic salmon *Salmo salar* embryos. J. Fish Biol., 23: 467-474.

Geffen, A.J. 1992. Validation of otolith increment deposition rate. In: D.K. Stevenson & S.E. Campana (eds.). Otolith microstructure examination and analysis. Can. Spec. Publ. Fish. Aquat. Sci., 117: 101-103.

Hayes, J.W. 1995. Importance of stream versus early lake rearingfor rainbow trout fry in Lake Alexandrina, South Island, New Zealand, determined from otolith daily growth patterns. N.Z. J. Mar. Freshw. Res., 29: 409-420.

Hare, J. & R. Cowen. 1995. Effect of age, growth rate, and ontogeny on the otolith size-fish size relationship in bluefish, *Pomatomus saltatrix*, and the implications for back-calculation of size in fish early life history stages. Can. J. Fish. Aquat. Sci., 52: 1909-1922.

Hernández, E. & L. Castro. 2000. Larval growth of the anchoveta *Engraulis ringens* during the winter spawning season off central Chile. Fish. Bull., 98: 704-710.

Hindell, J.S. & G.P. Jenkins. 2004. Spatial and temporal variability in the assemblage structure of fishes associated with mangroves (*Avicennia marina*) and intertidal mudflats in temperate Australian embayments. Mar. Biol., 144: 385-395.

John, R., R.J. Williams, A.M. Fowler, R.C. Deborah & I.M. Suthers. 2010. Identifying critical estuarine seagrass habitat for settlement of coastally spawned fish. Mar. Ecol. Prog. Ser., 408: 181-193.

Jones, C. 1992. Development and application of the otolith increment technique. Can. Spec. Publ. Fish. Aquat. Sci., 117: 1-11.

Kohn, Y.Y. & K.D. Clements. 2011. Pelagic larval duration and population connectivity in New Zealand triplefin fishes. Environ. Biol. Fish., 91: 275-286.

Larsen, K.A. & R.R. Reisenbichler. 1993. The importance of estuarine habitats of the Skagit River, WA, to juvenile chinook salmon, *Oncorhynchus tshawytscha*. Presentation at the International Symposium of Fish Otolith Research and Application January, SC, 23-27.

Leonardi, M., R. Vega & E. Tarifeño. 1991. Efecto del nivel de lípido en la dieta de trucha arcoíris KAMLOOP *Oncorhynchus mykiss* Jordan, 1982 en el crecimiento, factor de condición y coeficiente de conversión del alimento durante la fase de agua dulce. Rev. Biol. Mar., Valparaíso, 26(2): 253-266.

Mosegaard, H., H. Svedang & K. Taberman. 1988. Uncoupling of somatic and otolith growth rates in Arctic Char. Can. J. Fish. Aquat. Sci., 45: 1514-1524.

Mugiya, Y. & H. Oka. 1991. Biochemical relationship between otolith and somatic growth in the rainbow trout (*Oncorhynchus mykiss*): consequence of starvation, resumed feeding, and diel variations. Fish. Bull., 89: 239-245.

Neilson, J.D. & G.H. Geen. 1982. Otoliths of chinook salmon (*Oncorhynchus tshawytscha*): daily growth increments and factors influencing their production. Can. J. Fish. Aquat. Sci., 39: 1340-1347.

Neilson, J.D. & G.H. Geen. 1985. Effects of feeding regimes and temperature cycles on otolith increment formation in juvenile chinook salmon (*Oncorhynchus tshawytscha*). Fish. Bull., 83: 91-101.

Ojanguren, A.F., F.G. Reyes & R. Rodríguez. 1999. Effects of temperature on growth and efficiency of yolk utilization in eggs and pre-feeding larval stages of Atlantic salmon. Aquacult. Int., 7: 81-87.

Peñailillo, J. & M. Araya. 1996. Momento de formación y periodicidad de los micro-incrementos de crecimiento en otolitos de larvas de pejerrey (*Austro-menidia regia*) mantenidas en laboratorio. Invest. Mar., Valparaíso, 24: 31-38.

Plaza, G., M. Ishida & D. Aoyama. 2008. Temporal patterns of growth in larval cohorts of the Japanese

sardine *Sardinops melanostictus* in a coastal nursery area. J. Fish Biol., 73: 1284-1300.

Plaza, G., S. Katayama & M. Omori. 2001. Otolith microstructure analysis of the black rockfish *Sebastes inermis.* Mar. Biol., 139: 797- 805.

Quiñonez-Velásquez, C. & J.R. López-Olmos. 2011. Juvenile growth of white mullet *Mugil curema* (Teleostei: Mugilidae) in a coastal lagoon southwest of the Gulf of California. Lat. Am. J. Aquat. Res., 39(1): 25-32.

Radtke, R.L. & J.M. Dean. 1982. Increment formation in the otolith of embryos, larvae and juveniles of the mummichong, *Eundulus heteroclitus*. Fish. Bull., 80: 201- 215.

Shafer, D.J. 2000. Evaluation of periodic and aperiodic otolith structure and somatic-otolith scaling for use in retrospective life history analisys of a tropical marine goby, *Bathygobius coalitus.* Mar. Ecol. Prog. Ser., 199: 217-229.

Sponaugle, S. & R.K. Cowen. 1994. Larval durations and recruitment patterns of two Caribbean gobies (Gobiidae): Contrasting early life histories in demersal spawners. Mar. Biol., 120: 133-143.

Turan, C. 2006. The use of otolith shape and chemistry to determine stock structure of Mediterranean horse mackerel *Trachurus mediterraneus* (Steindachner). J. Fish. Biol., 69: 165-180.

Van Der Walt, B. & R.A. Faragher. 2002. Thermal marking of rainbow trout (*Oncorhynchus mykiss*) otoliths Can. J. Fish. Aquat. Sci, 36: 883-888.

Victor, B.C. 1982. Daily otolith in increments and recruitment in two coral-reef wrasses *Thalassoma bifasciatum* and *Halichoetes biyittayus.* Mar. Biol., 71: 203-208.

Volk, E.C., D.G. Mortensen & A.C. Wertheimer. 1995. Nondaily otolith increments and seasonal changes in growth of a pink salmon (*Oncorhynchus gorbuscha*) population in Auke Bay, Alaska. In: D.H. Secor, J.M. Dean & S.E. Campana (eds.). Recent developments in fish otolith research. University of South Carolina Press, Columbia, pp. 211-225.

Volk, E.C., S.L. Schroder & J.J. Grimm. 1996. Otolith thermal marking. In: Report of the study group on stock identification protocols for finfish and shellfish stocks. ICES CM 1996/M1: 95-129.

Wright, P.J., N.B. Metcalfe & J.E. Thorpe. 1990. Otolith and somatic growth rates in Atlantic salmon parr, *Salmo salar* L., evidence against coupling. J. Fish. Biol., 36: 241-249.

Wu, L., J.S. Liu, X.L. Wang, G. Zhang, Z.Y. Zhang, B.R. Murphy & S.G. Xie. 2011. Identification of individuals born in different spawning seasons using otolith microstructure to reveal life history of *Neosalanx taihuensis.* Fish. Sci., 77: 321-327.

Zhang, Z., R.J. Beamish & B.E. Riddell. 1995. Differences in otolith microstructure between hatchery-reared and wild Chinook salmon (*Oncorhynchus tshawytscha*). Can. J. Fish. Aquat. Sci., 52: 344-352.

Zimmerman, C. & R. Nielsen. 2003. Effect of analytical conditions in wave length dispersive electron microprobe analysis on the measurement of strontium-to-calcium (Sr/Ca) ratios in otoliths of anadromous salmonids. Fish. Bull., 101: 712-718.